

HOW RAW PEANUT MILK (VIGNA UNGUICULATA) AFFECTS THE VAGINAL pH AND URIC ACID LEVEL OF MENOPAUSAL WOMEN

Arika Indah Setyarini, Herawati Mansur, Eny Sendra, Mika Mediawati and Tanto Hariyanto*

Received: May 04, 2021; Revised: August 16, 2021; Accepted: August 25, 2021

Abstract

Menopause has been defined as the time in a woman's life when her period stops. It is a condition that often attributed to low levels of estrogen production and is related to hot flushes, sweating, insomnia, vaginal dryness, and discomfort. In this study, we examine how the consumption of raw peanut milk affects vaginal pH and uric acid levels among menopausal women.

Keywords: Raw peanut milk, Menopause, Uric acid, Vagina

Introduction

For the next 25 years, the population in Indonesia has been predicted to increase from 238.5 million in 2010 to 305.6 million in 2035. The country's average life expectancy has reportedly increased from 70.1 years (2010-2015) to 72.2 years (2030-2035) (Central Bureau of Statistics, 2013). Until now, Indonesian women with menopause status have been reported as 7.4% of the total population, which increased to 11% in 2005 and to 14% in 2015 (Indonesian Ministry of Health, 2020). Based on the 2000 Population Census, the number of women aged over 50 years is estimated to be about 15.5 million people, which is 7.6% of the total population. In 2020, this number is estimated to rise to 30.0 million or 11.5% of the total population (Indonesian Ministry of Health, 2020).

Menopause has been defined as a condition of decreased estrogen production and is associated with *hot flushes*, sweating, insomnia, vaginal

dryness, and discomfort, symptoms often experienced by 85% of menopausal women for

5 years or even longer (Goodman *et al.*, 2011). Palupi (Palupi *et al.*, 2010) reported that the results of qualitative research with a descriptive phenomenological design through in-depth interviews conducted in the popular traditional market, namely Pasar Rebo, East Jakarta, Indonesia to eight menopausal women who were participants revealed that various physical discomforts were experienced by all participants by expressing complaints of pain during sexual intercourse due to reduced vaginal lubrication.

Among menopausal women, one of the organs primarily affected by hypoestrogenism is the vagina. Painful sexual relations (dyspareunia) have been identified as one of the chief complaints during menopause, which can be attributed to the decrease in hormone estrogen production. This condition

Poltekkes Kemenkes Malang, Malang, Indonesia. E-mail: tanto_hariyanto@poltekkes-malang.ac.id

* Corresponding author

results in thinning, drying, and less elasticity of the vaginal walls due to epithelial atrophy and collagen reduction (Al-Baghdadi *et al.*, 2009; Alvisi *et al.*, 2019; Scavello *et al.*, 2019).

At a menopausal stage, various short-term and long-term problems can emerge because of decreased estrogen levels, termed as hypoestrogenism, but this can be managed by administering hormone replacement therapy (HRT). Moreover, this therapy has been determined with remarkable benefits in decreasing the risk of colorectal cancer and osteoporosis fractures. However, prolonged use can reportedly increase the risk of cardiovascular diseases and breast cancer (Stevenson, 2009). Taking these serious side effects into consideration, ethnobotanical resources such as plants are now being explored as a potent source of estrogen or its derivatives. There are already existing studies that provide evidence that *Pueraria lobata* and cowpea (*Vigna unguiculata*) contain high levels of estrogen as well as their derivatives; these plants are reported to be abundant in Indonesia (Wibisono, 2014).

The use of HRT, which aims to provide synthetic estrogen, has been identified as a solution to minimize complaints and/or disorders felt during *menopause*. However, its long-term administration can result in adverse side effects, for example, developing breast cancer (Manson *et al.*, 2013). Menopausal conditions, which also reflect a chronic inflammatory state, can cause oxidative stress on adipocyte cells; thus, HRT is used to reduce inflammation and oxidative stress. However, this method has its drawbacks and thus must be used under the supervision of a doctor, with drugs maintained in low doses and patients to be evaluated every 6 months (Rossouw *et al.*, 2002; U.S. National Library of Medicine, 2020). Recent research developments are now focused on utilizing phytoestrogens to help reduce the effects of oxidative stress and inflammation; these were performed using experimental animals such as ovariectomized rats.

