Eduvest – Journal of Universal Studies
Volume 6, Number 4, April, 2026
p- ISSN 2775-3735- e-ISSN 2775-3727

The Effect of Ethanol Extract of Purple Eggplant Peel (Solanum Melongena L.) on Estrogen Receptor-A Levels and Vaginal Epithelium Thickness in a Menopause Model Rat

Yunike Susilo, Ida Ayu Ika Wahyuniari*, I Wayan Sugiritama, Ida Sri Iswari, I Wayan Juli Sumadi
Universitas Udayana, Indonesia
Emails: yunikesdr@gmail.com, ikawahyuniari@unud.ac.id* , sugiritama@gmail.com, idasriiswari@yahoo.co.id, juli_sumadi@unud.ac.id

Keyword
Estrogen Receptor-, Menopause, *Solanum melongena L.*, Vaginal Epithelial Thickness

Abstract
Menopause is a natural phase in a woman's life marked by a decline in estrogen levels, which can lead to various health issues, including vaginal atrophy. One promising alternative treatment is phytoestrogens, such as those found in purple eggplant peel (*Solanum melongena* L.) extract. This study aims to analyze the effect of administering purple eggplant peel ethanol extract on estrogen receptor-α (ERα) levels and vaginal epithelial thickness in a menopause model rat. The study employed a posttest-only control group design with four groups: a control group (K) and three treatment groups that received the extract at doses of 25 mg/200 g BW, 50 mg/200 g BW, and 100 mg/200 g BW per day for 28 days. The results showed that the 100 mg/200 g BW per day dose significantly increased ERα levels and vaginal epithelial thickness compared to the control group ($p < 0.05$). The lower doses (25 mg/200 g BW and 50 mg/200 g BW) did not show significant differences from the control group. In conclusion, purple eggplant peel extract at a dose of 100 mg/200 g BW per day can increase ERα levels and vaginal epithelial thickness in menopause model rats, demonstrating its potential as an alternative therapy for menopause symptoms.

INTRODUCTION

Aging is a natural phenomenon characterized by a decline in biological and physiological functions with age. At the biological level, aging is caused by the progressive breakdown of various molecular and cellular structures over time. The effects of cellular degeneration can result from several factors, including internal factors—such as cellular changes due to inflammation and hormonal deficiencies—and external factors, such as smoking, alcohol consumption, and malnutrition. If these factors are not addressed for prevention or mitigation, the aging process will accelerate, increasing morbidity and mortality rates. Anti-aging medicine is a branch of medical science that utilizes advanced medical technologies for early detection, prevention, treatment, and rehabilitation of body systems to maintain optimal function, thereby improving the quality of aging.

Menopause is defined by the World Health Organization (WHO) as the permanent cessation of menstruation resulting from the loss of ovarian activity without any other pathological or physiological cause. Menopause is diagnosed after a woman experiences amenorrhea for 12 consecutive months. It typically occurs around the age of 50; however, in some cases, it may begin between the ages of 41 and 45 (early menopause) or even around the age of 40 (premature menopause). This age-related condition leads to endogenous estrogen

deficiency due to reduced ovarian function and alterations in the distribution of estrogen receptors (ERα and ERβ) according to menopausal stage. Aging reduces the activity of ovarian granulosa cells, which are the primary producers of estradiol. This reduction diminishes the negative feedback effect of estrogen on luteinizing hormone (LH) and follicle-stimulating hormone (FSH), resulting in a gradual decline in endogenous estrogen levels. This menopausal period can lead to a variety of symptoms that negatively affect a woman's health and quality of life (Ebrahimi et al., 2020).

The vagina undergoes atrophy due to decreased estrogen levels during menopause. Vaginal atrophy refers to changes in the vaginal tissue resulting from estrogen deficiency, with primary symptoms including vaginal dryness and pain during sexual activity. Decreased estrogen levels lead to several structural and functional changes in the vagina, including epithelial thinning, reduced epithelial cell maturation index, decreased collagen fiber content, reduced smooth muscle mass, and decreased vascularization (Alvisi et al., 2018; Xiao et al., 2025).

Numerous studies have shown that changes in estrogen levels during menopause correlate with ERα and ERβ expression levels. In premenopausal women, ERα and ERβ function optimally to maintain vaginal health and function. Generally, ERα expression is higher than ERβ in both the epithelium and stroma. In the vaginal epithelium, ERα plays a more significant role in epithelial cell proliferation, whereas its role in differentiation is not absolutely essential. Decreased ERα expression is associated with vaginal atrophy during the menopausal transition, as ERα is more dominant in vaginal tissue. Furthermore, with aging and reduced postmenopausal estrogen production, ERα expression declines significantly, resulting in vaginal atrophy characterized by symptoms such as dryness, irritation, and dyspareunia.

These changes manifest as vaginal atrophy, accompanied by disruptions in epithelial regeneration, mucus secretion, vaginal pH balance, and microbiota composition (Binder et al., 2019; Flores and Hall, 2020; Fithri et al., 2023). Several studies have demonstrated that estrogen therapy can restore vaginal health (Bleibel and Nguyen, 2023; Fithri et al., 2023). Therefore, estrogen supplementation is often considered necessary in postmenopausal women. Early studies primarily utilized hormone replacement therapy (HRT).

Hormone replacement therapy (HRT) is recommended by the International Menopause Society as the primary treatment for menopausal symptoms (Palacios et al., 2019; Vigneswaran & Hamoda, 2022). However, long-term use may increase the risk of breast cancer, particularly in women with a relevant family or medical history (Ahmad, 2019). The economic burden of HRT must also be considered, especially in developing countries. Consequently, researchers are exploring alternative therapies that are more natural and have fewer side effects, such as phytoestrogens.

Phytoestrogens are compounds with chemical structures similar to endogenous estrogens (estradiol) and can bind to ERα and ERβ within cells (Desmawati & Sulastri, 2019). Their binding can induce estrogen-responsive gene expression and stimulate cellular proliferation. One of the most extensively studied phytoestrogen groups is flavonoids. Several studies have demonstrated the effectiveness of phytoestrogens in reducing menopausal symptoms. Levis et al. (2011) investigated the effects of soy isoflavones in postmenopausal women and found a significant reduction in vasomotor symptoms, such as hot flashes and vaginal dryness,

compared to placebo. Similarly, Hallund et al. (2006) reported that flaxseed lignans reduced vasomotor symptoms and improved cardiovascular health in postmenopausal women.

Anthocyanins are natural pigments belonging to the flavonoid group, a class of polyphenols widely found in plants. Their structure and biosynthetic pathways are closely related to flavanones and isoflavones, which are known to exhibit estrogenic activity. Sugiritama and Adiputra (2019) reported that anthocyanins may help reduce menopausal symptoms. These findings suggest that anthocyanins possess phytoestrogenic properties and can bind to ERα and ERβ.

Several studies using postmenopausal or post-oophorectomy animal models support this theory, demonstrating that anthocyanin administration can increase vaginal epithelial thickness and significantly reduce apoptosis indices (Ferdina et al., 2019). Anthocyanins in mulberry extract have also been shown to significantly increase endometrial thickness (Pereira et al., 2022), as have anthocyanins from red dragon fruit peel.

One potential source of anthocyanins currently under investigation is purple eggplant. Purple eggplant (*Solanum melongena L.*) is widely consumed and known for its antioxidant properties. Its phytoestrogenic and antioxidant effects are attributed to anthocyanins. These compounds are predominantly found in the peel of purple eggplant (*Solanum melongena L.*), particularly in the form of delphinidin-3-rutinoside. The anthocyanin content in eggplant peel is notably high, with antioxidant activity reaching 74.26%. Azuma et al. (2008) reported that anthocyanins in eggplant skin, especially nasunin, exhibit strong free radical–scavenging activity and potential phytoestrogenic effects.

