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Efectiveness of kenitu leaf extract (*chyrysophyllum cainito l.*) on bone density and histopathology osteoblast-osteoklast in female mice

Nurdiana Tandi Pare[1], Gunawan Pamudji Widodo[2], Iswandi[3]

[1,2,3]Master of Pharmacy Study Program, Faculty of Pharmacy, Universitas Setia Budi, Surakarta, Indonesia

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ABSTRACT

Osteoporosis is a systemic bone disease characterized by a decrease in bone density and deterioration of bone microarchitecture so that bones become brittle and break easily. Osteoporosis due to long-term use of glucocorticoids causes osteoporosis. There is a lot of clinical evidence about the role of phytoestrogens in the treatment of osteoporosis. Kenitu (Chrysophyllum cainito) contains isoflavone phytoestrogen compounds. The aim of this research was to determine the anti-osteoporosis effect of ethanol extract of kenitu leaves (Chrysophyllum cainito L.), by looking at the increase in bone density as well as the number of osteoblast cells and the number of osteoclast cells in the femur trabecular bone of female mice induced by dexamethasone. In this study, 49 female mice were grouped into 7 groups, namely normal control, negative control, positive control with cabone naturindo, and groups treated using 70% ethanol extract of kenitu leaves with varying doses of 100 mg/kg BW, 200 mg/kg BW, 400 mg/kg BW and 800 mg/kg BW. An increase in femur trabecular bone density, the number of osteoblast cells and the number of osteoclast cells were observed using the Histomorphometry method. The results showed that the ethanol extract of kenitu leaves has anti-osteoporosis activity. The ethanol extract of kenitu leaves has a dose of 400 mg/kg BW which is the optimal dose as anti-osteoporosis which is indicated by an increase in bone density, an increase in the number of osteoblast cells and a decrease in the number of osteoclast cells.

Corresponding Author:

Nurdiana Tandi Pare,
Faculty of Pharmacy,
Universitas Setia Budi,
Jl. Letjen Sutoyo, Mojosongo, Kec. Jebres, Kota Surakarta, Jawa Tengah, 57127, Indonesia
Email: tandiparenurdiana1995@gmail.com

INTRODUCTION

Osteoporosis is a systemic bone disease characterized by a decrease in bone mass density and the deterioration of bone microarchitecture, making bones brittle and prone to fractures (Jennifer, 2008). Osteoporosis is often referred to as a "silent disease" because it can progress without noticeable symptoms until a fracture occurs (Misnadiarly, 2013). Bones can fracture due to frequent bending, lifting heavy loads, falling from a certain height, or even during everyday activities

(Schwinghammer, 2015). Osteoporosis occurs when an individual's bone metabolism is disrupted. Normal bone metabolism involves a balance between osteoclast and osteoblast activity. Osteoporosis develops when osteoclast activity exceeds that of osteoblasts, preventing osteoblasts from sufficiently refilling the bone resorption cavities. Long-term therapy with glucocorticoid drugs (such as dexamethasone) suppresses the production of gonadotropin hormones, leading to a decrease in estrogen production (Wardhana, 2012). Estrogen deficiency disrupts the normal bone metabolism cycle, resulting in osteoclast activity dominating over osteoblasts, eventually causing bone tissue loss (Gallagher et al., 2013).

According to data from the International Osteoporosis Foundation (IOF), there are approximately 75 million osteoporosis sufferers in Europe, Japan, and America, and 84 million in China, contributing to a global total of around 200 million people at increased risk of fractures (Mithal, Ebeling, & Kyer, 2013). The World Health Organization (WHO) predicts that by the middle of this century, the number of femoral fractures worldwide will triple. By 2050, it is estimated that there will be 6.26 million femoral fractures annually worldwide, with 3.25 million cases occurring in Asia (Ministry of Health of the Republic of Indonesia, 2015).

The impact of osteoporosis has reached alarming levels, with one in three women and one in five men in Indonesia affected by osteoporosis or bone fractures (Riskesdas, 2013). In postmenopausal women, estrogen deficiency significantly increases the risk of osteoporosis and eventual fractures (Anies, 2006). Estrogen levels drop sharply during the postmenopausal period (Baziad, 2003). Moreover, elderly men are also at high risk of developing osteoporosis due to androgen hormone deficiency, particularly testosterone, which plays a crucial role in suppressing systemic inflammatory cytokines that stimulate osteoclast differentiation, such as Interleukin-1 (IL-1), Interleukin-6 (IL-6), and Tumor Necrosis Factor-α (TNF-α) (Malkin et al., 2004). Estrogen deficiency is comparable to testosterone deficiency, as testosterone, an androgen hormone, is metabolized by the cytochrome P450 aromatase enzyme to produce 17-β-estradiol, serving as a precursor to estrogen (Reid, 2000).

