Decreased cholesterol levels from variation infusion breadfruit leaves against rats wistar and manufacturing herbal tea steeping

Hesti Riasari*, Deby Tristiyanti and Dea Alldila Yuniar
Indonesian School of Pharmacy (STFI), Jl. Soekarno Hatta no 354 (Parakan Resik) Bandung, Jawa Barat, INDINESIA
*hmm_riasari@yahoo.com

Abstract
Breadfruit is one of the traditional crops and widely used by people to treat various diseases especially it can reduce hypercholesterolemia. Biosynthesis process can cause discoloration of the breadfruit leaves from Green Leaves (GL), Yellow Leaves (YL), Fallen Yellow (FY), Fallen Dry (FD) as well as the fermentation process of fresh green produces Fermented Green (FG).

This study aims was to know the effect of variations infusion of the leaves of breadfruit to decreased levels of cholesterol using high fat diet foods and to determine the content of compounds contained in steeping tea leaves of breadfruit. This study used 30 male wistar rats divided into positive control group (simvastatin), the negative control group (Na-CMC 0.5%) and control group test (the variation breadfruit leaf infusion dose of 15g/kg). On the seventh day, all groups were induced by high-cholesterol foods and PTU. Measurement of cholesterol levels using a tool easy touch was analyzed using one-way ANOVA. The results showed a significant decrease in cholesterol levels, but on the leaves of yellow foliage fell the best decrease ($p \leq 0,05$)

Keywords: Breadfruit leaves, decreased cholesterol levels, steeping tea.

Introduction

Traditional medicine in Indonesia is used since time immemorial. Traditional medicine is widely used by the public, especially the lower middle class. Utilization of traditional medicine is no longer based on experience, but time supported by efficacy testing, security testing and toxicity testing, so that the quality of traditional medicine can be assured.[15]

One of the plants used as traditional medicine for lowering cholesterol is plant leaves of breadfruit. Leaves of breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) as the traditional crops are widely used by people to treat various diseases like hepatitis, dental pain, lower blood cholesterol levels and can treat kidney disease. Almost all parts of this plant have been used as medicine (leaves, fruit, root bark and sap).[18] Leaves of breadfruit have been shown to contain various flavonoids derived compounds. *Artocarpus altilis* (Parkinson) Fosberg contains several potent compounds such as saponins, polyphenols, hydrocyanic acid, acetylcholine, tannin, riboflavin and phenol. Breadfruit leaves also contain kuercetin, chaporol and artoindonesianin. Artoindonesianin and kuercetin are flavonoid compound.[25]

Biosynthesis process can cause discoloration of the breadfruit leaves of green leaves (GL), yellow leaves (YL), fallen yellow (FY), fallen dry (FD), as well as the fermentation process of fresh green leaves produces fermented green (FG). Leaf fermentation is done by piling leaves during 5 days after the process of picking and washing.[22]

Hypercholesterolemia is the incidence of elevated levels of cholesterol in the blood caused by the synthesis of cholesterol in the intestine and liver excessively.[17] Cholesterol is part of lipids, increased cholesterol in the body will result in oxidative accompanied by lipid peroxidation. Lipid peroxidation continuously bonded with free radicals will trigger the formation of (Malondyaldehide) MDA. Increase of lipid peroxides in the jejunum will result in damage to the jejunal mucosa.[23]

Research on the leaves of breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) had much to do as in previous studies using the water extract of leaves of breadfruit for the treatment of hypercholesterolemia. The content of leaves of breadfruit according to Siddesha et al[26] based on the results of phytochemical analysis, mentions the existence of flavonoids, tannins etc.

Tea is an infusion that is made by brewing the leaves, shoots, or leaf stalks of the plant dried with hot water.[28] The preparation of tea made from the leaves of breadfruit can be called herbal tea. According to Yudana,[29] herbal tea is a drink that is made using a material other than leaves of tea (Camellia sinensis). Infusion technique has several advantages when compared with the technique of making extracts for infusion technique is cheaper, faster and tools are simple. The separation of the active substance from the solvent is easier to obtain pure active substance.

