

# Test of Antidiarrheal Effect of Pomegranate (*Punica Granatum L*) Leaf Infusion to the Mature Male White Mice (*Rattus Norvegicus*) Which Induced by Oleum Ricini

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## Abstract

Pomegranate (*punica grantum L*) leaf has been used in traditional medication which potential to cure diarrhea, because it contains of tannin compound. However, the application is only based on empirical experience. This research aims to know antidiarrheal effect and the most effective concentration from pomegranate leaf infusion which affected as antidiarrheal. This research type was experimental research by using 15 mice as research subject which divided in 5 test group, group which given by pomegranate leaf infusion with concentration 12%w/v, 24%w/v, 36%w/v. Positive control group which given by suspension of Loperamide HCl and negative control given by distilled water. The solutions and induction were given by per oral. After the treatment, it was conducted by observation and also records effect which occurred to each mice using many test parameters such as diarrhea frequency, feces consistency, and diarrhea duration. To analyze research result, it was used by ANAVA test and continued by using SNK test. The research result showed that from three concentrations (12%w/v, 24%w/v, 36%w/v) of pomegranate (*punica granatum L*) leaf infusion which tested to the white mice, it could be concluded that in the concentration 36%w/v showed the most effective effect due to it was equal with loperamide HCl.

**Keywords:** Antidiarrheal, Pomegranate (*punica granatum L*) Leaf Infusion, White Mice

## I. INTRODUCTION

Today, diarrhea still becomes one of health problems. Million cases are reported, in every year it is predicted that there are about 4-5 million people die due to severe diarrhea. Diarrhea commonly found in geographic area either developed country or developing country such as Indonesia. In the developed country, although it has health improvement and high socio economic, but the incident (disease number) of diarrhea is still high and still becomes a health problem (Loehoeri, 1998).

Based on data of Indonesia health profile 2010, it shows that diarrhea is still high, which is from the largest 10 disease patterns, diarrhea includes into second rank after ISPA (Acute Respiratory Infection). The number of diarrhea case is about 213,435 people with mortality number about 1,289, and about 70-80% from the amount suffered by children especially under 5 years old. From the data above, it can be predicted that for 20-30 years in the future, diarrhea and many other infection diseases will still be a concern as the cause of health problem in the world.

Potential plant to cure diarrhea, for example, is pomegranate. Pomegranate mostly planted in the farm or yard as ornamental plant and medical plant (Tjitrosoepomo, 1994). It is also supported by Winarno and Dian (1996) who reported that pomegranate leaf is one of 117 plants which used by people in many regions in Indonesia to cure diarrhea. It is conducted by boiling 20 grams pomegranate leaf in 300 mL water. Compound content that predicted as active agent for antibacterial in healing diarrhea to the pomegranate leaf is tannin compound.

## II. RESEARCH METHOD

### 1. Independent variable

Independent variable in this research was pomegranate leaf infusion with concentration 12% w/v, 24%w/v, 36%w/v.