According to Kawiyana (2019), estrogen plays an essential role in bone metabolism and remodeling. In bone metabolism, estrogen inhibits the secretion of IL-1, IL-6, and TNF-α, which maintain osteoclast development. Estrogen also stimulates the production of Transforming Growth Factor-β (TGF-β). Clinical evidence has shown that Hormone Replacement Therapy (HRT) using synthetic estrogen at adjusted doses effectively prevents bone mass loss in postmenopausal women and reduces the incidence of osteoporosis. However, HRT use lowers the risk of fractures by 24%, but increases the risks of breast cancer by 26%, heart disease by 29%, and stroke by 41% (Cosman, 2009). In addition to these risks, HRT is also costly (Pertamawan and Hestiantoro, 2002). Due to the side effects and high costs of HRT, many people seek alternative treatments, particularly traditional medicines derived from natural sources, mainly plants (Anggraini, 2008). Phytoestrogens are compounds found in plants that have a structure similar to estrogen, can bind to estrogen receptors, and function similarly to estrogen (Yang et al., 2012). Previous studies have shown that phytoestrogens have a protective effect in preventing bone density loss caused by estrogen deficiency (Nurrochmad et al., 2010).

Najib (2021) reported that *in silico* studies of compounds in 96% ethanol extracts of kenitu (Chrysophyllum cainito) leaves showed agonist binding to 1A52 (ER-α) and 3OLS (ER-β) proteins, predicting a role as phytoestrogens due to their structural similarity to 17β-estradiol. Six compounds demonstrated agonist interactions with 1A52 and seven compounds with 3OLS. All of these compounds are predicted to have parameters very similar to 17-β-estradiol and could be accepted by the body as anti-osteoporosis therapies.

According to research by Fatimatuz (2015), kenitu leaf extract using 70% ethanol solvent had the highest total flavonoid content compared to 50% and 96% ethanol extracts. Various types of plant-derived estrogens include isoflavones, coumestans, lignans, glycosides, triterpenes, and other phytoestrogenic compounds such as diterpenoids, triterpenoids, flavones, chalcones,

coumarins, and acyclic compounds (Hoffman, 2004). Polyphenolic compounds offer numerous health benefits, including antioxidant properties, cancer prevention, cardiovascular disease prevention, anti-diabetic effects, osteoporosis prevention, and neurodegenerative disease prevention (Vauzour et al., 2010).

The flavonoid compounds contained in kenitu leaves (*Chrysophyllum cainito* L.) include isoflavones. Isoflavones are one of the compounds with significant phytoestrogenic properties (Grippo et al., 2007). Methanol extracts and fractions from kenitu leaves have also been studied for their potential as anti-hypersensitivity and anti-inflammatory agents in mice induced with carrageenan (Meira et al., 2014). Leaf infusions have also been traditionally used for the treatment of diabetes and joint rheumatism (Das et al., 2010). Moreover, research conducted by Utaminingtyas (2017) and Mustofa (2018) investigated the anti-osteoporosis effects of 70% ethanol and ethyl acetate extracts of *C. cainito* leaves on increasing trabecular bone density in female mice induced with dexamethasone, using bone thickness as the measured parameter. The studies showed positive results, indicating that both extracts possess anti-osteoporosis activity. Dexamethasone is a corticosteroid medication, and long-term use of corticosteroids can increase bone resorption and trigger osteoporosis (Mazziotti et al., 2006). Corticosteroids inhibit osteoblast activity, leading to reduced bone formation. The combined effect of increased osteoclast activity and decreased osteoblast function results in osteoporosis (Wardhana, 2012).

This research will be conducted *in vivo* using female mice as the test subjects. The mice will be induced with corticosteroids in the form of dexamethasone to develop osteoporosis. Measurements will be conducted using the histomorphometry method. Bone histomorphometry is a method used to assess bone quality and to evaluate the effects of treatments on bone mineralization and microarchitecture (Chavassieux, 2000).

Based on previous research findings, this study will continue using 70% ethanol as the extraction solvent. The obtained extract will then be tested *in vivo* on dexamethasone-induced mice to observe the phytoestrogen activity in improving bone density, the number of osteoblast cells, and the number of osteoclast cells in the femoral trabecular bone of female mice. The mice will be divided into several groups: a normal control group, treatment groups receiving various doses of 70% ethanol extract of kenitu leaves (*Chrysophyllum cainito* L.), a positive control group treated with the herbal product Cabone Naturindo, and a negative control group that will not receive any treatment.