This study aims to determine the effect of variations breadfruit leaf infusion to the decrease in total cholesterol levels in male rats and to investigate the activity of flavonoid compounds contained in herbal tea preparations of breadfruit leaves.

Material and Methods

Plant Material: The plant materials used in this study are the leaves of breadfruit (*Artocarpus altilis* (Parkinson.) Fosberg) with a variety of green leaves (GL), yellow leaves (YL), fallen yellow (FY), fallen dry (FD) and fermentated green (FG) obtained from Cipamokolan, Riung Bandung, Bandung, West Java, Indonesia.

Tools used: The tools used are waterbath, funnel, beaker, stir bar, filter paper, volume pipette, Erlenmeyer measuring cups, bowls vaporizer, syringes, oral sonde, analytical balance, toolcheck cholesterol (EasyTouch® GCU) and test strips (EasyTouch®), as well as tools used in the process and characterization skrinning such as porcelain cup, oven, furnace, desiccator, wood clamps, test tube, tube rack, drip plate, spatula ,spiritus burner etc.

Materials used: The material used are: aquadest, Na-CMC, simvastatin, propylthiouracil and equipment cholesterol test as MDLT (Food Diet High Fat) as a material inducer used in the study consisting of a mixture of beef fat, egg yolk quail and vegetable oil, reagent chemicals used for the phytochemical screening are: ammonia, chloroform, HCl, gelatin solution, amyl alcohol, ether, vanillin solution, H_2SO_4, KOH. Reagents used are Mayer Dragendorff and Lieberman-Burchard.

Preparation of Material: Materials such as variations leaves of green leaves (GL), yellow leaves (YL), fallen yellow (FY), fallen dry (FD), as well as the fermentation are green leaves collected, separated from impurities and cleaned under running water; select the leaves, chop and dry.

Phytochemical screening is conducted on variation fresh green leaves of breadfruit, green fermentation, stuck yellow, yellow fall and tumble drier to determine the content of secondary metabolites. In general, compounds tested include testing of alkaloids, flavonoids, tannins, phenolics, triterpenoids, steroids, quinones, monoterpenes, sesquiterpenes and saponins.

Pulverized dried leaves of breadfruit from each variety were weighed as much as 350 g, boiled in 3500 ml of distilled water (1:10) for 15 minutes at 90°C while stirring few times. The filtrate is filtered, stored and collected. Then evaporate on a water bath for concentrated infusion.

Testing of Activities

1. Preparation of Animal Test: Before being used for research, animals are acclimatized for 2 weeks in order to adjust to the new environment. During acclimatization, the mice are given drinking and food standards and health and weight control mice. Once acclimatized, rats are given high-cholesterol foods orally for 14 days.

2. Making Foods of High Cholesterol: Fat cows 1%, Quail egg yolks 5%, Cooking oil 1%. Weighing: Beef fat 1% = 1% x 1000 g = 1 egg yolk quail 5% = 5% x 1000 g = 5 g. Cooking oil 1% = 1% x 1000 g = 1 g standard rat feed ad 1000 g.

3. Induction Animal Experiments: One group of test animals that consists of three rats was weighed on the first day. After that feed high-cholesterol diet (MDLT) Fat cow 1%, 5% Yellow quail eggs and cooking oil 1%. Then give PTU diluted with water and then give to the stomach probes for 7 days and observe routine; weigh and check cholesterol levels on day 14 the mice were fasted beforehand for 12 hours after taking blood.

4. Testing hypercholesterolemia Total: At the start of the experiment specified weight and normal cholesterol levels of all animals were randomized.

Group I: Negative control group. Rats fed with high cholesterol diet and drinks containing propylthiouracil 0.01% for 7 days. Granted suspension 0.5% Na-CMC through a stomach sonde for 14 days.

Group II: Positive control group. Rats fed with high cholesterol diet and drinks containing propylthiouracil 0.01% for 7 days. 1 mL suspension given simvastatin (simvastatin contains 0.18 mg / 200 g bw / day) was administered through a gastric sonde for 14 days.