Another study found that purple eggplant peel extract contains phytoestrogenic compounds capable of binding to estrogen receptors, suggesting potential benefits in reducing menopausal symptoms such as hot flashes and maintaining bone health (Etta et al., 2018). Hodek et al. (2009) evaluated the estrogenic activity of flavonoids from eggplant skin through in vitro assays and found that these compounds can mimic estrogen activity. These findings highlight the potential of eggplant peel as an alternative therapy for women unable to undergo HRT. Despite this, no studies to date have specifically examined the effects of purple eggplant peel on vaginal atrophy in menopausal animal models.

This study aims to investigate the effects of anthocyanins derived from purple eggplant (*Solanum melongena L.*) peel on ERα levels and vaginal epithelial thickness in menopausal model rats. This research seeks to demonstrate the role of anthocyanins in alleviating vaginal atrophy by increasing ERα expression and promoting epithelial regeneration. Additionally, this study addresses a gap in the literature, as no prior research has evaluated the effects of purple eggplant peel extract on these parameters.

This study formulates several key research questions regarding the administration of ethanol extract of purple eggplant peel (*Solanum melongena L.*) in menopausal model rats. Specifically, it examines whether ERα levels in rats receiving doses of 25 mg/200 g body weight/day, 50 mg/200 g body weight/day, and 100 mg/200 g body weight/day orally are higher than those in the control group. Additionally, it evaluates whether vaginal epithelial thickness is increased in treated groups compared to controls.

The general objective of this study is to determine whether orally administered ethanol extract of purple eggplant peel (*Solanum melongena L.*) can reduce symptoms of vaginal atrophy in menopausal model animals. Specifically, this study aims to demonstrate that ERα

levels and vaginal epithelial thickness are significantly increased in treated groups compared to controls.

This study provides theoretical contributions by expanding scientific knowledge regarding the effects of purple eggplant peel extract on ERα levels and vaginal epithelial thickness. Practically, the findings are expected to serve as preliminary data for developing alternative therapies for menopausal symptoms, particularly vaginal atrophy. Furthermore, this research may support the use of purple eggplant peel as a natural therapeutic agent, potentially improving the quality of life of postmenopausal women while also contributing to agricultural and economic development, particularly for eggplant farmers in Indonesia, including regions such as Bali.

METHOD

This study is a purely in vivo experimental study with a randomized post-test only control group design pattern using a female white rat (Rattus norvegicus) strain of Wistar. The experimental animals were divided into four experimental groups with details of one control group that was given standard feed, bilateral oophorectomy and normal saline 1 mL/day for 28 days, and three treatment groups each of which was given ethanol extract of purple eggplant peel (*Solanum melongena L.*) orally with different doses, namely 25 mg/200 grBB/day, 50 mg/200 grBB/day, and 100 mg/200 grBB/day for 28 days after bilateral oophorectomy. The research was carried out at the Integrated Biomedical Laboratory Unit of the Faculty of Medicine, Udayana University for the study of experimental animal treatment and examination of ERα levels of vaginal epithelium and vaginal epithelium, while the phytochemical test of purple eggplant was carried out at the Integrated Research Laboratory, Faculty of Mathematics and Natural Sciences, Udayana University in the period November 2024 to February 2025.

The population of this study was a female white rat (Rattus norvegicus) of the Wistar strain aged 10-15 weeks with a weight of about 180-220 grams in good health who had undergone bilateral oophorectomy. The target population includes all female white rats of the Wistar strain aged 10-15 weeks weighing 180-220 grams, while the affordable population is mice with the same characteristics that are kept in an Integrated Biomedical Laboratory. The sample size was determined using Federer's formula with the results of the calculation of 6 mice per group, then added 10% to anticipate drop out so that it became 7 mice per group with a total of 28 mice. The sample inclusion criteria included female white rats of the Wistar strain in healthy condition, having undergone bilateral oophorectomy, aged 10-15 weeks, weighing about 180-220 grams, and eating, drinking, and moving behaviors that did not change within one week of observation, while the exclusion criteria were mice that showed signs of infection on the skin or died during the study.

Data collection was carried out through a series of procedures starting with the adaptation of mice for 7 days, followed by bilateral oophorectomy using ketamine anesthesia 50 mg/kgBB with xylazine 5 mg/kgBB, and a recovery period of 14 days with the administration of amoxicillin antibiotics 150 mg/kgBB/day. After the recovery period, the mice were randomly grouped into four groups and given a treatment in the form of aquaades for the control group or ethanol extract of purple eggplant peel with three dose variations for the treatment group for 28 days. Purple eggplant peel extract is made through maceration method using a 96% ethanol solvent with a ratio of 1:5 for 3x24 hours, then evaporated with a rotary evaporator at a

temperature of 40°C until a thick extract is obtained stored at a temperature of 2-8°C. Measurement of vaginal epithelial ERα levels was carried out by the Enzyme-Linked Immunosorbent Assay (ELISA) method using the Rat malondialdehyde ELISA Kit from Bioassay Technology Laboratory with results expressed in nanomoles per milliliter (nmol/mL), while the measurement of vaginal epithelial thickness was carried out through histological preparation examination with hematoxilin-eosin (HE) staining using an Olympus CX 41 microscope with a magnification of 400 times in five fields of view, where each field of view is measured on three areas of the vaginal epithelium and the result is expressed in micrometers (μm).

The collected data was recorded on a special research sheet and analyzed using the SPSS version 26.0 program through several stages of testing. The first stage is a descriptive test to obtain an overview of the characteristics and frequency distribution of all research variables presented in the form of tables and narratives. The second stage is the normality test using the Shapiro-Wilk test because the number of samples is less than 50, followed by a homogeneity test using the Levene test to determine the homogeneity of the variance of the variables tested. The last stage was a bivariate test using the one-way ANOVA method to determine the difference in the average level of ERα and vaginal epithelial thickness in each treatment group, with the difference considered significant if it had a p< value of 0.05, and followed by a post hoc analysis of LSD (least significant difference) to determine specific groups that had significant differences in data with normal distribution and homogeneous variants.

RESULT AND DISCUSSION

Descriptive Analysis

In the control group (K), the number of experimental animals analyzed was 6. Initially, each group was prepared 7 heads according to the calculation with Federer's formula of 6 heads plus 10%, which is 1 head as a reserve. In the course of the study, 1 head could not be included in the analysis because at the time of treatment it experienced signs of illness and then died. Thus, the number of samples K is 6 samples and still meets the minimum criteria required for statistical analysis. Meanwhile, in the P1, P2, and P3 treatment groups, there were 7 samples each.

Descriptive analysis includes mean, standard deviation, median, minimum and maximum. The results of the analysis of ERα levels and the thickness of the vaginal epithelium of rats are presented in Table 5.1 and Table 5.2. The highest average ERα levels were found in the P3 group (7.94 □ 1.07 ng/mL) and the lowest average ERα levels were found in the P1 group (6.14 □ 0.41 ng/mL). The minimum value of ERα level is 4.22 ng/mL and the maximum value is 9.77 ng/mL. The average thickness of the highest rat vaginal epithelium was found in the P3 group (70.15 □ 7.01 □m) and the lowest average thickness of the rat vaginal epithelium was found in the P2 group (60.33 □ 4.13 □m). The minimum value of the thickness of the vaginal epithelium of rats is 47.02 □m and the maximum value is 84.00 □m.

