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Original Research Article

Improvements of Vaginal Atrophy in Menopause with the Utilization of Natural Ingredients of Ambones Banana Blossom Extract (*Musa acumintacolla)* in Ovariectomy Rats

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ABSTRACT

The menopausal period causes vagina atrophy due to a decrease in estrogen. This condition is associated with symptoms such as dryness, burning, irritation, discomfort, pain during sex, and impaired sexual function. The use of natural ingredients as therapy in menopause is currently being widely developed. Flavonoids can bind to estrogen receptors because they have a similar structure and activity to endogenous estrogens. Banana Ambones blossom (*Musa acuminata Colla*) contains flavonoid compounds that have phytoestrogenic activity. This study evaluated the improvement of vaginal atrophy with the use of natural ingredients in menopausal rats. This study used female Wistar rats aged 10-12 weeks, weighing 200-250 g, 25 rats were divided into 5 groups, and the rats were subjected to ovariectomy. The normal group (KN), and negative control group (K-) were given standard food and drinking water; the positive control group (K+) received 0.01125 mg/day of conjugated estrogen. The P1 group was given banana blossom extract 200 mg/kg b.w, and the P2 group was given banana blossom extract 400 mg/kg b.w. After 21 days, vaginal tissues were collected and examined immunohistochemically. The results showed that banana flowers (*Musa acuminata* Colla) increased the expression of actin (p=0.036), Claudin-1 (p=0.029), TGF-β (p = 0.018), and collagen (p=0.001). The use of natural ingredients as an alternative therapy to correct vaginal atrophy should be further developed because phytoestrogens can reduce the negative effects of synthetic estrogen use which adversely affects health during menopause.

***Keywords*:** Actin, Claudin-1, Collagen, *Flavonoids*, Menopause, TGF-β, Vaginal atrophy.

Introduction

Menopause occurs due to the cessation of estrogen production activity by ovarian follicles and causes a decrease in estrogen levels and an increase in FSH concentrations. The menopause period is also interpreted as a marker of the end of reproduction in women.[1,2] The hormone estrogen plays an important role in growth, repair, and reproduction. Decreased estrogen levels in the body cause various complaints in menopause such as cardiovascular disease, osteoporosis, and genitourinary syndrome.[3] Genitourinary syndrome includes burning sensations, dryness, irritation, urinary tract infections, and pain during sexual intercourse (dyspareunia). Vaginal tissue undergoes many changes during menopause.

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The vaginal wall experiences a decrease in the thickness of epithelial cells in the superficial, intermediate, and parabasal layers to become thinner. In addition, there is also a decrease in glycogen which is important for maintaining acidic pH in the vagina.[4] This condition is associated with decreased estrogen levels in menopause leading to histological and anatomical changes in urogenital tissue leading to reduced vaginal elasticity, pH changes, reduced lubrication, and susceptibility to infections.[5,6]

Vaginal atrophy is closely related to vaginal elasticity which involves the proliferation of epithelial cells involving proteins in the cell, namely the cytoskeleton and cell junction. Cell junctions connect cells with other cells and extracellular matrices. Cell junctions are found in the epithelium of the vaginal mucosa, namely tight junctions consisting of transmembrane proteins.[7] Claudin-1 is a member of the protein family where claudin plays a role in the formation of tight junctions between adjacent cells. Claudin-1 is most commonly seen in the superficial epithelium. Changes in estrogen indicate a decrease in Claudin-1 expression.[8]The decrease in estrogen results in a disturbance of the vaginal homeostasis system, which has an impact on the reduction of collagen in vaginal muscle cells resulting in reduced vaginal elasticity. In addition, it can also reduce smooth muscle contractions due to decreased actin and myosin.[9]

A further element associated with vaginal elasticity is the TGF-β cytokine, which plays a role in the synthesis and control of collagen and elastin. Several signalling mechanisms, such as Mitogen-activated protein kinase (MAPK) that governs collagen metabolism, play a role in regulating collagen synthesis. Increased expression of TGF-β has a

significant impact on collagen production in different cell types, especially fibroblasts. TGF-β increases the expression of genes encoding different types of collagen, especially type I and III collagen.[10]Estrogen affects collagen synthesis and metabolism in the vaginal wall by stimulating collagen degradation by increasing the metalloproteinase-2 matrix. In menopausal conditions, collagen levels decrease significantly due to a decrease in the hormone estrogen.[11]