### 2. Dependent variable

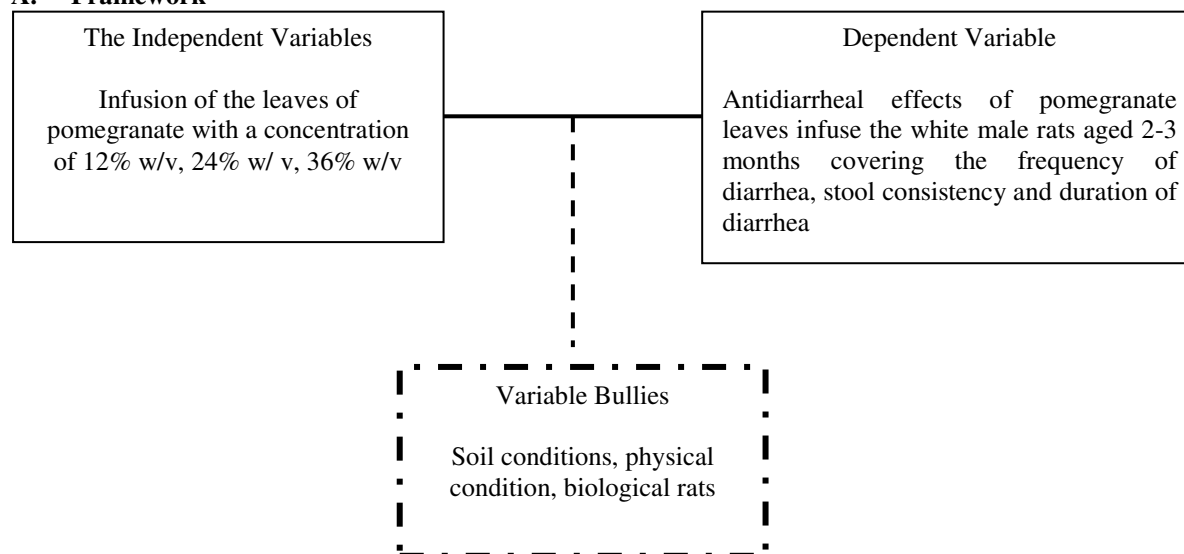
Dependent variable in this research was antidiarrheal effect of pomegranate leaf

### 3. Confounding variable

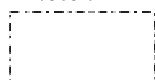
Confounding variable was soil condition for plant growth, physical and biological condition of mice.


Laboratory animal that used in this research was mice with criteria as follow: family muridae, genus rattus, species 2-3 months male *R.novergicus* with the weight 200-250 g which obtained from Biara Karmel Kupang.

## A. Framework



### Note :

 Variables examined

 Variables that are not investigated

## B. Operational Definition

1. Test of antidiarrheal infusion of pomegranate leaf to the 2-3 months male white mice is test that conducted to know antidiarrheal effect seen from diarrhea frequency, feces consistency, and diarrhea duration.
2. Feces consistency to the white mice is feces types which including thin, mushy, and less mushy.
3. Pomegranate leaf infusion is extraction process of active agent from pomegranate leaf with water solvent at the temperature 90°C for 15 minutes.

## C. Data Collection

### 1. Concentration Design

Calculation of pomegranate (*punica granatum L.*) leaf concentration

Made by using three concentrations. The application of pomegranate leaf as antidiarrhea empirically is by boiling the green and fresh pomegranate leaf for 20 g/300 mL and then strained and ready to drink (Winarno, 1996).

To determine concentration, it uses formulation as follow:  $Y_n = Y_1 \times R$

Note:

$Y_n$ : the n-dosage of the first dosage;

$Y_1$ : pacemaker factor (1, 2, 3, and so on);

$R$ : the first line dosage (Analisis Hayati: 60).

Based on the reference above, pomegranate leaf concentration made as follow:

#### a. First Concentration

Concentration that used for human is 20 g/300 mL to drink once.

Mice dosage: 20 g x 0.018 – 0.36 g/3 mL

Material that needed to make pomegranate leaf infusion in 100 mL:

$(100 \text{ mL} / 3 \text{ mL}) \times 0.36 \text{ g} = 12 \text{ g}/100 \text{ mL} = 12\% \text{ w/v}$

#### b. Second Concentration

The second concentration would be determined as follow:

$Y_n = Y_1 \times R$ ;  $Y_n = 0.36 \text{ g} \times 3$ ;  $Y_n = 0.72 \text{ g}/3 \text{ mL}$

Material that needed to make pomegranate leaf infusion in 100 mL:

$(100 \text{ mL} / 3 \text{ mL}) \times 0.72 \text{ g} = 24 \text{ g}/100 \text{ mL} = 24\% \text{ w/v}$

#### c. Third Concentration

The third concentration would be determined as follow:

$$Y_n = Y_1 \times R; Y_n = 0.36 \text{ g} \times 3; Y_n = 1.08 \text{ g}/3 \text{ mL}$$

Material that needed to make pomegranate leaf infusion in 100 mL:

$$(100 \text{ mL} / 3 \text{ mL}) \times 1.08 \text{ g} = 36 \text{ g}/100 \text{ mL} = 36\%w/v$$

d. Design for positive control

As positive control that would be used was dosage Loperamide HCl for human, which is 2 mg then for mice dosage was  $2 \times 0.018 = 0.036 \text{ mg}/3 \text{ mL}$ .