Group III: The test group breadfruit leaf variations. Mice were fed high cholesterol diet and drinks containing propylthiouracil 0.01% for 7 days. After treatment, the mice were fasted ± 12 hours. Then the blood sampling was done 3 times i) before the experiment (day 0), ii) after induction of high-cholesterol diet for 7 days iii) after administration of infusion variation breadfruit leaf suspension on day 21 repeat determination of the same blood cholesterol levels.[2] Blood sampling done by the end of the rat tail was cleaned with 70% alcohol, then cut approximately 1-2 mm and then massage slowly until blood flows out and then check in by Easy Touch Strip Test.

Data Analysis: Data was obtained on the test hypercolesterolemia activities using Analysis of Variance (ANOVA) followed by Tukey's test using SPSS version 16.2:10. Data was obtained on the test hypercholesterolemia activities, dianilisis using Analysis of Variance (ANOVA) followed by Tukey's test using SPSS version 16.

Thin Layer Chromatography (TLC): Each sample was spotted on the plates of silica gel, mobile phase used was a solvent mixture of ethyl acetate and methanol, then the developer is saturated. After the sample is spotted on the plates of silica gel chromatography, insert into the chamber and then the chamber chromatography is closed. After that, wait and observe to seep mobile phase and rises to the surface of the plate boundary. Spot the rise observed with plate TLC using reagent chromogenic and observed under ultra violet light with wavelengths of 254 and 366 nm and

then calculate Rf by measuring the distance traveled dissolved compounds and distance travelled by solvent.[27]

Preparation of Tea Steeping Breadfruit Leaves: After getting one of the variations of breadfruit leaf that has the effect of hypercholesterolemia, perform the manufacture of breadfruit leaves steeping dry powder in the following manner:

a. Breadfruit leaves are wilted fresh made by heating so that the moisture content is reduced, so that the breadfruit leaf tea can be easily milled.

Results and Discussion

Preparation of Crude: The plant material leaves of breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) with a variety of fresh green leaves, yellow leaves, fallen yellow leaves, fallen dry and green leaves fermentation were obtained from districts Cipamokolan, Riung Bandung, Bandung, West Java, Indonesia. Variations in the breadfruit leaves can be collected and cleaned with water. And then selected, chopped and dried at 40°C for 15 minutes. It aims to separate the dirt on the leaves of breadfruit to be used and prevents the growth of bacteria or fungi on plants that can damage the compounds contained in the plant.

Extraction Results: Breadfruit leaf extract variation is obtained by applying heat or is by using a water extract or infusion. The solvent used is water, water is selected as solvent for the water extract form or infuse in traditional medicine using breadfruit leaves. Water is a polar solvent with viscosity values of more than 1. Water has a characteristic that is the boiling point of 100ºC and viscosity of 1,005 at a temperature of 20ºC.

From the results of the collection and processing of breadfruit leaves, extract the highest yield obtained from the leaves of breadfruit fallen yellow (FY) amounted to 13.27% and the lowest yield of fallen dry (FD) extract 6.7%. These results indicate that the component compounds on fallen yellow leaves of breadfruit (FY) with solvent extracted more water than other breadfruit leaves.

Table 1
Yield of Breadfruit Leaf Extract with Water Variation

S.N.	Leaf Breadfruit	%Yield
1.	Green Leaves(GL)	11,18
2.	Yellow Leaves (YL)	7,9
3.	Yellow Fall (YF)	13,27
4.	Fallen Dry (FD)	6,7
5.	Fermented Green (FG)	8,48

Screening Phytochemical Crude and Infusion Variation Breadfruit Leaves: Phytochemical is an early stage to identify the content of secondary metabolites found in plants.

Table 2
Phytochemical Screening Variation Crude Breadfruit Leaf

Group	GL	FG	YL	FY	FD
Alkaloids	-	-	-	-	-
Flavonoids	+	+	+	+	+
Tanin	+	+	+	+	+
Phenolics	+	+	+	+	+
Monoterpenes and Sesquiterpenes	-	-	-	-	-
Steroids	+	+	+	+	+
Triterpenoids	+	+	+	+	+
Kuinon	+	+	+	+	+
Saponin	-	-	-	-	-

Note: GL = Leaf Green Fresh Breadfruit; FG = Breadfruit Leaf Green Fermentation; YL = Yellow Leaves Breadfruit stuck; FY = Yellow Fall Leaves Breadfruit; FD = Breadfruit Falling Leaves Dry; (+) = Identified and (-) = Not identified

Based on the results of phytochemical screening crude breadfruit leaf variation is the fifth positive breadfruit leaves containing flavonoids, tannins, phenolics, steroids, triterpenoids and quinones.