Table 1 Results of Descriptive Analysis of *Estrogen Receptor Levels*-α (ng/mL) Data

Groups	Average	Simpang Baku	Median	Minimum	Maximum
K	6,15	1,03	6,60	4,22	6,99
P1	6,14	0,41	6,13	5,48	6,76

P2	6,29	0,87	6,64	5,14	7,39
P3	7,94	1,07	7,54	7,03	9,77

Data Source: Experimental results from the descriptive analysis of estrogen receptor levels in Wistar rats, as per the study's methodology

Table 2 Descriptive Analysis Results of Vaginal Epithelial Thickness Data (μm)

Groups	Rerata	Simpang Baku	Median	Minimum	Maximum
K	60,87	7,04	62,85	47,07	66,45
P1	60,48	9,86	63,73	47,02	73,36
P2	60,33	4,13	59,94	55,33	65,83
P3	70,15	7,01	68,77	63,17	84,00

Data Source: Experimental results from the descriptive analysis of vaginal epithelial thickness in Wistar rats, as per the study's methodology

Data Normality Test

Data normality test of ERα levels and vaginal epithelial thickness of rats in each group using *the Shapiro-Wilk test.* The results of the data normality test can be seen in Table 3.

Table 3 Results of the Data Normality Test between Groups

Variabel	Groups	n	p	Remarks
Kadar *Estrogen Receptor-α*	K	6	0,054	Normal
	P1	7	0,989	Normal
	P2	7	0,322	Normal
	P3	7	0,075	Normal
Thickness of the vaginal epithelium	K	6	0,091	Normal
	P1	7	0,614	Normal
	P2	7	0,504	Normal
	P3	7	0,194	Normal

Data Source: Results of the Shapiro-Wilk normality test applied to the experimental data for estrogen receptor levels and vaginal epithelial thickness in Wistar rats, as part of the study methodology

Description: n = sample size, p = significance

Based on the results of the *Shapiro-Wilk* test, it was found that all groups had a normal data distribution ($p < 0.05$).

Data Homogeneity Test

The homogeneity of the data from the ERα levels and the thickness of the vaginal epithelium of rats in each group was tested using *the Levene statistical test*. Data variants of ERα levels and the thickness of the rat vaginal epithelium can be seen in Table 5.4. The data variant is said to be homogeneous if the p value > 0.05.

Table 4 Results of the Data Homogeneity Test Between Groups

Variabel	n	p	Remarks
Kadar *Estrogen Receptor-α*	27	0,110	Homogeneous
Thickness of the vaginal epithelium	27	0,125	Homogeneous

Description: n = sample size, p = significance

Data Source: Results from the Levene's homogeneity test applied to the experimental data for

estrogen receptor levels and vaginal epithelial thickness in Wistar rats, as part of the study methodology

Comparability Test

To compare the average ERα levels and the thickness of the vaginal epithelium of rats between groups K, P1, P2, and P3, a comparability analysis was performed. The one-way ANOVA test was chosen on the variables of ERα levels and the thickness of the vaginal epithelium of rats because the data from the study amounted to four groups of unpaired groups, the data were distributed normally and homogeneously.

a. Test the comparability of estrogen receptor levels-α

The comparability test aimed to compare the average levels of ERα between the four groups. The results of the comparative analysis of ERα levels can be seen from Table 5.

Table 5 Average Intergroup *Estrogen Receptor*α Levels (ng/mL)

Groups	n	Rerata	F	p
K	6	6,15 ± 1,03	6,902	0,002
P1	7	6,14 ± 0,41		
P2	7	6,29 ± 0,87		
P3	7	7,94 ± 1,07		

Remark: F = *ANOVA one-way* test result, n = sample size, p = significance

Data Source: Results from the one-way ANOVA test comparing average estrogen receptor levels across different treatment groups in Wistar rats, as part of the study methodology

Significance analysis using *one way ANOVA* obtained a value of F = 6.902 and a value of p = 0.002. This shows that there are at least two groups that have significantly different average ERα levels ($p < 0.05$). The average levels of ERα between groups can be seen in Figure 1.

Furthermore, to find out the differences between groups, *the least significance difference test* is used which can be seen in Table 5.6. The table shows a significant difference between the P3 group and the other groups ($p < 0.05$). However, there was no significant difference between the P1 and P2 groups and the K group ($p > 0.05$). This suggests that administering purple eggplant peel ethanol extract at a dose of 100 mg/200 grams/day can make ERα levels in rats undergoing bilateral oophorectomy higher. However, this effect was not found in the administration of purple eggplant peel ethanol extract at doses of 25 mg/200 grams/day and 50 mg/200 grams/day.

Table 6 Comparative Analysis of *Estrogen Receptor Levels* -α Intergroup

Groups	Groups	p
K	P1	0,982
	P2	0,769
	P3	0,001
P1	P2	0,742
	P3	0,001
P2	P3	0,002

Data Source: Results from the least significant difference (LSD) post hoc analysis following the one-way ANOVA test to compare estrogen receptor levels between

experimental groups in Wistar rats, as part of the study methodology

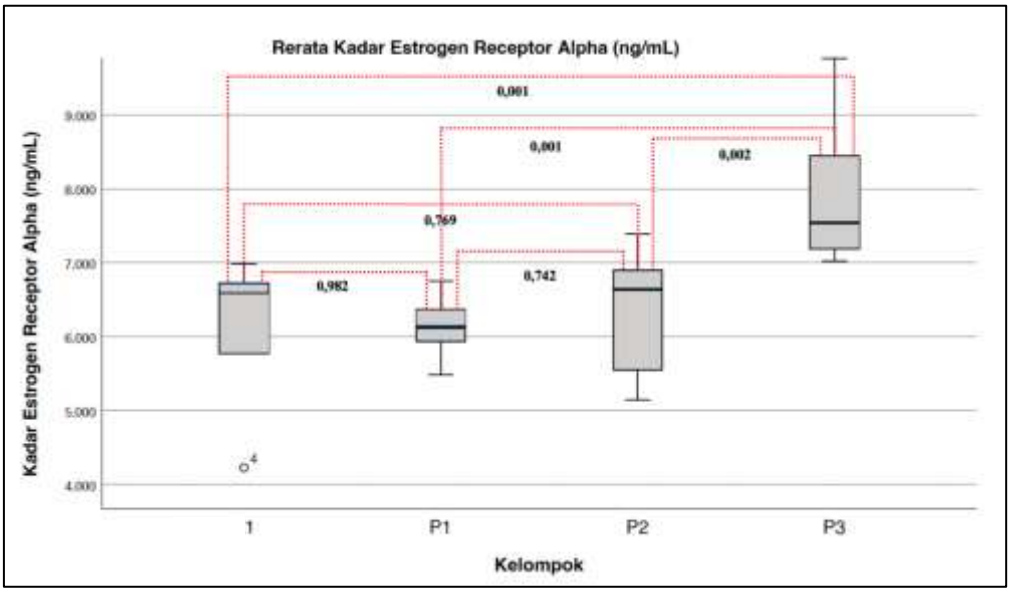


Figure 1 Difference in Average Estrogen Receptor Levels Between Groups

Description: p = significance

Remark: K = control group, P1 = dose group 25 mg/200 grams/day, P2 = dose group 50 mg/200 grams/day, P3 = dose group 100 mg/200 grams/day

Test of the thickness comparability of the vaginal epithelial of rats

The comparability test aimed to compare the average thickness of the vaginal epithelium of mice between the four groups. The results of the comparative analysis of the thickness of the vaginal epithelial of rats can be seen from Table 7.