Based on our findings (Setyarini *et al.*, 2019), the hypoestrogenic model mice experienced an increase in vaginal pH from acidic to alkaline. Thirty rats were divided into five groups, namely negative control (K-), positive control (K+), the treatment group by giving phytoestrogens in the form of raw peanut milk (*Vigna unguiculata*) at a dose of 1.25 mg/kg, 2.5 mg/kg, and 5 mg/kg, indicating that hypoestrogenic mice have decreased oxidative stress and increased fibroblast proliferation at a concentration of 2.5 mg/kg. Cowpea (*Vigna unguiculata*) is well known in Indonesia. Its nutritional value is comparable to that of soybeans; besides, the fat content in cowpea is

also lower when compared to soybeans (Kholis *et al.*, 2010). By identifying isoflavone components using high-performance liquid chromatography, concentrations of genistein and daidzein from cowpea were determined to be higher than that in local soybeans (Darmadi *et al.*, 2011).

Phytoestrogens, which are proteins found in cowpea, were identified to be very beneficial for hypoestrogenic individuals, but other ingredients are thought to increase uric acid, for example, purine. Although cowpea's purine content was classified as moderate, further research is needed to investigate the nucleotide pathways according to capacity reduction due to the high uric acid levels. The purpose of this study was to evaluate the effect of giving raw peanut milk on vaginal pH and uric acid levels of menopausal women.

Materials and Methods

Sample and Experimental Design

This study was carried out for 30 days in housing around one of public health center in Malang, East Java, Indonesia. This quasi-experimental study was designed with pretest-posttest control group examining the following five groups: (a) younger women (under 40 years old) who have occasional and/or routine period (s) (K-); (b) menopause group without cowpea treatment (K+); (c) menopause group treated with 200 cc/day (P1) of cowpea; (d) menopause group treated with 200 cc/two-days of cowpea (P2); and (e) menopause group treated with 200 cc/three-days of cowpea (P3). In total, 25 respondents (n=5 each group) were included in this study. The inclusion criteria were as follows: women (± 50 years old) who had menopause (a year after the end of the menstrual cycles), vaginal pH in alkaline conditions, willing to participate as respondents, had no history of nuts allergies and has a normal uric acid level (2.4-6.0 mg/dL). Meanwhile, the exclusion criteria were as follows: menopausal women who were senile and those who relocated during the study period. Sample replications could be increased to 6 each group (n total=30) to avoid a decrease in the number of samples due to illness, death, or relocating the study. This study has been approved by the Ethics Review Committee of the Health Polytechnic of Malang, Ministry of Health (No.:374/KEP-K/POLKESMA/2019).

Preparation of Raw Peanut Milk

Cowpea was washed and soaked in water for 8 h. After it was drained, it was then placed into boiling water (85°C-90°C) for 20 minutes and was removed and cooled under running water. The beans

were then mashed in a blender until it looked like porridge after adding water eight times the weight of the dry beans. The porridge was filtered and squeezed. The liquid obtained was called raw peanut milk; then sugar, salt, ginger, pandan or screw pine (*Pandanus amaryllifolius*) leaves, and lemongrass were added to the mixture. Raw peanut milk was heated at 95°C for ± 30 minutes and stirred.

Vaginal pH Measurement

Procedure for measuring vaginal pH using the MERCK pH indicator strips:

The strips were given to the respondents; these were inserted into their vagina with researcher assistance for 1 to 3 seconds (a maximum of 10 minutes). These strips were then examined according to the standard pH indicator strips of MERCK (pH 0-14).

Uric Acid Level Measurement

The uric acid level in each respondent was measured from the blood using the EasyTouch® GCU meter. The procedure was performed according to the instructions listed on the kit.

Data Analysis

Data for this study were collected from direct observation of the vaginal pH and uric acid levels of the respondents. Data were analyzed using one-way ANOVA test, multiple comparison test of least significant difference (LSD), and regression analysis by SPSS software for Windows.

Results

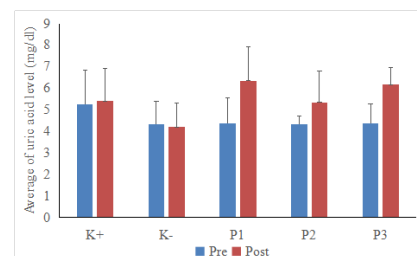
Measuring Uric Acid Levels of Menopausal Women After Cowpea Treatment

Further testing was done to determine how raw peanut milk can affect the uric acid levels of the respondents parametrically using the ANOVA test. At the pre-test, a p-value of 0.603 was obtained, greater than $\alpha=0.05$ ($p<0.05$). From this test, it can be concluded that there was no significant difference in uric acid levels in all groups (both the control group and the treatment group). At the time of the post-test, a p-value of 0.128 was obtained,