Based on the results of phytochemical skrinning both of simplicia as well as from breadfruit leaf infusion have the same positive results contain flavonoids, tannins, phenolics, steroids, triterpenoids and quinones because all the chemical compounds contained in the infusion may be attracted by the water solvent.

Table 3
Phytochemical Screening Variation Infusion Breadfruit Leaves

Group	GL	FG	YL	FY	FD
Alkaloids	-	-	-	-	-
Flavonoids	+	+	+	+	+
Tanin	+	+	+	+	+
Phenolics	+	+	+	+	+
Monoterpenes and Sesquiterpenes	-	-	-	-	-
Steroids	+	+	+	+	+
Triterpenoids	+	+	+	+	+
Kuinon	+	+	+	+	+
Saponin	-	-	-	-	-

Note: GL = Green Leaves; FG = Fermented Green; YL = Yellow Leaves; FY = Fallen Yellow; FD = Fallen Dry; (+) = Identified and (-) = Not identified

Characterization Results of Breadfruit Leaf Crude: Quality parameters simplicia are made of breadfruit green leaves, yellow leaves, fallen yellow, fallen dry and fermented green. Characterization simplicia aimed to find out the quality requirements simplicia to be processed into drugs.

Table 4
Results Characterization of Breadfruit Leaf Variations

Breadfruit Leaves	Total Ash Content (%)	Water Soluble Material (%)	Ethanol Soluble Material (%)	Drying shrinkage (%)
GL	13,50	20	4	7
YL	26,00	20	15	5
FY	27,36	20	15	4
FD	21,50	11	12	2.5
FG	20,50	23	17	8

Note: GL = Green Leaves; FG = Fermented Green; YL = Yellow Leaves; FY = Fallen Yellow and FD = Fallen Dry.

Total ash content results in table 4 show the percentage of different ash content, the value of total ash content in breadfruit leaves FY higher, indicating that the breadfruit leaf FY contains inorganic compounds, minerals and alkali and alkaline earth metals.

The results of the water-soluble extract content in breadfruit leaves FG are very high indicating FG-soluble secondary metabolites in both the solvent and the water levels in the ethanol-soluble extract of leaves of FG higher resulting in a high yield in the extract.

It is stated that simplicia variation breadfruit leaves meet the requirements of drying shrinkage of less than 10 %, drying shrinkage aims to reduce the water content contained in the bulbs, so bulbs are not easily damaged, avoid the growth of mold and can be stored for long periods.

Monitoring Extract: Monitoring patterns of chromatograms with TLC to variations infusion of the leaves of breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) green leaves, yellow leaves, fallen yellow, fallen dry and fermented green using a solvent the same developer that is with ethyl acetate, methanol and water gave ratios.

Based on the results of the chromatogram pattern by thin layer chromatography of all variations of infusion of the leaves of breadfruit, spot blue fluorescence at 366 nm uv and after steam ammonia into bluish green fluorescence showed flavonoid compounds of flavones and flavanones. Result of factor retention from thin layer chromatography show green leaves (GL) rf 0.75, fallen yellow (FY) by rf 0.4, fallen dry (FD) with rf 0.6, yellow leaves (YL) rf 0.47 and fermented green (FG) rf 0.52.

Testing anticholesterol: Anticholesterol used 30 male Wistar rats were divided into positive control group (simvastatin), the negative control group (Na-CMC 0.5%), the control group test (variation infusion of the leaves of breadfruit) dose of 15g / kg body weight per day. Each group was divided into 3 rats. All groups performed acclimatization for 7 days, this is done to adapt the mice with the state of the new environment such as the ambient temperature and humidity of the room so that the outside factors that may interfere with the results of the research can be minimized. Check the weight and physical condition of the mice during the acclimatization rats so that health can be maintained when the research is conducted. All rats were weighed before for the initial weight.

