

Original Article

FORMULATION AND PRODUCTION OF GRANULE FROM *ANNONA MURICATA* FRUIT JUICE AS ANTIHYPERTENSIVE INSTANT DRINK

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ABSTRACT

Objective: The present study aims to formulate instant granule drink from soursop (*Annona muricata* Linn) fruit juice and investigate its antihypertensive effect on Sprague-Dawley rats suffer from level 1 hypertensive disease.

Methods: Soursop instant granule was formulated using the wet granulation method, comprising of soursop fruit juice as the main ingredient with the addition of natrium carboxymethyl cellulose (CMC), citric acid, saccharalose, and maltodextrin as excipient and flavoring substances. The obtained granule, then administered orally to natrium chloride (NaCl)-induced hypertensive Sprague-Dawley rats and the systolic and diastolic blood pressure of rats was measured subsequently on the day 4th, 8th, 11th and 14th of treatment.

Results: The granule obtained from soursop fruit juice has very good physical characteristics, colored white, has fresh sweet and sour taste with the typical smell of soursop fruit. Other physical characteristics of the granule were it has 2.9% water content (<5%), 1.91 % ash content, 3.13 ml/s solubility, 29.3% angle of repose, and stable in the storage at 15 °C for two months. The oral administration of soursop granule significantly decrease the systolic and diastolic blood pressure in both male and female Sprague-Dawley NaCl-induced hypertension rats.

Conclusion: The granule produced from the juice of soursop fruit has acceptable physical features and proved effective to decrease high blood pressure, hence the soursop granule could be produced as an antihypertensive instant fruit drink in an industrial scale to substitute synthetic antihypertensive drugs.

Keywords: *Annona muricata* Linn, Antihypertensive medicinal herbal, Antihypertensive instant granule, Soursop fruit herbal drink

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INTRODUCTION

Hypertension has become a mayor cause of mortality throughout the world and constitutes a mayor risk factor for several cardiovascular diseases such as atherosclerosis, heart failure, renal insufficiency, coronary artery disease and stroke. The risk factor increases with the age in both sexes. According to World Health Organization, about one-third of the world's population suffers from hypertension and incidence has been increasing at a rapid rate due to human modern lifestyle [1]. Many efficacious synthetic drugs have been developed for the treatment of hypertension but these drugs have been to cause side effects such as insomnia, angioedema, cough, and fetal abnormalities [2]. In response to this circumstance, herbal medicines with hypotensive activity, therefore have become the object of interest in the recent because of their ease of availability, less adverse side effects and cost-effective [3, 4].

Annona muricata, a precious healthy fruit widely known as graviola or soursop is easily found in most of the tropical countries and has long been used as a natural remedy for a variety of illnesses and subject of countless medicinal uses. The decoction leaves and bark of soursop traditionally believed to treat high blood pressure and diabetes [5]. These folkloric uses have been scientifically validated since the 1940s and many studies reported that the bark and leaves of the soursop tree contain antioxidant, insecticide, antipyretic, and antibacterial as well [6, 7]. The properties and actions of soursop mostly documented by traditional uses were its ability to lower high blood pressure [8] meanwhile recent studies by different researchers demonstrated that the bark, as well as the leaves, have anti-hypertensive, vasodilator, antispasmodic (smooth muscle relaxant) and cardio depressant (slowing of heart rate) activities in animals [9]. Beside the bark and leaves, other data revealed that soursop fresh fruit also shows anti-depressive effect and toxicity against prostate PC-3 cancer cells [10].

Despite the fact that effects of medicinal plants have scientifically been proven and widely used in the modern world, very little work has been done to process these natural ingredients into more practical and easy to drink formula. Therefore, preparation of soursop juice to obtain a pleasant instant granule beverage, which also has an antihypertensive effect was considered as the main topic of this study.

Granules are agglomerates of powdered materials prepared into larger, free-flowing particles or a small compact particle of a substance. The shape of granules is generally irregular with the size typically fall within the range of 850 µm (no. 20 sieve) to 4.75 mm (no. 4 sieves) size. Granules have a smaller surface area than a comparable volume of powders which makes this form more stable physically and chemically than the corresponding powders and are less likely to cake or harden upon standing than are powders [11]. Granulation may be defined as size enlargement process which converts fine or coarse powder into physically larger and stronger particles. Granulation also produces particle-size uniformity, thus content uniformity.