Loperamide HCl that needed to make 100 mL as follow:  $(100/3) \times 0.036 = 1.2 \text{ mg}/100 \text{ mL}$

e. Design for negative control

As negative control, it was used by distilled water in which each mice given by per oral for 3 mL.

**2. Pomegranate (*Punica granatum L.*) leaf infusion making**

a. Infusion solution making

1) Fresh pomegranate (*punica granatum L.*) leaf that been washed well and chopped.

2) Then weighted for each concentration that needed

3) Each is soaked into erlenmeyer

4) Add water until 100 mL into erlenmeyer.

5) Heat each container and pay attention until the temperature reaches 90°C and then cooled for 15 minutes while it is stirred, then strained using flannel fabric. The decrease of volume due to evaporation is added by 100 mL distilled water.

**3. Test procedure**

Test procedure was conducted through steps as follow:

a. Mice were adapted for 2 weeks.

b. Mice were fasted for 3 hours before it given by treatment.

c. Preparing pomegranate (*punica granatum L.*) leaf infusion with many concentrations

d. Preparing Oleum ricini

e. Preparing loperamide HCl

f. Each group of mice given by treatment as follow: all mice were induced by oleum ricini for 1 mL orally. After 30 minutes, it was given by treatment as follow:

Negative control: Given by 3 mL distilled water

Positive Control: Given by loperamide HCl solution with concentration 12%w/v.

Group A1: Pomegranate (*punica granatum L.*) leaf infusion with concentration 12%w/v

Group A2: Pomegranate (*punica granatum L.*) leaf infusion with concentration 24%w/v

Group A3: Pomegranate (*punica granatum L.*) leaf infusion with concentration 36%w/v

g. Each tested animal was located in individual container on filter paper to ease the observation. Response that occurred to the mice was observed every 30 minutes for 7 hours.

h. Observe response that occurred to the mice for starting time of diarrhea until it is stopped, diarrhea frequency, and feces consistency.

i. Analyzing the obtained data.

**4. Data analysis**

The obtained data was calculated its average and then analyzed statistically using One Way Variance Analysis (ANOVA) and Complete Random Design (RAL) and then continued by using SNK test.

### III. RESULT AND DISCUSSION

#### A. Preparation of Pomegranate Leaf Infusion

It had been conducted by antidiarrheal effect from pomegranate (*punica granatum L.*) leaf infusion to the male white mice. The use of pomegranate leaf in this research was made in infusion that obtained by using infudation way. This infudation method selection related to tannin compound, which is content of pomegranate (*punica granatum L.*) leaf that useful as antidarrheal (astringent). Based on theory, this compound is soluble in the water. Pomegranate leaf that taken in this research was not so young or old. Pomegranate leaf that would be used as sample then processed with many steps: wet sortation, washing, chopping, and directly made as infusion.

Factors that affect infudation process are water temperature (90°C) and infudation period (15 minutes). This infudation period will be affected by solvent level, color intensity, and aroma. Infudation product that been obtained is easy contaminated by micro organism, thus it is not stored for more than 24 hours.

#### B. Tannin Identification

##### 1. Organoleptic

From infudation product of pomegranate (*punica granatum L.*) leaf, it was obtained three concentrations as follow:

**Table 1. Organoleptic Observation Result of Pomegranate Leaf Infuson**

Pomegranate ( <i>punica granatum L</i> ) Leaf Infusion	Concentration	Organoleptic
I	12%b/v	Color : dark brown Smell : typical poemgranate Taste : less bitter astringent taste
II	24%b/v	Color : dark brown Smell : typical poemgranate Taste : less bitter astringent taste
III	36%b/v	Color : dark brown Smell : typical poemgranate Taste : less bitter astringent taste

(Source : Data Primer Peneliti, 2015)

Pomegranate leaf infusion color that produced from this infudation process should be greed because it used fress pomegranate leaf. It was caysed by pomegranate leaf which had oxidation, especially for its chlorophil then the chlorophil content was decrease and resulted in brown pomegranate leaf infusion.