RESEARCH METHOD

This study is a laboratory experimental research. To determine the appropriate dose and effects of the 70% ethanol extract, female mice were used as test subjects, induced with dexamethasone to develop osteoporosis. Osteoporosis occurs due to the inhibition of osteoblast cell formation and increased osteoclast activity. The study was conducted using 7 experimental groups, each consisting of 7 female mice, to evaluate the effectiveness of the 70% ethanol extract of kenitu leaves (*Chrysophyllum cainito* L.) in improving bone density, the number of osteoblast cells, and the number of osteoclast cells.

The research subjects were female mice obtained from Surakarta Animal Market. The selected mice were adult females aged 5 months, weighing between 20–30 grams, in healthy condition as assessed through visual observation, and displaying normal activity. The research was carried out at Setia Budi University. Sample preparation and bone trabecular histopathology reading were performed at the Laboratory of the Department of Anatomical Pathology, Faculty of Medicine, Public Health, and Nursing, Gadjah Mada University, Yogyakarta.

The population in this study refers to all objects targeted by the research. The population consisted of kenitu leaves (*Chrysophyllum cainito* L.) obtained from the UPT Medika Laboratory in Batu City, East Java. Samples are a subset of the population used in the study. The part of the plant used as the sample was the leaves. Young leaves were selected, characterized by a green upper

surface and a golden-brown lower surface. Young leaves were chosen because they contain higher levels of flavonoids, whereas flavonoid concentrations decrease in older leaves (Mustofa, 2018).

RESULTS AND DISCUSSIONS

Results of the qualitative chemical content test of kenitu leaf extract

The chemical content tested is a compound that has osteoporosis activity, namely flavonoids, alkaloids, and terpenoids.

Table 1. Results of identification of chemical compound groups with tube tests

Compound	Testing Method	Literature Result	Test Result	Note
Flavonoid	+ powder sample + 2N HCl (2 ml) + amyl alcohol (5 ml)	Formation of red or brown color	Formation of brown precipitate	+
Alkaloid	+ 2 ml water, heated until residue forms + 5 ml 2N HCl, then into three separate tubes: 3 drops of Dragendorff reagent into the first tube, 3 drops of Mayer reagent into the second tube, and 3 drops of Wagner reagent into the third tube	First tube: Formation of orange precipitate; Second tube: Formation of white-yellowish precipitate; Third tube: Formation of brown precipitate	First tube: Formation of orange precipitate; Second tube: Formation of white-yellowish precipitate; Third tube: Formation of brown precipitate	+
Terpenoid	+ 2 mg sample with 3 ml acetic anhydride, heated until boiling then cooled + 1 ml H_2SO_4 using Liebermann–Burchard reagent	Formation of red–orange color	Formation of red–orange color	+

Description:
(+) = color change occurs
(-) = no color change occurs

Table 2. Results of identification of chemical compound groups using TLC

	flavonoid	alkaloid	terpenoid
Ethanol extract of kenitu leaves	+	+	-

Based on the results of phytochemical screening, flavonoids, alkaloids, and terpenoids were found. According to previous research journal references (Mustofa., 2018), phytochemical screening was carried out on kenitu leaf extract powder, flavonoids, alkaloids, and terpenoids were found, but in the TLC test, terpenoid compounds were not clearly visible. 8.1 Flavonoids. Flavonoids are one of the largest polyphenol compounds found in nature. Flavonoids have a basic framework consisting of 15 C atoms, where two C6 groups (substituted benzene rings) are connected by C3 (three-carbon aliphatic chain) to form a C6-C3-C6 arrangement (Harbone, 1987). The basic structure of flavonoid compounds is presented in Figure 1.

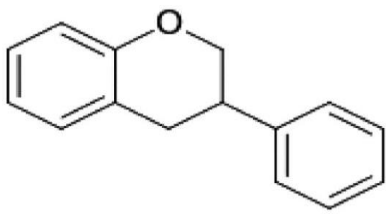

Figure 1. Basic structure of flavonoids (Robinson., 1995)

The extract of kenitu leaves that has been identified for compound content using TLC obtained positive results. Kenitu leaves have flavonoid content as evidenced by compound identification using TLC. Flavonoid compounds contained in kenitu leaves (Chrysophyllum cainito L.) include isoflavones. Isoflavones are one of the compounds that have quite high phytoestrogenic properties (Grippo et al., 2007, The structure can be seen in Figure 2.