Herbs in form of concentrated granules has gain its popularity as hot and cold instant drinks to take medicines in many markets. Individual sachets contain a single dose of granules that dissolves in water, making it easy to dose and stored for subsequent use and other advantages. Compare to other forms such as syrup or powder, the medicinal herbal in form of granule offer many advantages such as having good flow property, better compression characteristics and uniformity, easy to dose and swallow, remain stable in hot water, dissolve quickly and without leaving residues, even in cold water [12]. The granules also easily combined with colourants, flavorants, and other pharmaceutical ingredients, so the resulting solution or suspension has all the desired medicinal and pharmaceutical features of a liquid pharmaceutical.

MATERIALS AND METHODS

Chemicals and apparatus

Tools used in the study were digital scales (AND G-120®), non-invasive blood pressure monitor (CODA, Kent Scientific Corp.), evaporator (Buchi Healthy Bath B-490, Syncore®), moisture balance (AND MX 50®), freeze dryer (GVD-12, Girovac Ltd), granule flow tester (EFT-10, Electrolab India), powder tap densitometer (USP Bulk Density Tester® 315-2E), sieved (Forward Filter and Fitting Co. Ltd), oven (Ney®), and glass tools. All chemicals were of analytical grade, a product of Merck and Co., Inc. and Sigma-Aldrich Inc.

Preparation of plant material

100 kg of mature soursop fruits was bought from the local market in Bogor, Indonesia. The soursop fruits were then washed carefully

under flowing tap water, peeled, cut into halves, and deseeded. Fruit flesh obtained were put in heat resistant containers and steamed for 4 min over boiling water. The steamed fruit flesh then keep in the refrigerator for subsequent uses.

Preparation of instant granule

The fruit flesh was placed on a plain cotton cloth and squeezed using wringer to obtain pure fruit juice. The fruit juice was dried in freeze dryer machine with the addition of 20% of maltodextrin as a filler to obtain instant fruit powder (active ingredient). The formula of instant granule was produced based on author's experimental works. The active ingredient was mixed with CMC, citric acid, sakaralose, and maltodextrin as excipient and flavouring substances as listed in the table (1). The mixture then granulated by the wet granulation method.

Table 1: Instant granule formula

Ingredients	Percentage (%)	Weight (g)
Soursop juice powder	63	12.6
CMC	1	0.2
Citric acid	2	0.4
Saccharalose	0.1	0.02
Maltodextrin	33.9	6.78
Total Weight	100%	20 g
Active ingredients per sachet	12.6 g (equivalent to 100 g of soursop fresh weight)	

Soursop juice powder, CMC, citric acid, sucralose and maltodextrin were weighed according to the formula, mixed, and sieved through a 30-mesh sieve. The mixture was stirred until homogenous before sprayed with 70% ethanol sufficiently to form granule mass and dried in an oven at 40 °C-50 °C for 30 min. The coarse granule obtained from this process was sieved through a 12-mesh sieve, sprayed with 70% ethanol and dried in an oven at 40 °C-50 °C for 3 h and received through a 12-mesh sieve. The obtained granule was evaluated for physical characteristics such as color, water content, ash content, solubility, the angle of repose, and stability in storage. Finally, product was packaged in a small sachet containing 20 gr of soursop instant drink. Based on the formula above, the content of the active ingredient in each sachet drink is 12.6 gr which was equivalent to 100 g of soursop fresh fruit weight.

Experimental design and animals acclimatisation

The experiment in this study was conducted using randomized controlled design. Adult male and female Sprague-Dawley rats weighing between 200-250 g were prepared separately as test animals. The rats were randomly divided into 10 experimental groups (five female groups and 5 male groups, each group consisting of 10 rats). Prior to the experiment, the rats acclimatised for a period of 2 w under a controlled environmental condition in accordance with the standard of the care and use of laboratory animals. The systolic and diastolic pressure of rats during acclimatisation period were measured using non-invasive rat tail blood pressure

instrument to ensure the rats had normal blood pressure ranging from 120/80 to 130/90 mm Hg.