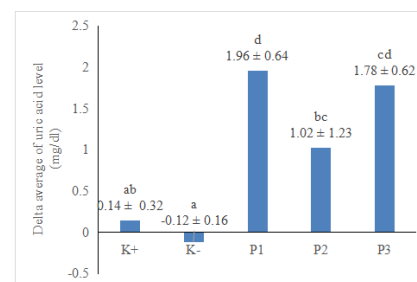
greater than $\alpha=0.05$ ($p>0.05$). From this test, it can be concluded that there was no significant difference in uric acid levels in all groups (both the control group and the treatment group) after administering cowpea (Table 1).

The average histogram shows the uric acid levels of both the control and treatment groups during the pre-test and post-test. Starting from the K+ group, it was observed that the average uric acid level increased at the time of the post-test, whereas in the K- group, uric acid levels reportedly decreased. In the treatment group, P1, P2 and P3 an increase in uric acid levels were noted at the time of the post-test (Figure 1a).

To compare the increase/decrease in uric acid levels that occurred at the time of the post-test, a comparison test of pre-and post-delta values was performed between groups. Based on these findings, the p-value was determined to be 0.000, which is smaller than $\alpha=0.05$ ($p<0.05$). From this test, it can be concluded that there are significant differences in the acid level changes between groups. By using the 5% LSD test, it was shown that K- had a negative delta value, which means that there was a decrease in uric acid levels at the time of the post-test; meanwhile, in the K+ group and the treatment groups P1, P2 and P3 there was an increase in uric acid levels at the time of the post-test. Comparing



(a)



(b)

Table 1. The effect of raw peanut milk on uric acid levels

Treatment	Pre	Post
K+	5.26 ± 1.59	5.4 ± 1.52
K-	4.32 ± 1.08	4.2 ± 1.1
P1	4.36 ± 1.18	6.34 ± 1.58
P2	4.32 ± 0.38	5.34 ± 1.47
P3	4.38 ± 0.88	6.16 ± 0.8
p-value	0.603	0.128

Figure 1. Histogram (a) Average of uric acid levels. (b) Changes in uric acid levels of both the control and treatment groups. The different letters mean a significant difference ($p<0.05$), and the same letter means no significant difference ($p>0.05$).

the K⁺ group with the treatment groups P1, P2 and P3 it is shown that the value means $\pm$ SD groups P1 and P3 contain letters that differ from K⁺. This proves that a significant increase in uric acid levels occurred in groups P1 and P3. The highest average delta value was found in group P1 (Table 2). This proves that giving 200 cc of cowpea once a day can increase uric acid levels, which was deemed the highest compared to other treatment groups.

Figure 1b shows the average delta histogram or changes in uric acid levels of both the control and treatment groups. Based on these figures, it can be concluded that all treatment groups had a significant increase in uric acid levels. The highest increase in uric acid levels was noted in the P1 treatment group.

Effect of Raw Peanut Milk Against pH

As explained in the results of testing the assumptions of normality and homogeneity, the pH variable has been fulfilled. Furthermore, testing is done to determine how raw peanut milk affects pH parametrically using the ANOVA test. At pre-test, a p-value of 0.139 was obtained, which was greater than $\alpha=0.05$ ($p>0.05$). Thus, it can be concluded that no significant difference was noted in the pH of all groups. Meanwhile, at post-test, a p-value of 0.609 was obtained, which was greater than $\alpha=0.05$ ($p>0.05$). Thus, it can be concluded that there was no significant difference in pH after administering cowpea (Table 3).

Figure 2a shows the histogram of average pH for both the control and treatment groups at pre-and post-test. Starting from the K⁺ group, it was observed that the average pH did not change at post-test; it was the same as in the K⁻ group. Meanwhile,

in the treatment groups P1, P2 and P3 a decrease in pH were noted at post-test.

To compare the increase/decrease in pH that occurred at the post-test, a comparison test of pre-and post-delta values was performed between groups (Table 4). Based on these results, a p-value of 0.067 was obtained, which was greater than $\alpha=0.05$ ($p>0.05$). Thus, it can be concluded that there were no significant differences in terms of pH changes between groups.

Figure 2b shows the average delta or change in pH of both control and treatment groups. Based on these figures, it can be concluded that in all treatment groups, there was a decrease in pH. However, it was statistically proven that the decrease in pH did not occur significantly.