Figure 2. Estrogean 17β estradiol and phytoestrogens (Anonymous, 2014)

Flavonoids identification was carried out using quercetin as a reference compound because it is a flavonol glycoside commonly found in plants. After the TLC plate was sprayed with ammonia vapor, faint brown or bright yellow spots appeared, visible under UV light at 254 and 366 nm, indicating the presence of flavonoids in the kenitu leaf extract.

Alkaloids piperine was used as a reference compound, as it is a well-known type of alkaloid. After spraying the TLC plate with Dragendorff's reagent, purple spots appeared under visible light, confirming the presence of alkaloids. According to Bingfeng Lin et al. (2022), alkaloids found in kenitu have anti-osteoporosis activity. TLC testing of the kenitu leaf extract yielded a positive result for alkaloid compounds. Terpenoids sitosterol was used as a reference compound because it is one of the most widely recognized terpenoids. After spraying with Liebermann-Burchard reagent, the terpenoid spots in the sample were unclear. However, according to Niliestria et al. (2015), terpenoids in kenitu plants possess activity that can increase bone density.

Results of Measurement of Average Number of Osteoblasts - Osteoclasts

The study on the activity of 70% ethanol extract of kenitu leaves was conducted on female rats induced with dexamethasone as an osteoporosis model. Osteoporosis is characterized by a disruption in bone turnover, an imbalance between osteoclast (bone resorption) and osteoblast (bone formation) activity, leading to a decrease in bone mass. In this study, the osteoporotic condition was visually indicated by the rats developing a kyphotic posture (forward bending), suggesting reduced trabecular femur bone density. The average measurement of osteoblast cell count showed that the normal control group had a stable number of osteoblasts due to the absence of dexamethasone induction, while the negative control group experienced a significant decrease as a result of oral dexamethasone induction. This shows that dexamethasone induction was successful. Dexamethasone is a synthetic corticosteroid with very high glucocorticoid activity. Consumption of this drug for 4 weeks in mice is equivalent to 3-4 years of use in humans (Manolagas, 2000). In fact, according to the Indonesian Ministry of Health (2015), the use of this class of drugs for more than 3-6 months can inhibit the bone formation process in osteoblasts.

Table 3. Results of the average number of osteoblast cells in each test group

Group	Mean ± SD
Normal	115 ± 4 b
Negative	78 ± 7 ac
Positive	127 ± 8 b
Kenitu leaf extract 100 mg/kg BW	95 ± 8 c
Kenitu leaf extract 200 mg/kg BW	109 ± 7 b
Kenitu leaf extract 400 mg/kg BW	125 ± 7 b
Kenitu leaf extract 800 mg/kg BW	113 ± 5 b

a: significantly different from normal
b: significantly different from negative
c: significantly different from positive

Numbers followed by different letters in the same column indicate significantly different test results ($p<0.05$). Based on the results of statistical analysis, the number of osteoblast cells in the positive

control group showed a very significant increase in the number of osteoblast cells, this increase was due to the administration of cabone naturindo. Measurement of the number of osteoblast cells in the kenitu leaf ethanol extract group increased. The results of the statistical test showed that there was a significant difference between the negative group and each test group given kenitu leaf ethanol extract. From the results of the statistical test, the positive control treatment group had a significant difference with the negative control and the 100mg/kg BB kenitu leaf ethanol extract group but did not have a significant difference with the normal control, the 200mg/kg BB kenitu leaf ethanol extract group, 400mg/kg BB and 800mg/kg BB.

Meanwhile, the ethanol extract group of kenitu leaves 400mg/kg BB had a significant difference with the negative control and ethanol extract of kenitu leaves 100mg/kg BB, but did not have a significant difference with the normal control, positive control, ethanol extract of kenitu leaves 200mg/kg BB and ethanol extract of kenitu leaves 800mg/kg BB. In the measurement results, the average number of osteoblast cells in the ethanol extract group of kenitu leaves 400mg/kg BB had a significantly higher increase in the number of osteoblasts. This shows that giving ethanol extract of kenitu leaves 400mg/kg BB to mice has a very good effect on increasing the number of osteoblast cells that function as bone formation.