High blood induction and animals treatment

In order to increase the blood pressure, rats of all experimental groups were orally administered with 2 ml of 4.5% NaCl daily for ten consecutive days. The increase in rats' blood pressure was then observed and measured. After high blood pressure induction, each group of experimental rats was treated daily with different doses of soursop granule as follows (see table 2):

Group 1 administered with 90 mg/200 g b. wt. soursop instant granule

Group 2 administered with 180 mg/200 g b. wt. soursop instant granule

Group 5 administered with 270 mg/200 g b. wt. soursop instant granule

Group 4 (positive control) administered with 0.45 mg/200 g b. wt. captopril (common antihypertensive drug)

Group 5 (normal control) administered with 2 ml aquades/200 g b. wt.

The blood pressure of rats was measured subsequently on the day 4th, 8th, 11th and 14th.

Table 2: Grouping of experimental rats based on dose of soursop granule

Groups of rat	Dose of soursop granule
Group 1	90 mg/200g b. wt.
Group 2	180 mg/200g b. wt.
Group 3	270 mg//200g b. wt.
Group 4	captopril 0.45 mg/200 g b. wt.
Group 5	2 ml aquades/200 g b. wt.

Statistical analysis

All the values of systolic and diastolic pressure were expressed as mean±SD (Standard Deviation). Statistical differences between the means of various groups were evaluated by Duncan's multiple range test (MRT) where means followed by the same letter in the same column are not significantly different at P≤0.05.

RESULTS AND DISCUSSION

Formulation and production of soursop juice fruit instant granule

The soursop juice powder obtained from 10 kg of soursop fruit with the addition of 20% maltodextrin was 1.575 kg or equivalent

to 1.26 kg pure soursop juice powder without addition of maltodextrin. This data shows that fresh soursop fruit effectively could produce 12.6% dry active ingredients. The instant drink obtained from processing soursop juice powder as the main ingredient has good physical characteristics as shown in fig. 1 appeared as bright white granule with typically fresh sour sweet taste and smell, 2.9% water content (<5%), 1.91 % ash content, 3.13 ml/s solubility, 29.3% angle of repose, and stable in the storage at 15 °C for two months.

The physical characteristics of soursop granule produced by wet granulation method meet the industrial standard and may be considered for large-scale production of high quality anti-hypertensive instant fruit drink.

NaCl induction

Average of systolic and diastolic on male rats before and after 5.5% NaCl induction is presented in the table (3).



Fig. 1: Instant granule produced from juice of soursop fruit

Table 3. Average of systolic and diastolic pressure of male rats after NaCl induction

Groups	Before induction		After induction	
	Systolic (mmHg)	Diastolic (mmHg)	Systolic (mmHg)	Diastolic (mmHg)
Group 1	106.8±5.4	86.3±8.3	151.8±7.2	101.0±6.6
Group 2	113.5±7.6	85.5±5.8	149.8±7.5	106.3±9.2
Group 3	118.3±5.8	88.0±2.7	152.0±6.85	112.0±8.2
Group 4	124.3±2.4	85.3±4.8	146.5±3.4	98.5±8.75
Group 5	114.0±4.75	88.5±8.3	151.5±3.8	107.3±6.4

Data are expressed as mean±SD of ten rats in each group.

Both of systolic and diastolic pressure of all groups appear to increase after induction with 5.5% NaCl for ten consecutive days. Average of systolic and diastolic pressure before NaCl induction were 115,4±7,4 mmHg and 86,7±4 mmHg respectively meanwhile systolic and diastolic pressure after NaCl induction increased to 150,3±3,2 mmHg and 105±5,8 mmHg respectively. This condition indicated that all experimental rats suffer from level 1 hypertensive disease.

Hypotensive effect of soursop instant granule on male rats

The decrease of systolic and diastolic blood pressure in experimental male rats during treatment was presented in table (4) and table (5).