After weighing, all rats were fasted and checks carried out for initial cholesterol. All groups were induced by MDLT (High Fat Diet Foods) and PTU 0.01% in the drinking water of mice is to get the state of hypercholesterolemia in rats. Induction provided consisted of standard feed mixed with cow fat 1%, 5% quail egg yolk and cooking oil 1%, feeding; this induction is given for 7 days by measuring the weight and cholesterol levels of mice prior to administration of induction.

Examination of cholesterol levels is important to determine the uniformity of the white rat cholesterol levels of all the treatment groups. All successfully treated animals can be induced and all treated animals had high cholesterol levels not only seen on cholesterol levels but on the physical condition of rats, namely body weight of mice rose.

After knowing the cholesterol levels after induction, each of the control group was given treatment that is positive controls were given the drug simvastatin, the negative control group were only given 0.5% CMC-Na, the control test given variation infusion breadfruit leaves (fresh green, yellow leaves, fallen yellow, fallen dry and fermented green) at a dose of 15g / kg. All treatments were carried out for 14 days.

On day 21, all treatment groups were fasted for 12-14 hours, take the blood sample, use touch easy tool to determine total cholesterol levels after treatment in all groups and all test data is seen as the ANOVA results.

Results of analysis using one way ANOVA on first day prior to induction in all groups of the experiment should not differ significantly for all groups of experimental animals in a normal state or not at induction. The high homogeneity may be significantly different between treatment groups. Data must be homogeneous, but in fact the data is not homogeneous.

On the 7th day after induction dose, the data showed the result is homogeneous. In a comparison between the positive control group, negative control group and the control group, test should not differ significantly because all experimental animals can be induced. The results showed that the positive control group, negative control group and the control group test results showed no significant difference in means of induction done with high-cholesterol diet and 0.01% PTU can successfully raise cholesterol levels of mice.

Table 5
Average total cholesterol levels.

Treatment Group	Average Cholesterol Levels (mg / dL)		
	Day 0	**Day 7, before treatment**	**Day 21, after treatment**
Negative	142±5,09	157,66±4,92	160,33±4,18[a]
Positive	179,33±11,61	339,33±29,78	231,33±18,92[b]
Dose to Infuse fresh green leaves	174 ±6,16	230±22,01	194,66±17,21 [ab]
Dose to Infuse yellow leaves	159,33±6,79	237,33±25,69	175±10,70 [a]
Dose to infuse fallen yellow	161,33±10,20	248,33±19,68	164±6,48 [a]
Dose to infuse fallen dry	137,33±14,88	234,33±11,72	179±26,19 [a]
Dose to infuse fermented green	163,66±11,84	226±21,27	185±12,68 [a]

Information day 21: a: significantly different with the positive control and b: significantly different with the negative control

After the 21st day after being given treatment with fresh green leaf infusion test preparation, yellow stuck, yellow fall, dried falls and fermentation green, the positive control group showed significantly different results with all variations of the leaves of breadfruit but more significant to the yellow leaves of breadfruit fall. The negative control showed significantly different results with all variations of breadfruit leaves and positive control group, this indicates that the Na-CMC does not affect the decrease in cholesterol levels.

In all variations of the infusion of the leaves of breadfruit, it shows a decrease in cholesterol levels. Breadfruit leaf flavonoid compounds have the effect as anticholesterol. Flavonoids are phenolic compounds that act as natural antioxidants.[17] Antioxidants serve to reduce free radicals, thus breadfruit leaf flavonoid is able to suppress the formation of excess free radicals in the body.[9] But a significant decrease is seen in the fallen yellow leaves of breadfruit infusion that have the effect of anti-cholesterol best compared to breadfruit leaf infusion of green leaves, yellow leaves, fallen dry and fermented green. Fallen yellow leaves of breadfruit have the best results in antihipercholesterolemia due to the biosynthetic process that occurs on the fallen yellow leaves undergoing a perfect secondary metabolite change as in the shikimat pathway of aromatic acid forming aromatic amino acids. At the time of the process of yellowing leaves, changes from aromatic amino acids to phenolic groups including flavonoids that act as antihipercholesterolemia.