Table 7 Average Thickness of Vaginal Epithelial Rats Intergroup (μm)

Groups	n	Rerata	F	p
K	6	60,87 ± 7,04	3,099	0,047
P1	7	60,48 ± 9,86		
P2	7	60,33 ± 4,13		
P3	7	70,15 ± 7,01		

Remark: F = *ANOVA one-way* test result, n = sample size, p = significance

Data Source: Results from the one-way ANOVA test comparing the average vaginal epithelial thickness across different treatment groups in Wistar rats, as part of the study methodology

Significance analysis using *one-way ANOVA* obtained a value of F = 3.099 and a value of p = 0.047. This shows that there are at least two groups that have significantly different average levels of vaginal epithelial thickness in mice ($p < 0.05$). The average thickness of the vaginal epithelium of mice between groups can be seen in Figure 5.2.

Furthermore, to determine the differences between groups, the *least significance difference test* is used which can be seen in Table 5.8. The table shows a significant difference between the P3 group and the other groups ($p < 0.05$). However, there was no significant difference between the P1 and P2 groups and the K group ($p > 0.05$). This suggests that

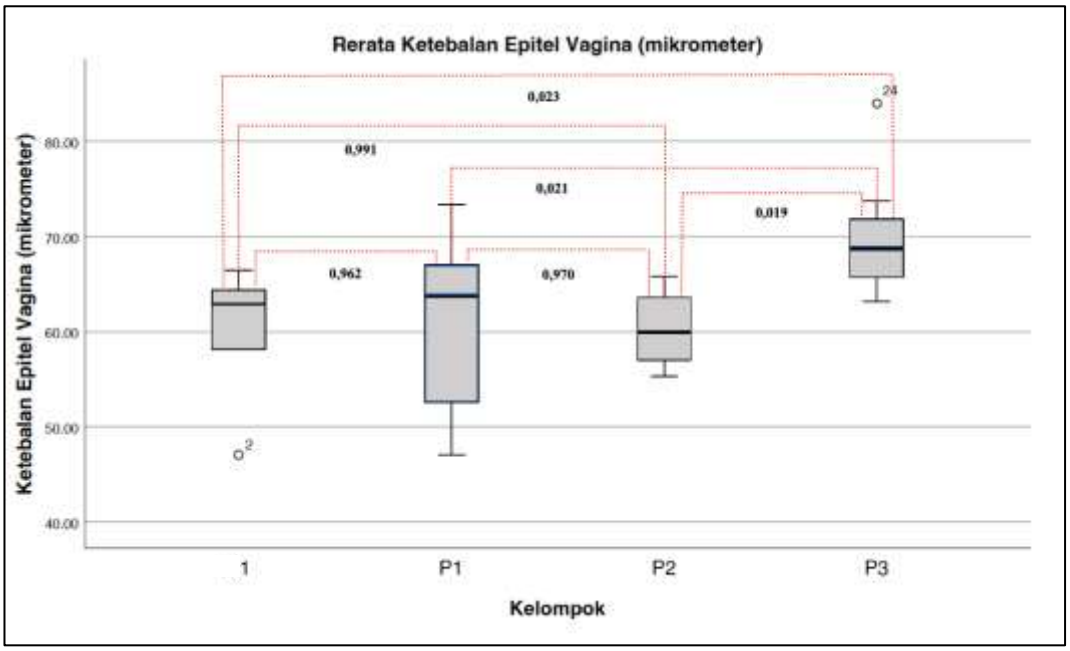
administering purple eggplant peel ethanol extract at a dose of 100 mg/200 grams/day can make the thickness of the vaginal epithelium in rats undergoing bilateral oophorectomy higher. However, this effect was not found in the administration of purple eggplant peel ethanol extract at doses of 25 mg/200 grams/day and 50 mg/200 grams/day.

Table 8 Comparative Analysis of Vaginal Epithelial Thickness of Rats Between Groups

Groups	Groups	p
K	P1	0,962
	P2	0,991
	P3	0,023
P1	P2	0,970
	P3	0,021
P2	P3	0,019

Description: p = significance

Data Source: Results from the least significant difference (LSD) post hoc analysis following the one-way ANOVA test to compare vaginal epithelial thickness between experimental groups in Wistar rats, as part of the study methodology



Remark: K = control group, P1 = dose group 25 mg/200 grams/day, P2 = dose group 50 mg/200 grams/day, P3 = dose group 100 mg/200 grams/day

Figure 2 Difference in Average Thickness of Vaginal Epithelium in Rats Between Groups

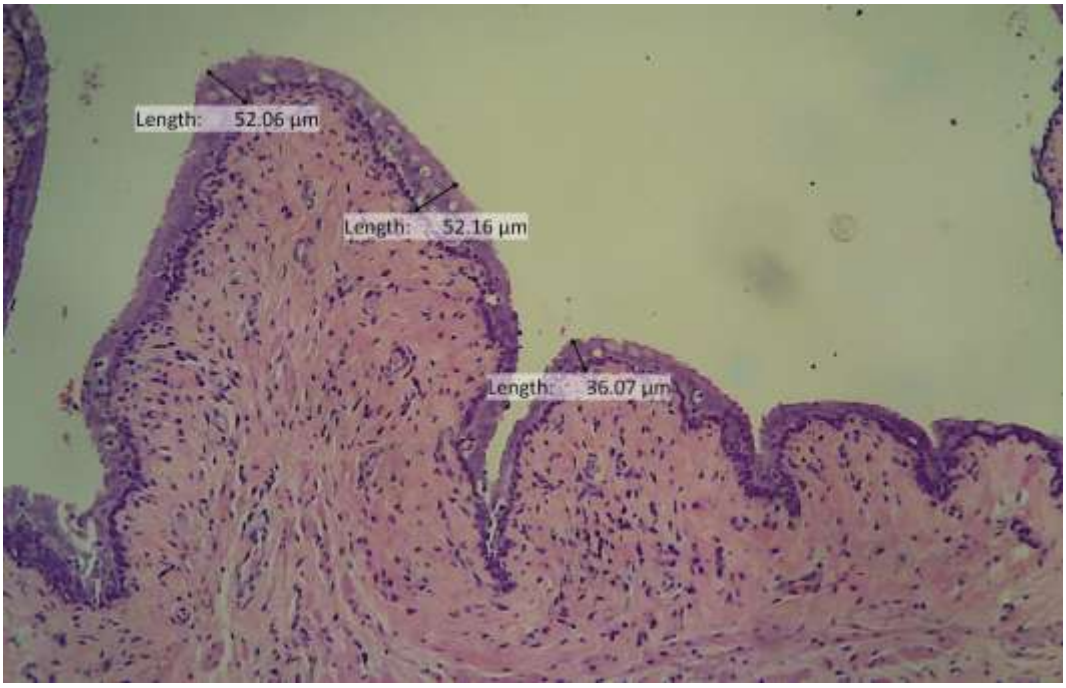


Figure 3 Histological Structure of Group K Vaginal Wall with 400x magnification HE painting. Epithelial thickness was measured at three locations in each field of view.