One of the treatments for hypoestrogen in menopause is hormone therapy. One of the best ways to address menopausal symptoms is with hormone therapy. Apart from its high cost, this hormone replacement therapy has enduring adverse consequences, including an increased susceptibility to endometrial cancer, cardiovascular disease, breast cancer, and several other ailments. Using phytoestrogens and antioxidant plants is now being researched as a safer option to treat menopausal symptoms. Phytoestrogens are endogenous steroids present in plants with a molecular structure like 17-β estradiol (E2). Flavonoids are a highly utilized category of phytoestrogens due to their ability to exert their effects via binding to the α estrogen receptor (ER-α) and the β estrogen receptor (ER-β).[12,13]

The banana blossom is a part of the banana plant that has a structure similar to the shape of the heart. The blossom of a banana also called a banana flower plays a role in the formation of bananas. The banana plant has many blossoms with brownish-red sheaths on each.[14]The composition of banana blossoms includes minerals, fatty acids, vitamins A, C, and E, potassium, as well as active chemicals like *alkaloids*, *flavonoids*, *saponins*, *tannins*, and *anthocyanins*[15] This compound has antiallergic, antiviral, anti-mutagenic, anti-microbial, and anticancer effects.[16] Anthocyanins consist of sugar groups (glycones) and (aglycones).[17]*Anthocyanins* are chemicals generated from polyphenols that possess a structure similar to estradiol and exhibit estrogenic action due to their ring arrangement resembling estradiol and the presence of two hydroxyl groups positioned at an optimal distance for binding to estrogen receptors.[18] In addition to *anthocyanin* compounds, *Genistein* also has beneficial effects on women's health. *Genistein* is a compound in the class of isoflavones that also has a structure similar to endogenous estrogen. *Genistein* is considered a phytoestrogen because it can bind to estrogen receptors (ER-α and ER-β) despite its lower affinity than estradiol.[19,20,21].

Prolonged administration of estrogen hormone treatment can elevate the susceptibility to breast cancer, endometrial cancer, cardiovascular disorders, and other adverse consequences. Therefore, it is necessary to find alternatives to manage menopausal symptoms using plants for safer treatments and hormone replacement therapy during menopause. Although there have been many studies on banana flowers for various purposes, its potential in addressing reduced vaginal elasticity after menopause remains unexplored. Therefore, the purpose of this study was to investigate the effects of administering ethanol extract derived from Ambon banana flowers on increasing vaginal elasticity in individuals facing menopause.

Materials and Methods

Drugs and chemicals used include ethanol 70%, Estrogen-conjugated 0.625 mg purchased from PT. SunthiSepuri, Claudin-1 Monoclonal Antibody Cat No ABP0072, Actin Monoclonal Antibody Cat No ABL1010, TGF-β Monoclonal Antibody Cat No ABP52598, Collagen Masson Trichome-kit code: 01MST100T.

Animals

Female Wistar rats weighing 200-250 g (10-12 weeks old) were obtained from the Laboratory of Animal Development, GadjahMada University. The rats were divided into 5 groups, namely Normal Group (KN), Negative Control (K-), Positive Control (K+), Treatment Group 1 (P1) and Treatment 2 (P2). The rats were acclimatized for 7 days in cages measuring 40 cm x 30 cm x 20 cm. Temperature and humidity were maintained at natural levels. The rats were fed a standard diet and had access to drinking water *ad libitum*. The experimental protocol was approved by the Ethics Committee of SebelasMaret University with approval number: 42/UN27.06.11/KEP/EC/2023.

Plant material

Ambones banana blossom (*Musa Acuminata* Colla) was obtained from banana plantations in Tanggamus Regency through the Makmur Hijau Farmer Producer Cooperative, Lampung, Indonesia on December 21, 2023. The Ambones banana blossom was identified at the Botany Laboratory, Faculty of Mathematics and Natural Sciences, University of Lampung, Indonesia, with the voucher specimen number: 1057/UN26.04/DT/2023. Banana blossoms were processed into simplicia.

Extraction

The banana blossoms were separated from foreign objects or dirt and then washed thoroughly in running water. The blossom of the ambon banana was cut into small pieces to make it easier to dry. The cut pieces were dried in an oven at 40ºC. Simplisia of dried banana blossom was then ground to form Simplicia powder, then stored in a dry container and tightly closed. About 300 g of the Simplicia powder was macerated in 1.5 L of 70% ethanol. The container was closed tightly and stored in sunlight for 3 x 24 hours with occasional stirring. After 3 days the mixture was filtered and the filtrate was taken. The marc was re-extracted with 1.5 L of ethanol and the combined macerate was evaporated to dryness using a rotary evaporator at a temperature of 60 °C until a thick extract was obtained.