## 2. Reaction

- Pomegranate leaf infusion + FeCl<sub>3</sub> → dark blue / black greenish
- Pomegranate leaf infusion + Potassium Bromide → brown

The reasons that affected success in this research were due to tannin content in pomegranate leaf which functioned as astringent. Besides that, tannin which can affect medicine absorption in the body also functioned as adsorbentia in giving antidiarrheal effect. Especially to adsorp toxins which secreted by bacteria thus it can destroy mucous celland results in the disturbance of liquid and electrolite resorption in intestines.

## C. Test of Antidiarrheal Effect

This research was experimental research by using animal laboratory of 15male white mice and grouped into five test groups and each group conducted in triplet. The purpose of replication for each concentration was in order to decrease error factor and to give better and more accurate assessment to the animal laboratory. Next, the mice made sure in health condition (not diarrhea) by observing the feces of normal mice before it given by sample treatment. The treatment was given per oral to 5 treatment groups which before given by oleum ricini. The first group was negative control (distilled water), second group was positive control which given by loperamide HCl, and third, fourth, also fifth group were sample group which given by sample treatment (pomegranate leaf infusion).

In this sample giving process, it was started by giving oleum ricini as substance which cause diarrhea. After that, it was time to wait until the mice got diarrhea with diarrhea frequency for 3 times and then given by sample, negative control, also positive control for each experiment group. In this research, oleum ricini was given previously and aims to make the mice got diarrhea, as occurred to human and then it was conducted by medication step by giving pomegranate (*punica granatum L*) leaf infusion.

This antidiarrheal effect was proven based on diarrhea frequency, thin feces consistency, and diarrhea duration variable. The reason to choose those test parameters based on diarrhea definition; which is Diarrhea is defecation with thin feces or half thin fecen (half solid), water content in the feces is more than usual, more than 200 grams or 200 mL/24 hours, that is the symptomps of diarrhea. To know antidiarrheal effect from the three concentrations of pomegranate (*punica granatum L*) leaf infusion thus it was conducted by observation of diarrhea frequency, feces consistency, and diarrhea duration to the mice which previously induced by oleum ricini. Diarrhea frequency was observed by calculating diarrhea spots on the pedestal. Feces consistency was observed by seeing feces type (thin, mushy, less mushy). Diarrhea duration was calculated from the beginning of diarrhea until the diarrhea was stopped. The observation was conducted for 7 hours.

## D. Observation Result

### 1. Diarrhea Frequency

**Table 2. Observation Data of Diarrhea Frequency**

Treatment	Replication			Total	Average	Percentage
	I	Ii	Iii			
Negative control	32	21	27	80	27	0,27 %
Positive control	5	7	6	18	6	0,06 %
Infusa 12%b/v (A <sub>1</sub> )	15	13	12	40	13	0,13 %
Infusa 24%b/v (A <sub>2</sub> )	9	10	8	27	9	0,09 %
Infusa 36%b/v (A <sub>3</sub> )	5	7	4	16	5	0,05 %

Based on data in table (2), the smallest percentage was pomegranate leaf infusion 36%w/v for 0.05%. From the table above, it can be concluded that the result that been obtained in this research showed the smaller percentage of diarrhea frequency, the stronger antidiarrheal effect. It showed that pomegranate leaf infusion that contains tannin compound could be affected as antidiarrheal, because tannin is secondary metabolic of astringent and the

mechanism can constrict intestine mucous membrane thus it can decrease liquid secretion (Winarno, 1996).