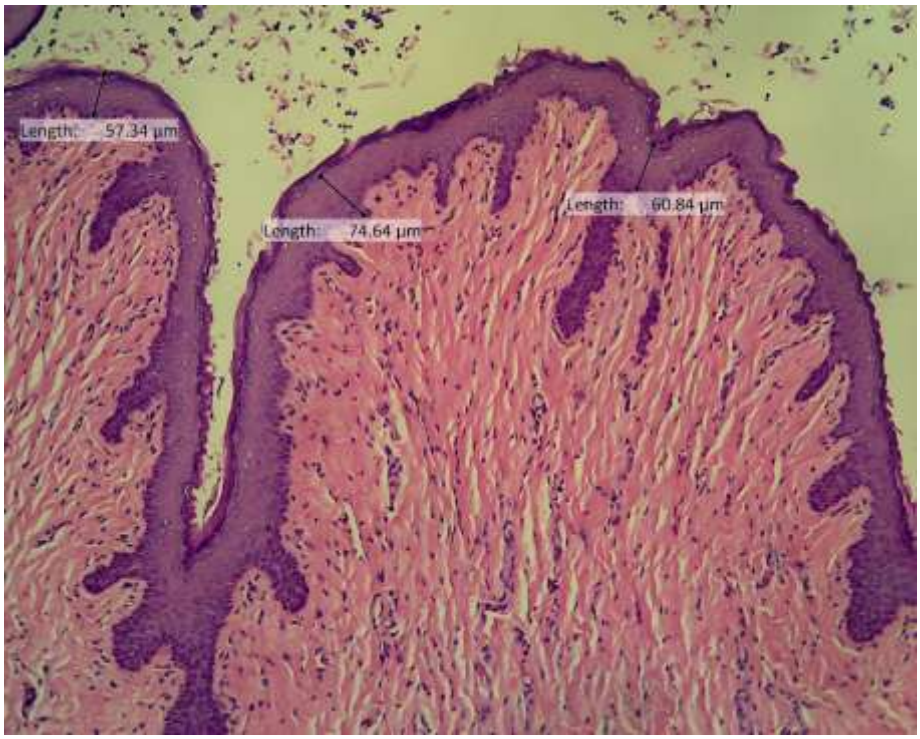


Figure 4 Histological Structure of the Vaginal Wall Group P1 with 400x magnification HE painting. Epithelium thickness was measured at three locations in each field of view

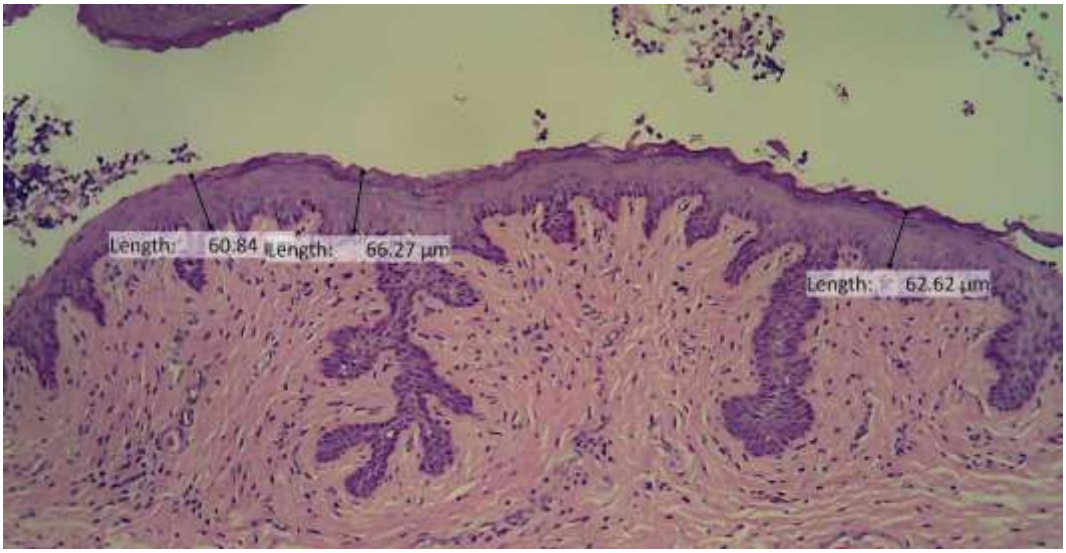


Figure 5 Histological Structure of the Vaginal Wall Group P2 with HE painting 400X magnification. Epithelium thickness was measured at three locations in each field of view

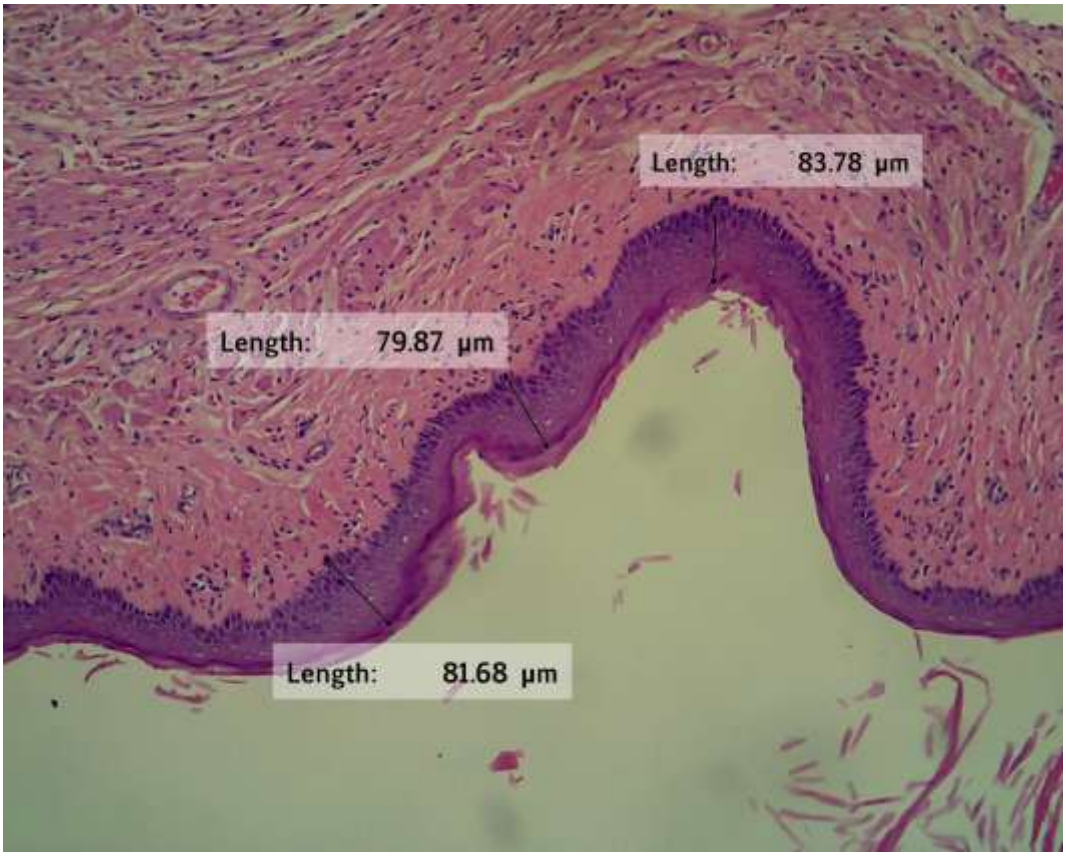


Figure 6 Histological Structure of the Vaginal Wall Group P3 with HE painting of 400X magnification. Epithelium thickness was measured at three locations in each field of view

Effects of Solanum melongene L. on Estrogen Receptor Levels-□

This study shows that the administration of purple eggplant peel ethanol extract at a dose of 100 mg/200 grams/day can make ERα levels in rats that underwent bilateral oophorectomy higher. However, this effect was not found in the administration of purple eggplant peel ethanol extract at doses of 25 mg/200 grams/day and 50 mg/200 grams/day. This may be due to the fact that the content of active compounds in purple eggplant peel ethanol extract, such as anthocyanins at doses of 25 mg/200 grams/day and 50 mg/200 grams/day, has not reached a sufficient concentration threshold to trigger a significant biological response to increased ERα expression. The effectiveness of phytochemicals in modulating estrogen receptor expression is highly dependent on the dose, bioavailability, as well as affinity of the active compound to the target receptor (Ayvaz et al., 2022). Therefore, a dose of 100 mg/200 grams/day is likely to have met the therapeutic threshold required to produce measurable physiological effects.