Discussion

In postmenopausal women, ovarian function is disrupted, which in turn causes the ovary to lose its

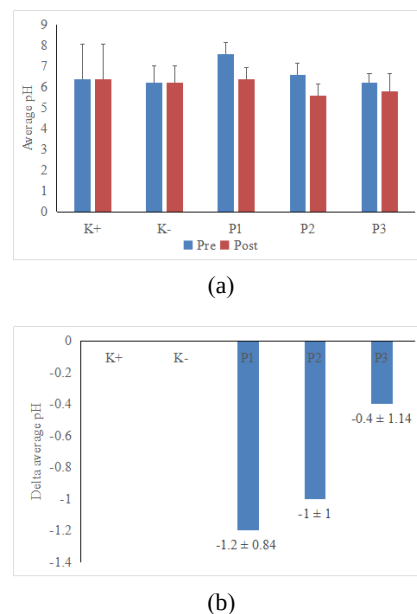


Figure 2. Histogram (a) Average of pH. (b) Changes in pH of both the control and treatment groups.

Table 2. Comparison of delta values in pre-post-test levels with ANOVA and LSD test 5%.

Treatment	Mean $\pm$ SD	p-value
K ⁺	0.14 $\pm$ 0.32 ^{ab}	0.000
K ⁻	-12.12 $\pm$ 0.16 ^a	
P1	1.98 $\pm$ 0.64 ^d	
P2	1.02 $\pm$ 1.23 ^{bc}	
P3	1.78 $\pm$ 0.62 ^{cd}	

a,b,c,d The different letters mean a significant difference ($p<0.05$), and the same letter means no significant difference ($p>0.05$).

Table 3. Testing how raw peanut milk affects pH via ANOVA test and LSD 5%.

Treatment	Pre	Post
K ⁺	6.4 $\pm$ 1.67	6.4 $\pm$ 1.67
K ⁻	6.2 $\pm$ 0.84	6.2 $\pm$ 0.84
P1	7.6 $\pm$ 0.55	6.4 $\pm$ 0.55
P2	6.6 $\pm$ 0.55	5.6 $\pm$ 0.55
P3	6.2 $\pm$ 0.45	5.8 $\pm$ 0.84
p-value	0.139	0.609

Table 4. Comparison of delta pH at pre-and post-tests with ANOVA and LSD test 5%.

Treatment	Mean $\pm$ SD	p-value
K ⁺	0.00 $\pm$ 0.00	0.067
K ⁻	0.00 $\pm$ 0.00	
P1	-1.20 $\pm$ 0.84	
P2	-1.00 $\pm$ 1.00	
P3	-0.40 $\pm$ 1.14	

ability to produce estrogen. Menopause can increase uric acid levels, which can be mitigated by administering estrogen as they can increase uric acid excretion via the kidneys (Moriwaki *et al.*, 2014). The hormone estrogen can increase uric acid excretion in the blood through the kidneys so that uric acid will not accumulate in the blood (McClory *et al.*, 2009). Raw peanut milk (*Vigna unguiculata*) has been identified to contain phytoestrogens, which are defined as compounds like estrogen but have lower activity than the latter (Prawirohardjo *et al.*, 2011).

Furthermore, the results of this study prove that uric acid levels of groups P1 and P3 have significantly increased. The highest average delta value was found in group P1. This proves that giving 200 cc of cowpea once a day can increase uric acid levels to the highest, compared to other treatment groups. We suggest that giving raw peanut milk can increase the estrogen levels of menopausal women. It is followed by an increase in uric acid production in menopausal women.

Aside from phytoestrogen, cowpea, including other legumes, has also been determined to contain adenine, hypoxanthine, xanthine, and guanine, which are known to increase blood uric acid levels. The content of purine bases in legumes that affect increasing blood uric acid levels are the purine bases adenine and hypoxanthine. Both adenine and hypoxanthine have been determined to increase blood uric acid levels, but hypoxanthine has a faster effect on raising blood uric acid levels compared to adenine (Budavari *et al.*, 2006).

A nationally representative sample of US women indicate that menopause is independently associated with higher serum uric acid levels, whereas postmenopausal hormone use is associated with lower uric acid levels among postmenopausal women. The age-associated increase in serum uric acid levels in women may be explained by menopause and other age-related factors (Hak *et al.*, 2008).

Besides, this research is deemed limited as the nutrient food intake of participants was not included for analysis, especially the consumption of high-purine foods that can affect uric acid levels.