## 2. Feces consistency

Antidiarrheal effect from pomegranate (*punica granatum L*) leaf infusion could be observed by using feces consistency. Feces consistency was divided into 3 criteria: thin, mushy, and less mushy

**Table 3. Observation Data of Feces Consistency**

Treatment	Replication												Average	Percentage
	I				II				III					
	C	L	AL	Total	C	L	AL	Total	C	L	AL	Total		
Negative control	16	3	0	19	14	1	0	15	15	2	0	17	17	0,17 %
Positive control	1	1	1	3	1	2	1	4	0	1	1	2	3	0,03 %
Infus 12%b/v (A <sub>1</sub> )	3	4	2	9	2	3	2	7	3	2	1	6	7,33	0,07%
Infus 24%b/v (A <sub>2</sub> )	2	1	2	5	1	1	1	3	1	1	2	4	4	0,04%
Infus 36%b/v (A <sub>3</sub> )	0	1	1	2	0	0	1	1	0	1	2	3	2	0,02%

Note :

C = cair (thin);

L = lembek (mushy);

AL = agak lembek (less mushy)

Based on data in table (3), the smallest percentage was pomegranate leaf infusion 36%w/v for 0.02%. From the table above, it can be concluded that the result that been obtained in this research showed the smaller percentage of feces consistency, the stronger antidiarrheal effect. It showed that pomegranate leaf infusion that contains tannin compound could be affected as antidiarrheal, because tannin is secondary metabolic of astringent and the mechanism can constrict intestine mucous membrane thus it can decrease liquid secretion (Winarno, 1996).

## 3. Diarrhea duration

**Table 4. Observation Data of Diarrhea Duration**

Treatment	Replication			Total	Averag	Percentage
	I	II	III			
Negative control	350	375	340	1.065	355	3,55%
Positive control	110	130	120	360	120	1,20%
Infus 12%b/v (A <sub>1</sub> )	230	170	220	620	206	2,06%
Infus 24%b/v (A <sub>2</sub> )	150	130	150	430	143	1,43%
Infus 36%b/v (A <sub>3</sub> )	120	100	110	330	110	1,10%

Based on data in table (4), the smallest percentage was pomegranate leaf infusion 36%w/v for 1.10%. From the table above, it can be concluded that the result that been obtained in this research showed the smaller percentage of feces consistency, the stronger antidiarrheal effect. It showed that pomegranate leaf infusion that contains tannin compound could be affected as antidiarrheal, because tannin is secondary metabolic of astringent and the mechanism can constrict intestine mucous membrane thus it can decrease liquid secretion (Winarno, 1996).

### a. Data of the average result

**Table 5. Average Result**

Treatment	The frequency of diarrhea ( $\bar{X}$ )	Liquid stool consistency ( $\bar{X}$ )	Old occurrence of diarrhea (Minutes) ( $\bar{X}$ )	Average	Percentage (%)
Negative control	27	17	355	133	1,33%
Positive control	6	3	120	43	0,43%
Infusa 12%b/v (A <sub>1</sub> )	13	7	267	96	0,96%
Infusa 24%b/v (A <sub>2</sub> )	9	4	143	52	0,52%
Infusa 36%b/v (A <sub>1</sub> )	5	2	110	39	0,39%

Therefore, it could be concluded that from data analysis it showed the increase of antidiarrheal effect to the larger concentration or the smaller percentage. It could be stated that from the three dosages of pomegranate (*punica granatum L.*) leaf infusion all had antidiarrheal effect. However, in the concentration 36%w/v, it had equal effect to the loperamide HCl (positive control).

## IV. CONCLUSION

From the three concentrations of pomegranate (*punica granatum L.*) leaf infusion that tested to white mice for

12% w/v, 24%w/v, 36%w/v, it could be concluded that concentration 36%w/v was the most effective in giving antidiarrheal effect due to it was equal with loperamide HCl.

#### Suggestion

1. It is suggested to conduct further research by using other extraction method and different dosage.
2. It is suggested to conduct further research to the acute toxicity test from pomegranate (*punica granatum L.*) leaf infusion to know its safety application.
3. It is suggested to develop infusion storage in suspension storage type in order to ease the application

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