The skin of the purple eggplant (*Solanum melongena L.*) is known to contain various bioactive compounds, especially the flavonoid and anthocyanin groups, with nasunin as the main component. Nasunin is a delphinidin-3-(p-coumaroylrutinoside)-5-glucoside-type anthocyanin, which has been shown to have high antioxidant activity and potential as a modulator of steroid hormones. This activity is possible through its ability to interact with estrogen receptors, especially ERα.

Estrogen receptor-α is one of the two main subtypes of estrogen receptors involved in the regulation of various physiological processes such as growth, differentiation, tissue homeostasis, and reproductive function. Under normal conditions, estrogen binds to ERα, activating the transcription of estrogen target genes. Phytoestrogen compounds in plants that have an estrogen-like chemical structure, can bind to these receptors and produce agonistic or antagonistic effects depending on the target tissue and its concentration.

In this study, the administration of purple eggplant peel ethanol extract at a dose of 100 mg/200 grams/day showed a significant increase in ERα levels. This suggests that the bioactive compounds in the skin of purple eggplant act as ERα agonists, where they can bind to ERα and activate it, but not as strong as natural estrogen (estradiol). So, the effect is similar to estrogen, but weaker. However, this effect is enough to increase ERα expression. This mechanism is similar to the effects of several other flavonoid compounds such as genistein from soybeans or schisandrol A from Schisandra chinensis which have been shown to increase ERα expression and activate signaling pathways such as PI3K/Akt and MAPK/ERK (Peiffer et al., 2020; Wang et al., 2021).

The ethanol extract of purple eggplant peel in this study is known to have an anthocyanin level of 113.78 mg/100 grams. This level is quite high compared to the levels in extracts of some other natural ingredients. For example, red dragon fruit peel extract has anthocyanin levels of around 58.07 mg/L (5.81 mg/100 grams) (Arsyad, 2021), and purple sweet potato that has anthocyanin levels ranging from 0.6 mg/g to 1 mg/g (60mg/100 grams to 100mg/100 grams), depending on the extraction method used (Cao et al., 2011). Other sources such as blueberries are recorded to have anthocyanin levels that range from 80–160 mg/100 grams (Ramdan and Lestari, 2023). This comparison shows that purple eggplant peel has a relatively higher potential as a source of anthocyanins.

Increased levels of ERα can be explained through two main mechanisms. First, the anthocyanin compounds in Solanum melongene L. acts as a direct agonist against ERα through ligand-receptor bonding. Research by Zhang et al. (2021) suggests that anthocyanins from Hibiscus sabdariffa can bind strongly to the binding domain of ERα ligands based on molecular simulations. Nasunin, which has a similar structure, has the potential to have a similar affinity to ERα (Zhang et al. 2021). Anthocyanins are able to attach precisely to the ERα Ligand Binding Domain (Ligand Binding Domain) attachment site (Nanashima et al., 2015). The attachment of anthocyanins as ligands to estrogen receptors is able to trigger the activation of estrogen receptors, and then followed by the translocation of complex ligands into the cell nucleus (Szymanski et al., 2021). In the cell nucleus, the receptor-ligand complex attaches to ERE (estrogen response elements) and acts as a transcription factor so that it is able to regulate the expression of estrogen receptor genes and genes involved in estrogen signaling (Nanashima et al., 2015; Horie et al., 2019). Activation of ERE by ligand-receptor complexes has been shown to be able to induce the induction of ERα expression (Zheng et al., 2019).

The second mechanism is through direct activation of estrogen receptors, the anthocyanin compounds in the skin of purple eggplant are thought to contribute to increased ERα expression through epigenetic modulation mechanisms. One of the main pathways that may be involved is the inhibition of DNA methyltransferase (DNMT), an enzyme that plays a role in adding methyl groups to the region of the gene promoter. Hypermethylation in the ERα promoter can suppress the transcription of the gene. Some flavonoids, including anthocyanins, have been shown to inhibit DNMT, thereby causing DNA hypomethylation and increasing the expression of target genes such as ERα (Wang et al., 2013). In addition, anthocyanins also play a role in the inhibition of histone deacetylase (HDAC), which contributes to increased chromatin accessibility for gene transcription. A more open chromatin structure allows for increased ERα transcription (Bayazid and Lim, 2024). Such anthocyanins may also improve the stability or expression of ERα genes through epigenetic modulation or activation of upstream signaling pathways such as PI3K/Akt and MAPK that are known to amplify ERα expression.

In this study, purple eggplant peel extract containing phenols in the form of gallic acid and other flavonoids, namely quercetin, is suspected to contribute to increasing ERα levels. Studies by Dogrul et al. (2016) show that galic acid can affect estrogen metabolism enzymes, such as CYP19 (aromatase), COMT, quinone reductase (QR), and glutathione S-transferase (GST). In normal fibroblast cells, gallic acid increases the expression of CYP19, which functions to convert androgens into active estrogen. This increased availability of local estrogen due to this metabolic modulation can indirectly increase ERα activation in target tissues such as the vagina. Quercetin, which is a phytoestrogen, has also been found to stimulate ERα and Erβ.

This effect was seen in line with the increase in ERα levels observed in this study after the administration of purple eggplant peel extract. With increased levels of ERα, the tissue's response to estrogen increases, which can provide physiological effects such as improved bone metabolism, cardiovascular protection, and neuroendocrine stability.

These findings suggest that active compounds such as anthocyanins (nasunin), quercetin, and galic acid act as phytoestrogens that can bind and activate ERα. This activation takes place through two main mechanisms, namely direct binding with estrogen receptors that trigger target gene transcription, as well as epigenetic modulations such as DNMT and HDAC inhibition that

increase the expression of ERα genes. The PI3K/Akt and MAPK/ERK signaling pathways are also thought to be involved in the amplification of the expression of these receptors. The combination of these mechanisms makes tissues more responsive to estrogen, so purple eggplant peel extract has the potential to be a natural agent to support the prevention of estrogen deficiency conditions. opens up the potential use of Solanum melongene L. as a natural ingredient in estrogen-deficient conditions such as menopause, osteoporosis, or neurodegenerative disorders.

Effect of Solanum melongena L. on Vaginal Epithelial Thickness

This study shows that the administration of purple eggplant peel ethanol extract at a dose of 100 mg/200 grams/day can make the thickness of the vaginal epithelium in rats that underwent bilateral oophorectomy higher. However, this effect was not found in the administration of purple eggplant peel ethanol extract at doses of 25 mg/200 grams/day and 50 mg/200 grams/day. This can be due to the fact that the concentration of active compounds in purple eggplant peel ethanol extract, such as anthocyanins at doses of 25 mg/200 grams/day and 50 mg/200 grams/day, is not enough to optimally stimulate the proliferation of vaginal epithelial cells.