Ovariectomy procedure

Ovariectomy was performed in the rats in all the groups to provide a model of menopausal rats. In the initial procedure of ovariectomy, anaesthesia was induced with Ketamine 0.2 mg and Xylazine 0.1 mL injected into the rat muscles. After the rats fell asleep, a small incision in the peritoneal was transversely made in the middle of the abdomen. This was done to show the abdominal transverse muscles. Once the muscle was cut, the peritoneal cavity and adipose tissue that covers the ovaries were visible. Ovariectomy was performed at the same time with one incision in the ventral part of the abdomen. The right and left ovaries were taken with a single incision. After the ovarian tissue was removed, the incision wound was treated, and stitched. The rats were left for 21 days to get the effects of menopause. Following 21 days of treatment, the following groups received standard feed and water for the normal group (KN) and negative control group (K-), conjugated estrogen therapy (0.625 mg/day) for the positive group (K+), banana blossom ethanol extract (200 mg/kg body weight) for the P1 group, and treatment group 2 (400 mg/kg body weight) for 21 days.

Statistical analysis

Statistical analysis was done using IBM SPSS Statistics program version 25.0. Data analysis included normality test (*Shapiro-Wilk*), homogeneity test (Levene test), and one-way *ANOVA* test (p<0.05). Homogeneous data was followed by the *Post Hoc Tukey* test. If the data were not normally distributed, the data was analyzed using the Kruskal-Wallis test. The significance level used was $p < 0.05$.

Results and Discussion

Results showed that the expression of Actin, Claudin-1, TGF-β, and collagen in the vagina of rats increased in the treatment group compared to the negative control group (K-) or the menopausal rat group after being treated with Ambones banana blossom extract for 21 days. The minerals magnesium, manganese, and zinc were found in the banana blossoms, along with active substances such as anthocyanins, saponins, flavonoids, and alkaloids. Flavonoid compounds such as anthocyanins and genistein have many beneficial effects on various diseases as anti-inflammatory, anticancer, anti-diabetic, and also anti-ageing. The main biological activity of flavonoids is their antioxidant potential that can prevent damage due to free radicals through inhibition of ROS formation, and activation of antioxidant defenses by increasing antioxidant enzymes in free radicals scavenging.[22,23] In addition to acting as antioxidants, flavonoid compounds can also act as phytoestrogens because they have a structure similar to 17-estradiol. Flavonoids that play a role as phytoestrogens in the research of ambon banana blossom extract are anthocyanins and genistein. This compound has a structural similarity to estrogen and can interact with estrogen

receptors in the body to elicit estrogenic effects.[24] Actin expression in the negative control group (K-) decreased after ovariectomy compared to the normal group. After treatment with conjugated estrogen 0.00625 mg/day in the (K+) group, actin expression increased. In the P1 group, rats treated with banana ethanol extract 200 mg/kg bb (P1), actin expression was 9.3%, and in the P2 group with a dose of 400 mg/kg bb (P2), actin expression increased compared to the P1 group with p=0.036 (Table 1). Actin plays a role in giving cell shape and various cellular processes including cytoplasmic division, motility, and muscle contraction. Actin can be either a globular protein (G) or a filament (F). Actin content is an important factor in determining intracellular elasticity.[25]Through alterations in globular actin, the hypoestrogenic state causes modifications to the vaginal wall's extracellular matrix and cell shape.

Table 1: Mean Rank of Actin expression in the vagina of menopausal rats

Group	Actin		P-value
	Median (Min-Max)	SW	
KN	180.00 (50-190)	0.012	
K-	120.00 (90-180)	0.234	
K+	160.00 (80-270)	0.814	0.036*
P1	120.,00 (60-180)	0.490	
P2	200.00 (160-285)	0.019	