Based on our results, it was determined that the K+ and K- groups on average did not experience changes in vaginal pH after administration of raw peanut milk. Meanwhile, in groups P1, P2 and P3 changes in vaginal pH were noted. Although the changes did not occur significantly, this research proves that raw peanut milk can reduce the pH of vaginal menopausal women.

Vaginal pH has been identified as a sensitive biological indicator of decreased and low estrogen levels in postmenopausal women. During menopause, two factors can affect vaginal pH: menopausal status and the presence of potentially pathogenic organisms. In normal fertile women, lactobacilli maintain the normal acidic vaginal pH that protects the vagina against colonization (Farage *et al.*, 2010). Some strains of lactobacilli produce hydrogen peroxide that prevents vaginal colonization of uropathogenic microorganisms (Terraf *et al.*, 2017). Therefore, exclusion of vaginitis is essential for the vaginal pH to reflect the state of the menopausal vagina (Panda *et al.*, 2014). The pH level of a woman's vagina will increase to six or more at post menopause due to decreased of *Lactobacillus* colonies in the vagina that occur secondary to the reduction of superficial cells, which can result in decreased glycogen production and thinning of the vaginal epithelium. As a result of this incident, the postmenopausal female vagina will be exposed to an increased risk of infection and inflammation, although an increased incidence of vaginal infection has not been supported by adequate data.

Phytoestrogens found in cowpea have been determined to have the same structure and function as endogenous estrogen. Phytoestrogens can bind to estrogen receptors, which in turn can increase endometrial thickness, increase elasticity and decrease vaginal pH, increase bone density, inhibit atherosclerosis, overcome hot flashes, and improve cognitive function. Therefore, phytoestrogens can reduce menopausal symptoms.

A decrease in vaginal pH has been observed in both the control and the treatment group after administration of cowpea, but it was deemed not significant. Meanwhile, a significant increase in uric acid levels was also noted in both the control group and the treatment group after cowpea administration. The highest increase in uric acid levels was noted in P1 treatment group, which was administered with 200 cc/day of cowpea.

The limitations of this study are daily diet for respondents was not managed well and it currently not evaluated with objectivity. Further research can focus on developing other variables that are more influential on the changes in the condition of menopausal women, especially in dealing with symptoms and complaints. This should be supported by a larger sample and a longer research time so as to get maximum results.