Table (4) and table (5) revealed significant differences between treatment groups and untreated control group in which the decrease of systolic and diastolic pressure occur in all treatment groups. Normal systolic and diastolic tension occurs on day 4 in group 4 and on day 11 in group 1, 2, and 3.

Hypotensive effect of soursop granule on female rats

The increase of systolic and diastolic blood pressure in experimental female rats after NaCl induction was presented in table (6) and a decrease of systolic and diastolic pressure during treatment were presented in table (6) and table (7).

Table 4: Average of systolic pressure of male rats during treatment period

Groups	Systolic pressure (mmHg)				
	Day 1	Day 4	Day 8	Day 11	Day 14
Group 1	151.8±1.9	147.5 ^d ±3.0	141.0 ^{bc} ±4.7	112.3 ^c ±4.4	109.5 ^c ±4.7
Group 2	149.8±1.3	144.3 ^c ±1.6	140.0 ^b ±1.7	110.0 ^{bc} ±5.4	107.3 ^b ±2.9
Group 3	152.0±2.7	141.8 ^b ±1.1	138.8 ^b ±1.1	106.5 ^{ab} ±3.6	104.0 ^{ab} ±2.2
Group 4	146.5±3.6	131.3 ^a ±0.4	113.8 ^b ±4.2	102.5 ^a ±1.7	101.8 ^a ±1.5
Group 5	151.5±1.5	148.5 ^d ±1.1	147.5 ^c ±2.2	147.8 ^d ±2.9	143.3 ^d ±2.4

Data are expressed as mean±SD of ten rats in each group, where the means followed by same letter in the same column are not significantly different at P≤0.05 according to Duncan's multiple range test.

Table 5: Average of diastolic pressure of male rats during treatment period

Groups	Diastolic pressure (mmHg)				
	Day 1	Day 4	Day 8	Day 11	Day 14
Group 1	101.0±0.7	92.3 ^a ±6.4	88.5 ^a ±5.7	81.4 ^a ±1.3	80.8 ^a ±0.83
Group 2	106.3±2.4	103.8 ^a ±4.2	89.5 ^c ±2.2	86.3 ^c ±1.9	83.5 ^b ±3.34
Group 3	112.0±5.5	104.3 ^b ±1.9	88.3 ^c ±2.2	85.8 ^b ±2.8	81.3 ^b ±1.6
Group 4	98.5±2.2	96.5 ^a ±2.3	87.3 ^{ab} ±1.9	85.0 ^b ±3.9	80.8 ^{ab} ±2.5
Group 5	107.3±2.8	104.0 ^b ±5.2	100.0 ^{bc} ±3	98.1 ^{ab} ±5.8	94.3 ^c ±1.1

Data are expressed as mean±SD of ten rats in each group, where the means followed by the same letter in the same column are not significantly different at P≤0.05 according to Duncan's multiple range test.

Table 6: Average of systolic and diastolic pressure of female rats after NaCl induction

Groups	Before NaCl induction		After NaCl induction	
	Systolic (mmHg)	Diastolic (mmHg)	Systolic (mmHg)	Diastolic (mmHg)
Group 1	109.25±7.3	87.50±5.4	148.5±7.5	112.75±6.6
Group 2	101.75±4.3	82.50±3.8	145.5±8.66	113.50±7.45
Group 3	113.0±2.24	91.75±7.4	144.75±6	104.25±8.7
Group 4	119.25±6.3	91.50±4.6	152.75±3.8	106.75±3.75
	106.75±6.3	88.64±3.4	144.35±5.3	114.50±6.7

Data are expressed as mean±SD of ten rats in each group.

Table 7: Average of systolic pressure of female rats during treatment period

Groups	Systolic pressure (mmHg)				
	Day 1	Day 4	Day 8	Day 11	Day 14
Group 1	148.5 ± 7.8	140.3 ^c ±5.3	129.5 ^b ±6.1	114.0 ^b ±8.4	110.8 ^b ±5.6
Group 2	145.5±2.2	139.4 ^{bc} ±4.2	130.6 ^b ±9.6	112.5 ^b ±2.2	107.8 ^b ±4.9
Group 3	144.8±8.7	134.5 ^b ±4.7	115.0 ^a ±9.9	105.5 ^a ±5.0	104.8 ^{ab} ±5.6
Group 4	152.8±8.8	127.8 ^a ±4.2	114.0 ^a ±4.2	103.8 ^a ±2.7	101.2 ^a ±1.5
Group 5	144.0±2.7	141.3 ^c ±1.3	139.2 ^b ±0.84	129.4 ^c ±4.1	124.2 ^c ±5.31

Data are expressed as mean±SD of ten rats in each group, where the means followed by same letter in the same column are not significantly different at P≤0.05 according to Duncan multiple range test.