Table 4. Results of the average number of osteoclast cells in each test group

Group	Mean ± SD
Normal	14 ± 1 b
Negative	25 ± 2 ac
Positive	12 ± 1 b
Kenitu leaf extract 100 mg/kg BW	23 ± 2 ac
Kenitu leaf extract 200 mg/kg BW	21 ± 2 c
Kenitu leaf extract 400 mg/kg BW	10 ± 2 b
Kenitu leaf extract 800 mg/kg BW	17 ± 2 b

a: significantly different from normal
b: significantly different from negative
c: significantly different from positive

Numbers followed by different letters in the same column indicate significantly different test results ($p<0.05$). The results of the statistical test of the number of osteoclast cells showed a significant difference between the kenitu leaf ethanol extract group and the negative control group. The positive control group had a significant difference with the negative control group, kenitu leaf ethanol extract 100mg/kg BB and kenitu leaf ethanol extract 200mg/kg BB but did not have a significant difference with the normal control group, kenitu leaf ethanol extract 400mg/kg BB and kenitu leaf ethanol extract 800mg/kg BB. In the kenitu leaf ethanol extract group 400mg/kg BB and kenitu leaf ethanol extract 800mg/kg BB, it was effective in reducing the number of osteoclast cells. The results of statistical analysis of the number of osteoclast cells in the 400mg/kg BB kenitu leaf ethanol extract group had a very significant difference with the negative control, the 100mg/kg BB kenitu leaf ethanol extract group, the 200mg/kg BB kenitu leaf ethanol extract group and the 800mg/kg BB kenitu leaf ethanol extract group, but did not have a significant difference with the positive control group and normal control.

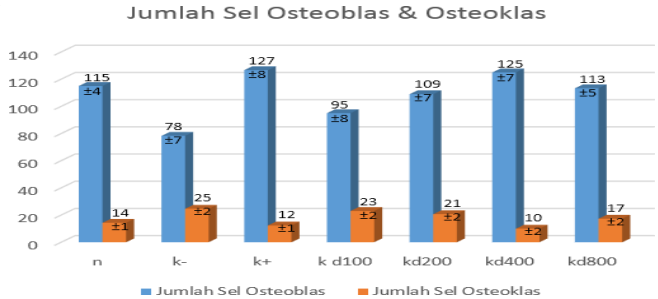

Figure 3. Graph of osteoblast and osteoclast cell count

Based on the graph above, the number of osteoclast cells in the negative control group showed a significant increase. This indicates that dexamethasone induction can increase the number of osteoclast cells in the test animals, which play a role in bone resorption, resulting in osteoporosis. This also shows that the group treated with 400 mg/kg BW of Kenitu leaf ethanol extract had a better pharmacological effect than Cabone Naturindo, which was used as a positive control in this study. The flavonoids contained in Kenitu leaves (Chrysophyllum cainito L.), such as isoflavones, are one of the compounds that exhibit a relatively high phytoestrogenic effect (Grippo et al., 2007). The in vitro mechanism of phytoestrogens in preventing osteoporosis is by stimulating osteoblast activity and inhibiting osteoclast formation (Branca, 2003).

Bone Density Histomorphometry Measurement Results

The test animals that underwent osteoporosis were induced using dexamethasone at a dose of 0.145 mg/kg BW with a volume of 0.34 ml once a day for 28 days. Dexamethasone consumption for 28 days in mice is equivalent to 3-4 years of use in humans. Long-term administration of dexamethasone results in decreased trabecular bone density or bone density by inhibiting estrogen from binding to estrogen receptors, leading to estrogen deficiency, which causes an imbalance in the bone remodeling process. In this process, osteoblast formation decreases, while resorption by osteoclast cells increases (Meeta, 2013).

Figure 4. Graph of average bone density histomorphometry

The graph data above can be seen that the results of the statistical test show that there is a significant difference between the negative group and each test group given ethanol extract of kenitu leaves. The results of the measurement of the average bone density or bone density in the normal control group are still normal because they are not induced by dexamethasone so they are still maintained in a healthy condition. The results of the Post Hoc statistical test using Tukey, the administration of cabone naturindo to the positive group was able to provide a significant effect of increasing bone density or bone density compared to the negative control group and the normal control. The positive control group did not have a significant difference with the kenitu leaf ethanol extract test group. The results of the statistical analysis of the graph of the average bone density measurement of the ethanol extract group of kenitu leaves 400mg/kg BW and the ethanol extract group of kenitu leaves 800mg/kg BW had a very significantly higher bone density than the positive control.