Results of phytochemical screening steeping Breadfruit Leaf Tea Fallen Yellow: Having obtained the results of infusion variation breadfruit leaves that have activity antihipercholesterolemia most good, namely the infusion of breadfruit fallen yellow, perform the manufacture of steeping tea leaves of breadfruit fallen yellow by weighing simplicia breadfruit leaves yellow fall as much as 15 g and dissolved in hot water of 500 mL.

Results obtained from steeping tea phytochemical screening breadfruit leaves yellow fall filtered and not filtered are positive containing flavonoids, tannins, phenolics, steroids, triterpenoids and quinones. These results indicate that the compounds are made by steeping tea leaves of breadfruit yellow fall as much as 15 g brewed with 500 mL of hot water with the result screening breadfruit leaves infuse phytochemical compounds get the same results.

Table 6
Results of phytochemical screening 15 g steeping tea leaves of breadfruit fallen yellow in strain and not filtered

Group	FY Filtered	FY Was Not Filtered
Alkaloids	-	-
Flavonoids	+	+
Tanin	+	+
Phenolics	+	+
Monoterpenes and Sesquiterpenes	-	-
Steroids	+	+
Triterpenoids	+	+
Kuinon	+	+
Saponin	-	-

Description: FY = Fallen Yellow Leaves Breadfruit; (+) = Identified and (-) = Not identified.

Conclusion

Based on the result of the research, it is concluded that the variations of fresh green breadfruit, green fermented, yellow and falling leaves can lower cholesterol in male wistar white rats. The yellow-fallen breadfruit leaves give the best anticholesterol activity in comparison to the variation of other breadfruit infusions, but the anti-cholesterol effect is not as good as the Simvastatin medication.

The results of infusion screening and foliage of leaves of yellow foliage showed positive flavonoid results in which flavonoid compound has an effect on anticholesterol. The results of thin layer chromatography showed flavonoid compounds that were marked with a bluish green spot after being evaporated with ammonia vapor.

References

1. Aulanni'am, Effect Des Fibres duriz Sur Le Profil Lipidique Du Rats Comparison Entre Le Riz Cargo Et Les Fibres du Son, These USTL, Montpellier, France **(1993)**

2. Adaramoye Oluwatosin A. and Akanni Olubukola O., Effect of Methanol Extract of Breadfruit (Artocarpus altillis) on Atherogenic Indices and Redox Status of Cellular System of Hypercolesterolemic Male Rats, *Advance in Pharmacological Sciences*, http://dx.doi.org/10.1155/2014/605425 **(2014)**

3. Blick K.E. and dan Liles S.M., Principles of Clinical Chemistry, Canada **(1985)**

4. Departemen Kesehatan Republik Indonesia, Farmakope Indonesia Edisi IV, Jakarta, 9 **(1995)**

5. Departemen Kesehatan Republik Indonesia, Materia Medika Indonesia Edisi V, Jakarta **(1989)**

6. Farnsworth N.R., Biological and Phytochemical Screening of Plants, *Journal of Pharmaceutical Sciences*, **55(3)**, 257-265 **(1966)**

7. Gani N., Momuat L.I. and dan Pitoi M.M., Profil Lipida Plasma Tikus Wistar yang Hiperkolesterolemia pada Pemberian Gedi Merah (*Abelmoschus manihot* L.), *Jurnal Mipa Unsrat*, **2(1)**, 44-49 **(2013)**

8. Ganong W.F., Buku Ajaran Fisiologi Kedokteran, Edisi ke-14, Editor Bahasa Indonesia, Jonatan Oswari, Jakarta, EGC Hal, 288 **(1992)**

9. Per-Giorgio Pietta, Flavonoids as Antioxidant, *J. Natural Product*, **63(7)**, 1035-1042 **(2000)**

10. Hanna K., Pengaruh air Tripang Pasir (Holothuria scabra) Terhadap kolesterol Total Pada Tikus Hiperlipidemia, Fakultas Farmasi Universitas Muhammadiyah Surakarta **(2012)**

11. Heyne K., Tumbuhan Berguna Indonesia II, Badan Penelitian dan Pengembangan Kehutanan, jilid III, Yayasan Sarana Wana Jaya, Jakarta, Hal, 775 **(1987)**