Table 8: Average of diastolic pressure of female rats treatment period

Groups	Diastolic pressure (mmHg)				
	Day 1	Day 4	Day 8	Day 11	Day 14
Group 1	112.8 ± 4.6	103.5 ^{bc} ± 4.1	93.5 ^{ab} ± 7.0	91.5 ^a ± 6.0	90.3 ^b ± 5.9
Group 2	113.5 ± 2.8	105.3 ^c ± 4.9	96.5 ^b ± 4.4	91.3 ^a ± 3.6	89.3 ^{ab} ± 4.9
Group 3	104.3 ± 3.9	95.8 ^a ± 4.9	93.8 ^a ± 4.0	88.5 ^a ± 3.88	88.5 ^{ab} ± 3.9
Group 4	106.8 ± 3.6	100.3 ^b ± 1.1	90.3 ^a ± 0.43	88.0 ^a ± 0.71	85.3 ^a ± 1.1
Group 5	104.5 ± 1.8	100.5 ^b ± 0.7	96.8 ^b ± 1.26	90.8 ^b ± 0.9	92.0 ^b ± 2.45

Data are expressed as mean±SD of ten rats in each group, where the means followed by same letter in the same column are not significantly different at P≤0.05 according to Duncan multiple range test.

Similar to the results of male rat groups, administration of the soursop granule show to decrease systolic and diastolic blood pressure of female rats. Both systolic and diastolic pressure reach its normal rate on day 4 in group 4, and on day 11 in group 1, 2, and 3. The concentration of granule affected the treatment period, the higher the concentration of granule, the shorter the treatment time. Overall, the data revealed an interesting fact that the effectiveness of soursop instant granule in decreasing systolic and diastolic pressure compares to the effectiveness of captopril were not significantly different. The difference only occurs in the treatment period in which captopril could decrease blood pressure in shorter treatment period than the granule.

The ability of soursop fruit to decrease high blood pressure possibly related to the high potassium content which found to range from 1.29% to 1.35% [13]. Potassium is important in maintaining fluid and electrolyte balance in the bodies of humans and animals where a high potassium intake will increase its concentration in the intracellular fluid. The high concentration of potassium in intracellular fluid tend to absorb the extracellular fluid which, at the end, cause to lower blood pressure [14, 15]. Recent studies on role potassium in regulating blood pressure strengthen this assumption and has proven that the hypotensive effects of soursop fruit are not mediated through muscarinic, histaminergic, adrenergic and nitric oxide pathways, but through peripheral mechanisms involving antagonism of Ca²⁺ [16].

Other studies, however, suggest that the hypotensive properties of soursop fruit probably related to high polyphenol and antioxidant compounds found in various part of soursop fruit. Antioxidant and phenolic compounds decrease high blood pressure by increasing nitric oxide and prostacyclin formation, increasing endothelium-derived hyperpolarizing factor, mediated vasorelaxation and decreasing low-density lipoprotein formation and endothelial dysfunction [17, 18].

Hence, further research should be conducted to elucidate compounds responsible for decreasing high blood pressure in soursop fruit.

CONCLUSION

The granule produced from the juice of soursop fruit proved effective to decrease systolic and diastolic pressure in both male and female NaCl-induced hypertensive Sprague-Dawley rats. The possible mode of action of the granule might be related to high potassium and phenolic content found in the soursop fruit. The results of this study indicated that soursop granule has a potent as an antihypertensive instant drink to prevent and treat high blood pressure diseases and can be produced in industrial scale using simple and inexpensive technology.

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CONTRIBUTION OF AUTHORS

The first and second author have participated in the work including participation in the concept, design, analysis, writing, and revision of the manuscript.