The phytoestrogen compounds in the extract are thought to work by binding to estrogen receptors, which then induce the transcription of genes involved in epithelial cell regeneration and differentiation. At lower doses, the affinity and amount of phytoestrogens available may not be sufficient to produce adequate estrogen receptor activation, so there is no increase in epithelial thickness. On the other hand, at a dose of 100 mg/200 grams/day, the concentration of the active compound seems to have reached an effective level to elicit a physiological response in the form of an increase in epithelial thickness as one of the indicators of estrogenic effects.

The vaginal epithelium is a tissue that is very sensitive to the influence of the hormone estrogen. The thickness and maturation of the vaginal epithelium is directly affected by estrogen levels, specifically estradiol, which interacts with estrogen receptors in the target tissue. In estrogen-deficient states, such as post-menopause or oophorectomy, there is a decrease in epithelial cell proliferation, a decrease in the superficial cell layer, and an increase in the proportion of parabasal cells, all of which indicate the occurrence of vaginal epithelial atrophy.

On the contrary, increased estrogenic activity will stimulate the division and differentiation of epithelial cells thereby increasing the thickness of the vaginal epithelium. Therefore, the thickness of the vaginal epithelium is often used as a biological parameter to assess the estrogenic effects of a compound or hormone therapy.

The skin of purple eggplant is rich in anthocyanin compounds, especially nasunin, a delphinidine-type anthocyanin. Nasunin has been studied to have high antioxidant potential and possible mimetic hormone activity, especially against estrogen. Nthocyanins from various plant sources have been shown to have estrogenic effects by binding to ERα and ERβ and triggering the expression of estrogen-responsive genes.

Some experimental studies have shown that polyphenol compounds and phytoestrogens from plants can increase the thickness of vaginal epithelium. For example, isoflavones from soy (Glycine max) and flavonoids from Colocasia esculenta successfully increased the

thickness of the vaginal epithelium in menopausal model mice. Nasunin, which has a similar chemical structure, has the potential to have a comparable mechanism of action.

Increased vaginal epithelial thickness after administration of purple eggplant peel extract involves the activation of ERα, especially as expressed in the basal and parabasal layers of vaginal epithelium. The interaction of phytoestrogen compounds with ERα will trigger the activation of genomic and nongenomic signaling pathways leading to the activation of transcription factors such as ERE, induction of cell-cycle gene expression (e.g., cyclin D1), increased synthesis of structural proteins and cellular metabolism-supporting enzymes. In addition to acting directly on ERE, phytoestrogens can also regulate the expression of estrogen receptors through their influence on transcription factors that act on estrogen receptor promoters.

These effects will morphologically be seen as an increase in the number of epithelial layers, thickening of the mucosa, and increased mitotic activity in the basal zone. In addition, estrogen also increases the accumulation of glycogen in the vaginal epithelium which will support the growth of lactobacillus flora and maintain the normal acidic pH of the vagina.

Although there have not been many direct studies on purple eggplant skin extracts, studies on similar compounds have shown consistent results. In a study by Cipolletti et al. (2018), polyphenols from foods such as berries and grapes have an agonistic ability to ERα which shows an increased estrogenic effect. Such effects include increased epithelial thickness, cervical mucus production, and mucosal vascularization.

Based on the assumption that bioactive compounds in the skin of purple eggplant such as nasunin have an affinity for estrogen receptors, the mechanism of increasing the thickness of the vaginal epithelium through this pathway is scientifically acceptable and physiologically relevant (Dugan et al., 2018).

The increase in the thickness of the vaginal epithelium due to the administration of ethanol extract of purple eggplant peel is thought to be closely related to the content of active compounds in it, especially anthocyanins, quercetin, and cholic acid, which work through anti-inflammatory and antioxidant mechanisms, although this study did not measure the antioxidant activity of purple eggplant peel The antiflamation effect of purple eggplant ethanol extract may also play a role in improving symptoms of vaginal atrophy. Clinical studies have found that postmenopausal women who experience vaginal atrophy show improved epithelial integrity after administration of sea buckthorn oil, confirming that the atrophic condition is initially susceptible to mild irritation and inflammation (Barnard et al., 2021; Davinelli et al., 2015)).

In hypoestrogenic conditions such as post-ovhorectomy, endogenous estrogen levels decrease drastically, causing increased oxidative stress due to the accumulation of reactive oxygen species (ROS) in vaginal epithelial tissue. Excessive ROS is known to damage cell membranes, proteins, and DNA, as well as inhibit the process of epithelial cell proliferation and differentiation. The presence of compounds with high antioxidant abilities is essential in supporting tissue regeneration.

Anthocyanins, especially delphinidin and nasunin types found in purple eggplant shells, act as free radical scavengers and are able to stabilize ROS through electron donation. This compound is also known to activate nuclear transcription factor erythroid 2-related factor 2 (Nrf2), which further increases the expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). In addition to its direct

antioxidant effects, anthocyanins also play a role in the activation of estrogenic signaling pathways such as PI3K/Akt and MAPK/ERK, as well as increased ERα expression, which contributes to the stimulation of epithelial proliferation, vascularization, and mucosal tissue regeneration.

The increase in the thickness of the vaginal epithelium due to the administration of ethanol extract of purple eggplant peel is suspected to be not only due to the estrogenic activity of phytoestrogen compounds such as anthocyanins, but also closely related to the antioxidant effects of other ingredients it has, such as quercetin and cholic acid.

Quercetin, another flavonoid in purple eggplant skin extract, also has an important role. In vivo studies show that quercetin has estrogenic activity, as evidenced by the increase in uterine weight and the prevention of endometrial thickness decline in prepubertal mice that experienced primary ovarian failure due to exposure to 4-Vinylcyclohexene dioxide/VCD. This effect was reinforced by the finding that the estrogenic effect of quercetin could be inhibited by fulvestrant (an ER antagonist), indicating its interaction with estrogen receptors, including ERα. In addition, quercetin also exhibits strong antioxidant effects through Nrf2 activation and increased expression of antioxidant enzymes, as well as inhibition of oxidative stress due to ROS.

Gallic acid in purple eggplant peel extract plays a role in strengthening the tissue protective effect through the Nrf2/HO-1 pathway and inhibiting the activation of NF-κB, which is often activated in chronic inflammatory conditions or mucosal atrophy. Research by Zhou et al. (2022) and Sohrabi et al. (2021) proves that galic acid lowers ROS levels, reduces macrophage infiltration, and suppresses the expression of inflammatory molecules such as IL-1β and TNF-α through Nrf2 activation and inhibition of the NF-κB pathway. Gallic acid is known to increase the activity of antioxidant enzymes such as SOD, CAT, GPx, and glutathione reductase (GR), as well as decrease lipid peroxidation.

Research by Kianitalei et al. (2022) shows that intravaginal administration of gallic acid from Alcea angulata extract improves hydration and the thickness of the vaginal epithelium through anti-inflammatory and protective effects. Gallic acid will increase estrogenic responses in the vaginal epithelium, including stimulation of cell division, increased vascularization, and mucus production. This effect contributes to the recovery of atrophied epithelial structures, as indicated by the increased thickness of the vaginal epithelium in the treatment group. Thus, the gallic acid content in purple eggplant peel extract has the potential to improve vaginal atrophy through the mechanism of activating estrogen metabolism, which ultimately strengthens the epithelial structure and maintains the function of the vaginal mucosa (Dogrul et al., 2016). Decreased ROS levels and increased local estrogenic activity, epithelial proliferation increases, so that the structure of the vaginal mucosa recovers and shows an increase in thickness.