Table 5. Paired samples test bone density table

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	kd8 - kd16	-7.66667	34.31229	19.81021	-92.90312	77.56979	-.387	2	.736

Based on the SPSS analysis, a Paired T-Test between the 400 mg/kg BW and 800 mg/kg BW kenitu leaf ethanol extract groups showed no significant difference, with a significance value of 0.736 (> 0.05). Although the correlation was significant (sig = 0.997), the difference between the two groups may be influenced by the hormonal mechanism of the kenitu leaf extract's activity. Hormonal activity has a special characteristic called a non-monotonic dose response, where the dose does not necessarily correlate with the expected activity as with typical drugs. Increasing the dose in hormonal activity does not necessarily increase the effectiveness of the dose, nor does a decrease (Beausoleil, 2013). The 400 mg/kg BW Kenitu leaf ethanol extract group had a very good effect on significantly reducing the number of osteoclast cells in the test animals (mice). The pharmacological effect exhibited by the Kenitu leaf extract across four dosage groups, with the 400 mg/kg BW group even exceeding the positive control, Cabone Naturindo. This pharmacological effect, in the form of increased femoral bone density in female mice, is suspected to be due to the estrogenic properties of the phytoestrogens in C. cainito leaves. Phytoestrogens are plant-derived substances that are structurally or functionally similar to 17β-estradiol (E2). The estrogenic properties arise because phytoestrogens also contain 2 hydroxyl groups (-OH) that are 1.0-11.5 A˚ apart, which enables them to bind to estrogen receptors (Benassayag, 2002).

This study showed that the 400 mg/kg BW Kenitu leaf ethanol extract group increased the trabecular bone density in the test animals. The increase in the trabecular bone mass density is due to the estrogenic activity of the phytoestrogen compounds in the Kenitu leaf extract, which bind to estrogen receptors and promote the homeostasis of bone remodeling (Urasopon et al., 2008). These findings are consistent with previous research by Utaminingtyas (2017), which indicated that the optimal dose of 70% ethanol extract of C. cainito leaves for increasing trabecular bone density in dexamethasone-induced female mice is 400 mg/kg BW.

Further research by Mustofa (2018) also found that the 400 mg/kg BW dose of ethyl acetate extract from C. cainito leaves had the highest activity in increasing the trabecular bone density in dexamethasone-induced female mice. Phytoestrogens are plant compounds with a chemical structure similar to estrogen, exhibiting estrogenic effects and working on estrogen receptors (Yang et al., 2012). Phytoestrogens represent a potential alternative to estrogen without harmful side effects (Villiers, 2009). The activity of phytoestrogen compounds is known to be significant in bone health (Pawitan, 2002). In vitro tests have shown that phytoestrogens can increase osteoblast formation and inhibit osteoclast formation, making them useful for preventing osteoporosis (Branca, 2003). Phytoestrogens can bind to estrogen receptors in the body, including both estrogen receptor alpha (ER-α) and estrogen receptor beta (ER-β).

Results of Trabecular Femur Bone Histomorphometry Examination

After 28 days of treatment, the test animals were dissected on day 29 at the Setia Budi University Laboratory. Histopathological preparations of the trabecular femur bone were conducted at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Gadjah Mada, using Hematoxylin and Eosin (HE) staining. Observations were made with an Olympus CX33 microscope and Optilab camera at 40x and 100x magnifications to ensure accurate visualization and selection of intact bone areas, as some sections may have been damaged during cutting or staining.

Bone mass density measurements were performed using Motic Image Plus 3.0 software on the metaphyseal region, which is the lower part of the epiphysis, an active area for bone growth that influences the formation of compact bone structure and bone cavities. This area is easily measured for bone density and is commonly used for identifying osteoporosis. The metaphyseal region was measured three times in replication on one side of the bone to obtain an accurate section and value for identification (Rizalah et al., 2016).

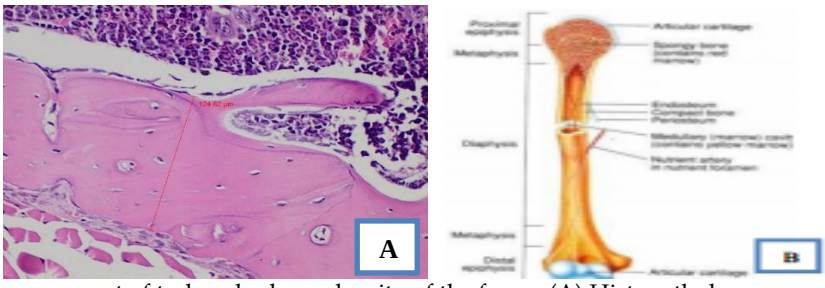

Figure 5. Measurement of trabecular bone density of the femur (A) Histopathology preparation sample; (B) Tabecular bone structure of the femur