*Significance level p <0.05

As a part of the cytoskeleton, globular actin is found in the cytoplasm of the cell where it is organised into filament networks or parallel filaments. Globular actin maintains the cell connections necessary for intercellular communication and regulates the stiffness of the cell in response to stimuli.[26,27]Ageing has an impact on globular reduction in actin, which changes actin from its stable state to polymerisation and the creation of a stiff cytoskeleton, which lowers epithelial permeability. Estrogen therapy through the activation of estrogen α-receptor (ER-α) will increase epithelial permeability and increase Globular actin.[27]The mean rank value shows the average rank of each treatment group presented in (Figure 1). The KN and K+ groups have a higher average rating than K- and P1 and the P2 group has the highest average rating compared to the KN, K-, K+, and P1 groups. A rise in the density of vaginal smooth muscle cells, which was accompanied by an increase in percentage actin expression, revealed the effects of actin expression. The expression of actin in the treatment group is presented in (Figure 2). The expression of actin was seen with the number of cells from the vaginal epithelium with 400x magnification.There was a significant difference in the expression of claudin-1 in treatment group 2 (P2) compared to the negative control group (K-). The P2 group showed a higher increase in claudin-1 expression compared to the treatment group 1 (P1). After being treated for 21 days, Claudin-1 expression in menopausal rats increased compared to the normal group (p=0.026). The administration of ethanol extract of ambones banana blossom at a dose of 400 mg/kg b.w increased the expression of claudin-1 in menopausal rats. The results of Claudin-1 are presented in (Table 2). The Claudin-1 family comprises membrane proteins that play a crucial role in the assembly of tight junctions, found in endothelial and epithelial cells. Claudin-1 regulates transepithelial and paracellulartransendothelial transport of molecules and ions and also plays a role in cell growth and differentiation.[28]The kind of tissue can influence how estrogen affects claudin 1 expression differently. A rise in claudin 1 aids in the creation and maintenance of tight junctions, which are critical for the integrity of the endometrial epithelium and have an impact on the function of the epithelial barrier. Estrogen is crucial in the development of the menstrual cycle and the regeneration of the endometrium in the reproductive tissue. Increased expression of claudin 1 can strengthen tight junctions, enhancing barrier integrity which is important in maintaining homeostasis and protection against pathogens and harmful bacteria.[29,30]

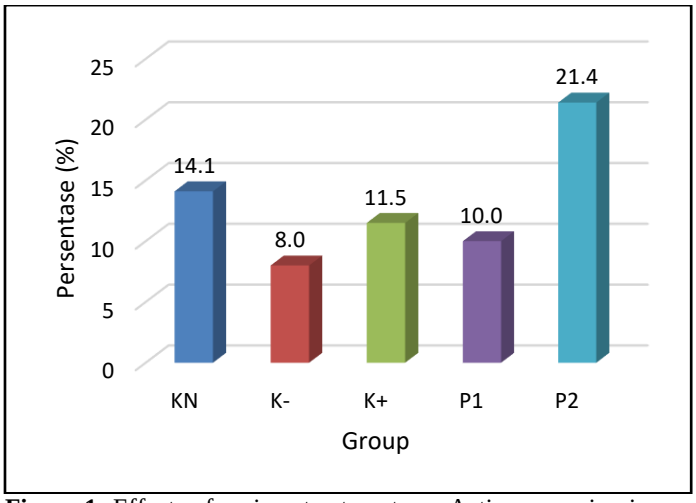

Figure 1: Effects of various treatments on Actin expression in the menopausal rats model.
Notes: The mean rank of Actin expression KN: normal group, K-: negative control, K+: positive control + CEs 0.625 mg/day, P1: banana blossom extract at a dose of 200 mg/kg b.w, P2: banana blossom extract at a dose of 400 mg/kg b.w

Table 2: Mean Rank of Claudin-1 expression in the vagina of menopausal rats model

Group	Claudin-1		P-value
	Median (Min-Max)	SW	
KN	160.00 (120-285)	0.021	
K-	180.00 (180-270)	0.490	
K+	270.00 (120-300)	0.135	0.026*
P1	180.00 (120-270)	0.146	
P2	285.00 (180-285)	0.006	