References

- Al-Baghdadi, O. and Ewies, A.A.A. (2009). Topical estrogen therapy in the management of post menopausal vaginal atrophy: An up-to-date overview. *Climacteric.*, 12(2):91-105
- Alvisi, S., Gava, G., Orsili, I., Giacomelli, G., Baldassarre, M., Seracchioli, R., and Meriggiola, M.C. (2019). Vaginal health in menopausal women. *Medicina (Kaunas).*, 55(10):615.
- Budavari, S., (1996). *The Merck Index-An Encyclopedia of Chemicals, Drugs, and Biologicals*, 12th ed., Whitehouse Station, NJ: Merck and Co. Inc
- Central Bureau of Statistics. (2020). *Proyeksi penduduk indonesia 2010-2035 [Indonesia population projection 2010-2035]*. Jakarta: BPS. Available from <https://www.bps.go.id/publication/2013/10/07/053d25bed2e4d62aab3346ec/proyeksi-penduduk-indonesia-2010-2035.html>. Accessed date: Aug 25, 2020.
- Darmadi, D., Nurdiana, and Eviana, N. (2011). Efek ekstrak kacang tunggak terhadap osteoblas dan osteoklas pada tikus dengan ovariectomi [Effect of *vigna unguiculata* extract on osteoblasts and osteoclasts in mice with ovariectomy]. *Brawijaya Med J.*, 26(3):151-5.
- Farage, M.A., Miller, K.W., and Sobel, J.D. (2010). The vaginal microbiota in menopause. In: *Textbook of aging skin*. Farage MA, Miller KW, Maibach HI. Springer, Berlin, p. 883-94.
- Goodman, N.F., Cobin, R.H., Ginzburg, S.B., Katz, I.A., and Woode, D.E. (2011). American association of clinical endocrinologists medical guidelines for clinical practice for the diagnosis and treatment of menopause. *Endocr Pract.*, 17(Nov-Dec):1-25.
- Hak, A.E. and Choi, H.K. (2008). Menopause, postmenopausal hormone use and serum uric acid levels in US women – The Third National Health and Nutrition Examination Survey. *Arthritis Res. Ther.*, 10(5):R116.
- Indonesian Ministry of Health. (2005). *Indonesia Health Profile 2001 towards healthy Indonesia 2020*. Jakarta: Indonesian Ministry of Health; Available from <https://pusdatin.kemkes.go.id/resources/download/pusdatin/profil-kesehatan-indonesia/profil-kesehatan-indonesia-2005.pdf> Accessed Date: Jul 15, 2020
- Kholis, M.N., Purwanti, S., Permata, A.T., Rizky, G., and Giyanti, D. (2020). Optimalisasi pemanfaatan kacang tunggak (*Vigna unguiculata* L.) dalam pembuatan tempe [Optimizing the utilization of cowpeas (*Vigna unguiculata* L.) in making tempe]. Central Java, Indonesia: Universitas Sebelas Maret. Available from: <https://sripurwanti.blog.uns.ac.id/files/2010/02/proposal-kacang-tunggak.pdf>. Accessed date: Aug 25, 2020.
- Manson, J.E., Chlebowski, R.T., Stefanick, M.L., Aragaki, A.K., Rossouw, J.E., and Prentice, R.L. (2013). The women's health initiative hormone therapy trials: update and overview of health outcomes during the intervention and post-stopping phases. *JAMA.*, 310(13):1,353-68.
- McClory, J. and Said, N. (2009). Gout in women. *Med. Health R.*, 92(11):363-68.
- Moriwaki, Y. (2014). Effects on uric acid metabolism of the drugs except the antihyperuricemics. *J Bioequivalence Bioavailab.*, 6(1):10-7.
- Palupi, P. (2020). *Pengalaman seksualitas perempuan menopause di wilayah kerja puskesmas kecamatan Pasar Rebo Jakarta Timur [Experience of menopause women's sexuality in the work area of Pasar Rebo district health center]*, [Master thesis]. Medical Faculty, University of Indonesia, Depok, Indonesia.
- Panda, S., Das, A., Singh, A.S., and Pala, S. (2014). Vaginal pH: A marker for menopause. *J. Midlife Health.*, 5(1):34-37.
- Prawirohardjo, S. (2011). *Ilmu kandungan [Gynecology]*. 3rd ed. Bina Pustaka Sarwono Prawirohardjo Foundation, Jakarta, 981
- Rossouw, J.E., Anderson, G.L., Prentice, R.L., LaCroix, A.Z., Kooperberg, C., Stefanick, M.L. (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the women's health initiative randomized controlled trial. *JAMA.*, 288(3):321-33.
- Scavell, I., Maseroli, E., di Stasi, V., and Vignozzi, L. (2019). Sexual health in menopause. *Medicina (Kaunas).*, 55(9):559.
- Setyarini, A.I., Wiyasa, I.W.A., Ratnawati, R., and Indrawan, I.W.A. (2019). Phytoestrogen in cowpea (*Vigna unguiculata* L. Walp) (Fabaceae) extract reduces vaginal oxidative stress and increases proliferation of fibroblast in ovariectomized rats. *Trop J. Pharm Res*, 18(10):2101-2107.
- Stevenson, J.C. (2009). Hormone replacement therapy and cardiovascular disease revisited. *Menopause Int.*, 15(2):55-7.
- Terraf, M.C.L., Tomás, M.S.J., Rault, L., Le Loir, Y., Even, S., and Nader-Macias, M.E.F. (2017). In vitro effect of vaginal lactobacilli on the growth and adhesion abilities of uropathogenic *Escherichia coli*. *Arch Microbiol.*, 199(5):767-74.
- U.S. National Library of Medicine. DHEA. (2020). US: NIH, U.S. National Library of Medicine. Available from: <http://www.nlm.nih.gov/medlineplus/druginfo/natural/331.html>. Accessed date: Nov 2, 2020.
- Wibisono, H. (2014). *Perbedaan Pengaruh Antara Ekstrak Kacang Tunggak Dan Genistein Berbagai Dosis Terhadap Rasio Bax dan Bcl2 dan Ekspresi PCNA pada Sel Epitel Kelenjar Payudara [Differences in Effect of Cowpea Extract and Genistein at Various Doses on Bax and Bcl2 Ratio and PCNA Expression in Epithelial Cells in Breast]*. [Master Thesis]. Specialist Doctor Education Program II Fertility Reproductive Endocrinology LAB/SMF Obstetrics and Gynecology, Faculty of Medicine, Universitas Airlangga/RSUD Dr. Soetomo-Surabaya, Indonesia.