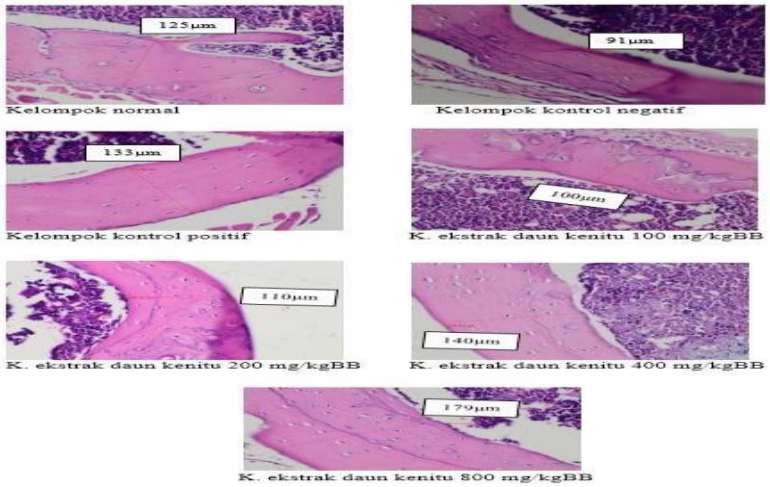

Figure 6. Photo results of bone organ preparations with 40X magnification

Note: The lines in the image indicate the distance between the thickness or density of the bone, meaning that the wider the distance between the thickness of the bone, the higher the bone density. Histomorphometric examination was conducted to determine the activity of the ethanol extract of kenitu leaves on increasing the density of femoral trabecular bone mass in µm units (see appendix 22). The average results of each test group were then compared with the negative control group to determine whether or not there was activity in the ethanol extract of kenitu C. cainito leaves. The results of the histomorphometric examination obtained can be seen in table 8 and figure 21 as follows.

Table 6. Average results of bone density or bone density for each test group

Group	Mean ± SD
Normal	123 ± 13 c
Negative	85 ± 10 c
Positive	132 ± 9 ab
Extract of Kenitu leaf 100 mg/kg BW	104 ± 4 c
Extract of Kenitu leaf 200 mg/kg BW	108 ± 12 c
Extract of Kenitu leaf 400 mg/kg BW	136 ± 12 ab
Extract of Kenitu leaf 800 mg/kg BW	144 ± 25 ab

a: significantly different from normal
b: significantly different from negative
c: significantly different from positive

The numbers followed by different letters in the same column indicate significantly different results ($p < 0.05$). Based on the results in the table above, the average bone density or thickness in micrometers per 10 field views, from the lowest to the highest, are as follows: the negative control group has 85; the ethanol extract of Kenitu leaf 100 mg/kg BW therapy group has 104; the ethanol extract of Kenitu leaf 200 mg/kg BW therapy group has 108; the normal control group has 123; the positive control group has 132; the ethanol extract of Kenitu leaf 400 mg/kg BW therapy group has 136; and the ethanol extract of Kenitu leaf 800 mg/kg BW therapy group has 144. This shows an increase in bone density after treatment. The negative control group has the lowest bone density compared to the other groups. Statistically, this group has a significant difference from all other six groups. The decrease in bone density in this group is due to the decreased estrogen hormone levels in the mice caused by dexamethasone induction. Estrogen hormone is supposed to accelerate the apoptosis of osteoclasts and stimulate the proliferation of osteoblasts. Since this group did not receive any fitoestrogen supplementation, there was nothing to replace the lost estrogen, resulting in fewer osteoblasts compared to the other treatment groups.

The significant difference in bone density between all the test groups and the negative control indicates the pharmacological effect of the Kenitu leaf extract across the four dosage groups, with even the 400 mg/kg BW ethanol extract group showing better results than the positive control (Cabone Naturindo) and the normal control. This pharmacological effect is believed to be due to the chemical compounds in the Kenitu leaf extract, particularly flavonoids. Flavonoids are known to function as fitoestrogens, structurally or functionally similar to 17β-estradiol (E2). This similarity allows fitoestrogens to bind with estrogen receptors, thus producing estrogenic activity, one of which is increasing homeostasis in bone remodeling (Urasopon et al., 2008).