*Significance level p<0.05

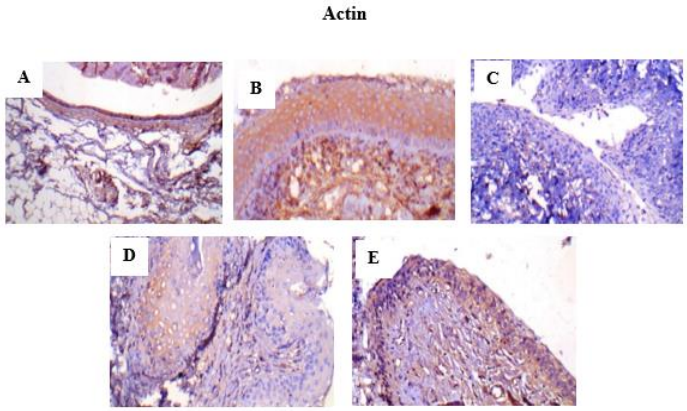

Figure 2: Expression of Actin in the vagina of a menopausal rats model (A) KN: normal group, (B) K-: negative control, (C) K+: positive control + CEs 0.625 mg/day, (D) P1: banana blossom extract at a dose of 200 mg/kg b.w, (E) P2: banana blossom extract at a dose of 400 mg/kg b.w

Estrogen can affect the expression of proteins involved in the formation of tight junctions including Claudin-1. Estrogen plays an important role in maintaining the integrity and function of the vaginal epithelium, helping to prevent infection and inflammation.[31]The mean rank value of each treatment group is presented in Figure 3. In the expression of

Claudin-1, the mean rank values of KN and K+ have the same average rating, and the P2 group has an average value that tends to be higher compared to the P1 group. The expression of Claudin-1 in the treatment group is presented in Figure 4. The expression of Claudin-1 was observed with the number of cells from the vaginal epithelium with 400x magnification. TGF-*β* expression changed significantly following a 21-day course of therapy. When comparing the treatment group to the negative control group (K), there was a greater expression of TGF-*β*. Based on data analysis, it was shown that all data on TGF-*β* had an abnormal distribution with the results of the Shapiro-Wilk test $p<0.05$

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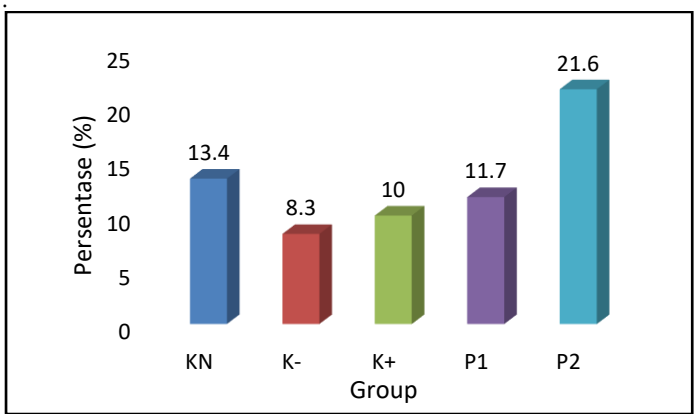

Figure 3: Effects of various treatments on Claudin-1 expression in the menopausal rats model. The mean rank of Claudin-1 expression KN: normal group, K-: negative control, K+: positive control + CEs 0.625 mg/day, P1: banana blossom extract at a dose of 200 mg/kg b.w, P2: banana blossom extract at a dose of 400 mg/kg b.w

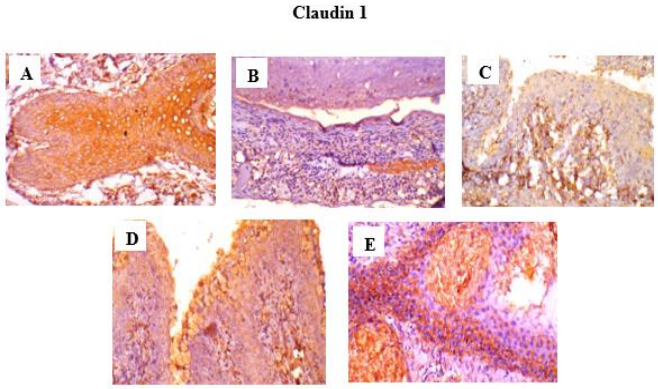

Figure 4: Expression of Claudin-1 in the vagina of a menopausal rats model (A) KN: normal group, (B) K-: negative control, (C) K+: positive control + CEs 0.625 mg/day, (D) P1: banana blossom extract at a dose of 200 mg/kg b.w, (E) P2: banana blossom extract at a dose of 400 mg/kg b.w