The synergistic effects of anthocyanins, quercetin, and gallic acid in purple eggplant peel extract work through estrogenic and antioxidant mechanisms. These compounds bind to estrogen receptors (especially ERα), activate estrogenic target gene transcription, and stimulate epithelial cell proliferation and differentiation. In addition, antioxidant effects through activation of the Nrf2 pathway and increased enzymes such as SOD, CAT, and GPx also support epithelial regeneration and suppress oxidative stress induced by estrogen deficiency. The synergistic activity between the estrogenic and antioxidant effects of purple eggplant peel

extract has the potential to be utilized as a natural agent in efforts to prevent early vaginal atrophy in hypoestrogenic conditions.

CONCLUSION

Ethanol extract of purple eggplant peel (*Solanum melongena L.*) 25 mg/200 grams/day orally could not make vaginal ERα levels higher in menopausal model mice compared to the control group. Ethanol extract of purple eggplant peel (*Solanum melongena L.*) 50 mg/200 grams/day orally did not produce higher vaginal ERα levels in menopausal model mice compared to the control group. Ethanol extract of purple eggplant peel (*Solanum melongena L.*) 100 mg/200 grams/day orally did not produce higher vaginal ERα levels in menopausal model mice compared to the control group. Ethanol extract of purple eggplant peel (*Solanum melongena L.*) 25 mg/200 grams/day orally did not produce a higher thickness of the vaginal epithelium in the menopausal model rats compared to the control group. Ethanol extract of purple eggplant peel (*Solanum melongena L.*) 50 mg/200 grams/day orally did not produce a higher thickness of the vaginal epithelium in menopausal model mice compared to the control group. Ethanol extract of purple eggplant peel (*Solanum melongena L.*) 100 mg/200 grams/day orally may make the vaginal epithelium thickness higher in menopausal model mice compared to the control group. Future research is recommended to explore higher dosages, longer treatment durations, alternative extraction methods to optimize bioactive compound availability, and additional molecular markers to better understand the mechanisms underlying the potential phytoestrogenic effects of purple eggplant peel.

REFERENCES

Ahmad, A.D. 2019. Hormone Replacement Therapy pada Wanita Menopause Meningkatkan Risiko Terjadinya Ductal Carsinoma In Situ. Jurnal Majority, 8(2), 205-207.

Alvisi, S., Baldassarre, M., Gava, G., Mancini, I., Gagliardi, M., Seracchioli, R., & Meriggiola, M. C. (2018). Structure of epithelial and stromal compartments of vulvar and vaginal tissue from women with vulvo-vaginal atrophy taking ospemifene. *The Journal of Sexual Medicine*, *15*(12), 1776–1784.

Ayvaz, H., Cabaroglu, T., Akyildiz, A., Pala, C.U., Temizkan, R., Ağçam, E., Ayvaz, Z., Durazzo, A., Lucarini, M. dan Direito, R. 2022. Anthocyanins: Metabolic digestion, bioavailability, therapeutic effects, current pharmaceutical/industrial use, and innovation potential. Antioxidants, 12(1), 48.

Barnard, N.D., Kahleova, H., Holtz, D.N., Del Aguila, F., Neola, M., Crosby, L.M. dan Holubkov, R. 2021. The Women's Study for the Alleviation of Vasomotor Symptoms (WAVS): a randomized, controlled trial of a plant-based diet and whole soybeans for postmenopausal women. Menopause (New York, NY), 28(10), 150.

Bayazid, A. B., & Lim, B. O. (2024). Therapeutic Effects of Plant Anthocyanin against Alzheimer's Disease and Modulate Gut Health, Short-Chain Fatty Acids. Nutrients, 16(11), 1554. https://doi.org/10.3390/nu16111554

Binder, R.L., Freedman, M.A., Sharma, K.B., Farage, M.A., Wang, Y., Combs, C., Moore, D., Tiesman, J.P., Bascom, C.C. dan Isfort, R.J. 2019. Histological and gene expression analysis of the effects of menopause status and hormone therapy on the vaginal introitus and labia majora. Journal of Clinical Medicine Research, 11(11), 745.

Bleibel, B., Nguyen, H. 2023. Vaginal Atrophy. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.

Cipolletti, M., Solar Fernandez, V., Montalesi, E., Marino, M., Fiocchetti, M. 2018. Beyond the Antioxidant Activity of Dietary Polyphenols in Cancer: the Modulation of Estrogen Receptors (ERs) Signaling. International Journal of Molecular Sciences, 19(9), 2624.

Davinelli, S., Bertoglio, J.C., Zarrelli, A., Pina, R. dan Scapagnini, G. 2015. A randomized clinical trial evaluating the efficacy of an anthocyanin–maqui berry extract (Delphinol®) on oxidative stress biomarkers. Journal of the American College of Nutrition, 34(sup1), 28-33.

Desmawati, D., Sulastri, D. 2019. Phytoestrogens and their health effect. Open Access Macedonian Journal of Medical Sciences, 7(3), 495.

Dogrul, B. N., Kiliccalan, I., & Asci, E. S. (2016). The protective effect of gallic acid against endocrine disrupting damages of bisphenol A and possible mechanism. Journal of Contemporary Medical Education, 4(1), 39–1–39–13. https://doi.org/10.5455/jcme.20160331115900

Dugan, S.A., Gabriel, K.P., Lange-Maia, B.S. dan Karvonen-Gutierrez, C. 2018. Physical activity and physical function: moving and aging. Obstetrics and Gynecology Clinics, 45(4), 723-736.

Ebrahimi, A., Tayebi, N., Fatemeh, A. dan Akbarzadeh, M. 2020. Investigation of the role of herbal medicine, acupressure, and acupuncture in the menopausal symptoms: an evidence-based systematic review study. Journal of Family Medicine and Primary Care, 9(6), 2638.

Ferdina, C.S., Ratnawati, R. dan Andarini, S. 2019. Pengaruh Antosianin terhadap Ketebalan Epitel, Indeks Apoptosis dan Ekspresi p53 Sel Epitel Vagina pada Tikus Putih (Rattus norvegicus) Ovariektomi. Jurnal Ners dan Kebidanan (Journal of Ners and Midwifery), 6(2), 126-133.

Fithri, A.N., Yueniwati, Y., Arsana, I.W., Khotimah, H. dan Nurwidyaningtyas, W. 2023. Epithelial thinning in vaginal atrophy related to lowering of calcitonin gene-related protein, vascular endothelial growth factor, and nerve growth factor expressions in a menopausal rat model. Journal of Pharmacy dan Pharmacognosy Research, 11(1), 110-116.

Flores, S.A. dan Hall, C.A. 2022. Atrophic vaginitis. Treasure Island (FL): StatPearls Publishing.

Grant, M D., Marbella, A., Wang, A T., Pines, E., Hoag, J., Bonnell, C J., Ziegler, K M., dan Aronson, N. 2015. Menopausal Symptoms: Comparative Effectiveness of Therapies.

Palacios, S., Stevenson, J. C., Schaudig, K., Lukasiewicz, M., & Graziottin, A. (2019). Hormone therapy for first-line management of menopausal symptoms: Practical recommendations. *Women's Health*, *15*, 1745506519864009.

Vigneswaran, K., & Hamoda, H. (2022). Hormone replacement therapy–Current recommendations. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *81*, 8–21.

Xiao, Z., Zhou, L., Wu, J., Yang, S., Wu, Y., Zhang, P., Li, A., Tang, A., Sang, N., & Yang, J. (2025). Age-Associated Collagen Remodeling in Vaginal Tissue: A Histomorphometric Analysis. *Aesthetic Surgery Journal*, *45*(12), 1263–1270.