CONFLICT OF INTERESTS

Authors hereby declare that there were no conflict of interests in the accomplishment of this study.

REFERENCES

1. World Health Organization in collaboration with the World Heart Federation and the World Stroke Organization. Global atlas on cardiovascular diseases prevention and control. Geneva Switzerland: WHO Press; 2011. p. CH-1211.

2. Yeo SK, Ooi TJ, Linn TJ, Liong MT. Antihypertensive properties of plant-based prebiotics. *Int J Mol Sci* 2009;10:3517-30.
3. Tabassum N, Feroz Ahmad F. Role of natural herbs in the treatment of hypertension. *Phcog Rev* 2011;5:30-40.
4. Najmi A, Nasiruddin M, Khan RA, Haque SF. Indigenous herbal product *Nigella sativa* proved effective as an antihypertensive in metabolic syndrome. *Asian J Pharm Clin Res* 2013;6:61-4.
5. Badrie N, Schauss AG. Soursop (*Annona muricata* L.): Composition, nutritional value, medical uses, and toxicology. In: Watson, R. R., and Preedy, V. R. (eds.), *Bioactive foods in promoting health*. Oxford; 2009. p. 621-43.
6. Kedari TS, Khan AA. Guyabano (*Annona muricata*): A review of its traditional uses, phytochemistry and pharmacology. *Am J Res Commun* 2014;2:247-68.
8. Kumar S, Venkatarathanamma, Saibabu N, Ram S. Antipyretic activity of annona plants leaves on brewer's yeast induced febrile rats. *Asian J Pharm Clin Res* 2015;8:210-12.
9. Sushmita C, Latika S, Manoranjan PS. Phytochemical and antimicrobial screening of *Annona muricata* leaf extracts against clinical important gastrointestinal pathogens. *J Natural Prod Plant Resource* 2012;2:524-9.
10. Moghadamtousi SZ, Fadaeinasab M, Nikzad S, Mohan G, Ali MH, Habsah Abdul Kadir HA. *Annona muricata* (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. *Int J Mol Sci* 2015;16:15625-58.
11. Patel S, Patel JK. A review on a miracle fruits of *Annona muricata*. *J Pharmacogn Phytochem* 2016;5:137-48.
12. Aulton M. *Pharmaceutic: The science of dosage form design*. Edinburgh: Churcill Livingstone; 2000.
13. Allen LV, Ansel HC. *Pharmaceutical dosage, forms and drug delivery system*. 10th ed. Philadelphia: Lippincott Williams and Wilkins, a Wolter Kluwer Business; 2005.
14. Degnon RG, Adjou ES, Noudogbessi JP, Metome G, Boko F, Edwige DA, *et al.* Investigation on nutritional potential of soursop (*Annona muricata* L.) from Benin for its use as food supplement against protein-energy deficiency. *Int J Biosci* 2013;3:135-44.
15. Waring SW, Shahana E. 2005. How should serum uric acid concentration be interpreted in patients with hypertension. *Curr Hypertens Rev* 2005;1:89-95.
16. Nwokocha CR, Owu DU, Gordon A, Thaxter K, McCalla G, Ozolua RI, *et al.* Possible mechanisms of action of the hypotensive effect of *Annona muricata* (soursop) in normotensive Sprague-Dawley rats. *Pharm Biol* 2012;50:1436-41.
17. Adefegha SA, Sunday IO, Ganiyu O. Distribution of phenolic contents, antidiabetic potentials, antihypertensive properties, and antioxidative effects of soursop (*Annona muricata* L.) fruit parts *in vitro*. *Biochem Res Int* 2015. <http://dx.doi.org/10.1155/2015/347673>
18. Jean-Claude S, Chataigneau T, Ndiaya M, Oka MH, Jasser BE, Chataigneau M. Vascular protection by dietary polyphenols. *Eur J Pharmacol* 2004;500:299-313.
19. Rajesh P. Antioxidant property of antihypertensive drugs (carvedilol, enalapril, and amlodipine) on liver function in animal (rats) models. *Asian J Pharm Clin Res* 2016;9:292-3.

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