There was a significant difference in TGF-*β* expression across groups, as indicated by the *Kruskal-Wallis* test findings (Table 3), with a value of p=0.018. The multifunctional cytokine knowXn as transforming growth factor-*β* (TGF-*β*) is involved in a variety of biological processes, such as cell proliferation, migration, differentiation, apoptosis, and extracellular matrix remodelling. It is also known to have a significant impact on tissue wound healing. Transforming growth factor-β regulates the production and breakdown of collagen. Numerous signalling pathways govern the production of collagen, with MAPK being involved in the regulation of collagen metabolism. The increased expression of TGF-β significantly affects the production of collagen in several cell types, especially fibroblasts. TGF-*β* increases the expression of genes encoding different types of collagen, especially type I and III collagen. TGF-*β* induces the differentiation of fibroblasts into myofibroblasts that have a high ability to produce collagen and other extracellular matrix (ECM) components. Estrogen can modulate TGF-*β* expression in various cells. The transcriptional ability of phytoestrogens can induce the proliferation and maturation of squamous epithelial cells, induce columnar epithelium, and produce thickening of epithelial cells in the vagina. Fibroblasts play a role in synthesizing collagen and stimulating the proliferation of epithelial maturation on the vaginal wall which can increase the thickness of the vaginal wall.[32]The mean rank value showing the average rank of each treatment group is presented in Figure 5.

Table 3: Mean Rank of TGF-*β* expression in the vagina of menopausal rats model

Group	TGF-*β*		P-value
	Median (Min-Max)	SW	
KN	140.00 (100-240)	0.731	0.018*
K-	240.00 (160-300)	0.062	
K+	180.00 (100-270)	0.453	
P1	160.00 (160-270)	0.033	
P2	270.00 (160-285)	0.033	

***Significance level p <0.05**

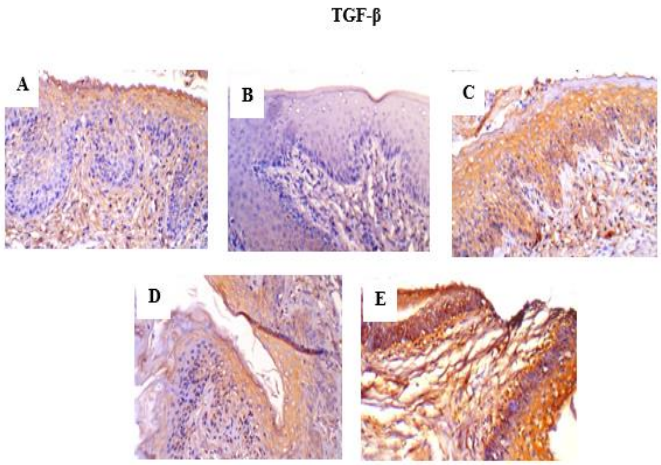

Figure 5: Expression of TGF-*β* in the vagina of a menopausal rats model (A) KN: normal group, (B) K-: negative control, (C) K+: positive control + CEs 0.625 mg/day, (D) P1: banana blossom extract at a dose of 200 mg/kg b.w, (E) P2: banana blossom extract at a dose of 400 mg/kg b.w

KN had a mean rank value that tended to be lower than the treatment group. K+ and P1 have lower average scores than the P2 group. While collagen KN has the highest average rating compared to other groups, P2 has a lower average rating compared to K+ and P1. The expression of TGF-*β*in the treatment group is presented in Figure 6 which showed a difference in collagen dissemination in the treated rats compared to the negative control group (K-). The P1 group showed a higher average collagen density compared to the negative control group (K-). The positive control group (K+) given estrogen therapy of 0.01125 mg/day, showed a collagen density of 32.83%, which was higher than the negative control group (K-). The average collagen density of menopausal rats model given banana blossom ethanol extract of 200 mg/kg bw was 30.83% compared to the negative control group (K-). Meanwhile, in treatment group 2 (P2) in the menopausal rats model, an average collagen density of 19.14% was obtained which was lower than that of the P1 group. The results of the *One-Way ANOVA* test (Table 4) indicated a statistically significant difference in the mean collagen density between the groups (p=0.001). The collagen density in the percentage of collagen fibers could be seen in blue in the vaginal