12. Katzung B.G., Farmakologi Dasar dan Klinik, Edisi X, Jakarta, EGC, 575-588 **(2010)**

13. Keele C.A., Neil E., Joels N. and Samson S., Right Applied Physiology, 13th ed., Oxford University Press, New York **(1984)**

14. Ko H.H., Lu Y.H., Yang S.Z., Won S.J. and Lin C.N., Cytotoxic prenylflavonoids from Artocarpus elasticus, *J. Nat. Prod*., **68(11)**, 1692-1695 **(2005)**

15. Ma'arifin H., Farmakologi dalam Pengembangan Obat Tradisional Risalah Simposium Penelitian Tumbuhan Obat III, 25-26 September 1980, Fakultas Farmasi Universitas Gadjah Mada, Yogyakarta **(1983)**

16. Murray R.K., Dryer R.L., Conway T.W. and dan Spector A.A., Biokimia Harper, Edisi ke-25, Alih Bahasa, Andry Hartono, Jakarta, EGC, hal, 260-262, 270-278, 581 **(2003)**

17. Metwally M., El- Gellal A. and El- Sawaisi S., Effect of silymarin on lipid methabolism in rats, *World Appl. Sci. J.*, **6**, 1634-1637 **(2009)**

18. Noveriza N.N.K. and Rizal Balittro M., Peluang Tanaman Obat sebagai Alternatif Bahan Obat Flu Burung, *Warta Penelitian dan Pengembangan Tanaman Industri*, **14(1)**, 20 **(2008)**

19. Nwokocha C.R., Owu D.U., Mc Laren M., Murray J., Delgoda R., Thaxter K., Mc Calla G. and Young L., Possible Mechanisms of Action of The Aqueous Extract of Artocarpus altilis (breadfruit) Leaves in Producing Hypotension in Normotensive Sprague-Dawley Rats, *Pharm. Biol.*, **50(9)**, 1096-102 **(2012)**

20. Phyu Medica, Penapisan Farmakologi, Pengujian Fitokimia dan Pengujian Klinik, Kelompok Kerja Ilmiah Yayasan Pengembangan Obat, Jakarta Pusat **(1993)**

21. Ragone D., Breadfruit Artocarpus altilis (Parkinson) Fosberg, Promotig the conservation and use of underutilized and neglected crops, 10, Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Roma **(1997)**

22. Riasari Hesti, Sukrasno and Komar Ruslan, Metabolite Profile of Various Development Bread Fruit Leaves (Artocarpus altilis. Parkinson. Fosberg) and The Identification of Their Major Componens, *IJPSR*, **6(5)**, 2170-2177 **(2015)**

23. Rosyid F.N., Peranan Lipoprotein Terhadap Terjadinya Aterosklerosis Pada Arteriokoronaria, *Jurnal Ilmu Kesehatan*, **2(4)**, 1979-3812 **(2009)**

24. Sancaya Rini, Pengaruh Pemberian Diet Tinggi Lemak Terhadap Kadar Trigliserida Pada Tikus, Skripsi Universitas Muhammadiyah Surakarta **(2012)**

25. Shabella R., Terapi Daun Sukun, Cetakan Pertama, Cable Book, Klaten, 33-44 **(2012)**

26. Siddesha J.M., Angaswang N. and Vishwanath B.S., Phytochemical Screening and evaluation of in vitro angiotensin-coverting enzyme inhibitory activity of *Artocarpus altilis* leaf, *Nat. Prod. Res*., **25(20)**, 1931-1940 **(2011)**

27. Sudjadi, Kimia Farmasi Analisis, Pustaka Pelajar, Yogyakarta, 27, 220-255, 353-362 **(2007).**

28. Sembiring Netti V.N., Pengaruh Kadar Air dari Bubuk Teh Hasil Fermentasi Terhadap Kapasitas Produksi Pada Stasiun Pengeringan di Pabrik The PTPN IV Unit Kebun Bah Butoh, Fakultas MIPA, Universitas Sumatra Utara, Medan **(2009)**

29. Yudana dan luize, Mengenal Ragam Dan Manfaat the, (http://www.indomedia.com/intisari/1998/mei/the.htm) **(1998)**.

*****