epithelium under 400x magnification (Figure 7).Collagen is the main structural protein in connective tissue that creates resistance to stretch and pull. Additionally, collagen plays a part in regulating cell migration, adhesion, and tissue healing.[33]The hypoestrogenic state in menopause results in decreased smooth muscle contractions, epithelial atrophy, and decreased collagen and elastin fibres.[34] Estrogen affects the synthesis and metabolism of collagen in the vaginal walls. The loss of rugae occurs due to the breakdown of collagen support tissue. Collagen turnover increases in line with the age of women.[9] Banana blossom ethanol extract contains flavonoids in the form of anthocyanins and genistein which can act as phytoestrogens and antioxidants in menopausal conditions, can activate the process of cell proliferation and maturation. This is in agreement with other studies findings which reported that phytoestrogens have been proven to be effective in improving urogenital symptoms shown through growth, proliferation and increased vaginal epithelial maturation index.[35,36] The results of this study revealed that the phytoestrogenic properties of flavonoids, anthocyanins, and genistein in blossom of the ambon banana (*Musa Acumnita Colla*) may be responsible for its activity in improving vaginal atrophy and increasing elasticity in the vagina. Increased vaginal elasticity can prevent sexual dysfunction and thus improve the quality of life in menopausal women.

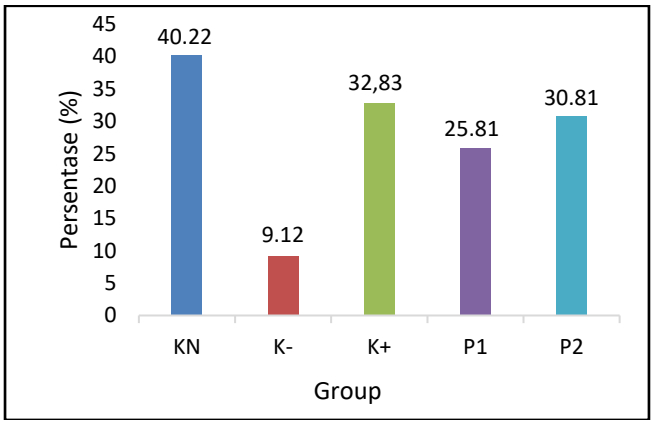

Figure 6: Effects of various treatments on Collagen expression in the menopausal rats model. The mean collagen expression KN: normal group, K-: negative control, K+: positive control + CEs 0.625 mg/day, P1: banana blossom extract at a dose of 200 mg/kg b.w, P2: banana blossom extract at a dose of 400 mg/kg b.w

Table 4: Mean of Collagen in the vagina of menopausal rats model

Group	Collagen		Homogeneity	P-value
	Mean ± SD	SW		
KN	40.22 ± 5.50	0.670	0.238	0.001
K-	10.51 ± 3.64	0.109		
K+	32.83 ± 12.40	0.992		
P1	30.64 ± 11.67	0.841		
P2	20.81 ±10.13	0.151		

*Significance level $p < 0.05$

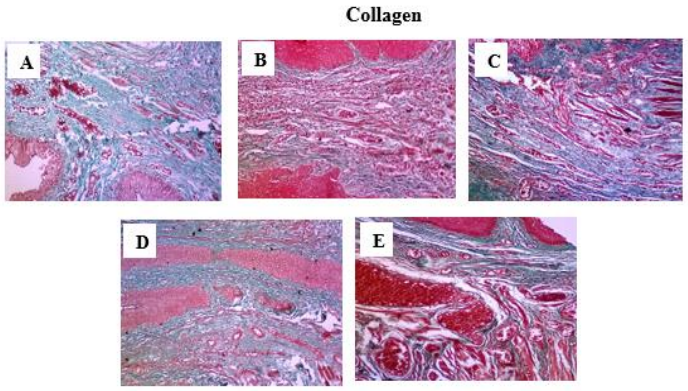

Figure 7: Expression of Collagen in the vagina of a menopausal rats model. KN: normal group, K-: negative control, K+: positive control + CEs 0.625 mg/day, P1: banana blossom extract at a dose of 200 mg/kg b.w, P2: banana blossom extract at a dose of 400 mg/kg b.w

Conclusion

These findings show that Ambones banana blossom (*Musa acuminata* Colla) exhibits antioxidant and phytoestrogenic effects by increasing the expression of Actin, Claudin-1, TGF-*β*, and collagen in the vagina, which may be responsible for increasing vaginal elasticity in menopausal rat models. Rich amounts of a*nthocyanins* and *genistein* (*flavonoids)* found in banana plants (particularly the hearts), could serve as a natural source and an alternative to hormone replacement therapy for menopausal women, obviating the risk of cancer, cardiovascular disease, and other adverse effects, associated with hormonal therapy.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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