The mechanism of fitoestrogens in enhancing homeostasis in the bone remodeling process begins with their binding to estrogen alpha and beta receptors in the cytosol of cells, reducing cytokine secretion such as IL-1, IL-6, and TNF-α. These cytokines affect bone resorption, so the presence of fitoestrogens helps reduce bone resorption. Fitoestrogens also enhance the secretion of Transforming Growth Factor β (TGF-β), which acts as a mediator to attract osteoblasts into the bone to repair the cavities formed by osteoclasts during resorption. Additionally, fitoestrogens increase apoptosis in osteoclasts, the bone-resorbing agents, through TGF-β production (Meeta, 2013).

These findings align with previous research by Utaminingtyas (2017), which stated that 70% ethanol extract of Kenitu leaf has activity in increasing trabecular bone density in female mice induced with dexamethasone, due to the presence of fitoestrogen compounds, specifically isoflavones. The optimum dose in that study was also 400 mg/kg BW.

CONCLUSION

The 70% ethanol extract of Kenitu leaf (Chrysophyllum cainito L.) has activity in increasing the number of osteoblast cells in the trabecular femur of female mice induced by dexamethasone. The 70% ethanol extract of Kenitu leaf (Chrysophyllum cainito L.) has activity in decreasing the number of osteoclast cells in the trabecular femur of female mice induced by dexamethasone. The 70% ethanol extract of Kenitu leaf (Chrysophyllum cainito L.) has an effective dose of 400 mg/kg body weight and activity in increasing the bone density (trabecular bone density) in the femur of female mice induced by dexamethasone.

The results of this study indicate that ethanol extract of kenitu leaves at an optimal dose of 400 mg/kg BW is able to increase bone density, increase the number of osteoblasts, and suppress the number of osteoclasts significantly in dexamethasone-induced osteoporosis models. These findings open up opportunities for further development into pre-clinical stages and first-in-human trials as a phytotherapy approach for osteoporosis. For this reason, dosage formulation strategies need to be directed towards increasing the bioavailability of isoflavone compounds, given that

phytoestrogen compounds generally have low stability and limited bioavailability by the oral route. The use of nanoencapsulation technology, liposomes, or complexation with carriers such as cyclodextrin can be considered in the preparation design. On the other hand, the design of early-stage clinical trials in postmenopausal women needs to be organized by considering aspects of safety, tolerability, as well as pharmacokinetic and pharmacodynamic parameters, especially related to hormonal responses and markers of bone metabolism. With a multidisciplinary approach and rigorous scientific validation stages, kenitu leaf extract has the potential to become a safe and effective alternative phytotherapy candidate in the management of osteoporosis.

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References

Anggraini, W. 2008. *Fitoestrogen sebagai Alternatif Alami Terapi Sulih Hormone untuk Pengobatan Osteoporosisi Primer pada Wanita Pascamenopause*. Volume 231, halaman: 25-31.

Anies, 2006. Waspada Ancaman Penyakit Tidak Menular Solusi Pencegahan dari Aspek perilaku dan Lingkungan. Gramedia : Jakarta.

Baziad, A. 2003. *Menopause dan Andropause*. Jakarta: Yayasan Bina Pustaka Sarwono Prawirohardjo.

Beausoleil C., Jean-Nicolas Ormsby, Andreas Gies, Ulla Hass, Jerrold J. Heindel, Marie Louise H., Pia Juul N., Sharon Munn, Gilbert Schoenfelder. 2013. *Low dose effects and non-monotonic dose responses for endocrine active chemicals: Science to practice workshop: Workshop summary.* Elsevier. Chemosphere 93 (2013) 847–856.

Chavassieux, P.M., Arlot, M.E., Roux, J.P., Portero, N., Daifotis, A., Yates, A.J., and Hamdy, N.A.T. 2000. —Effects of Alendronate on Bone Quality and Remodeling in Glucocorticoid- Induced Osteoporosis: A Histomorphometric Analysis of Transiliac Biopsies. *Journal of Bone and Mineral Research.*Volume 15, Nomor 4: 754–62.

Cosman, F. 2009. *Osteoporosis: Panduan Lengkap agar Tulang Anda Tetap Sehat.*Yogjakarta: B-First

Graham, J., Gallagher, R. and Bothe, J. (2013) *discharge planning and risk assessment: behaviours, understanding and barriers'*, Journal of Clinical Nursing, 22.

Grippo A, Capps K, Rougeau B and Gurley BJ. 2007. *Analysis of flavonoid phytoestrogens in botanical and ephedra-containing dietary supplements*. Ann Pharmacother. 41: 1375-82.

Jennifer JW. 2008. *Metods in Molecoler Biology:Osteoporosis Methods and Protocol,* Human Press. *Journal of Agricultural and Food Chemistry*.

Kawiyana I.K.S. Osteoporosis Patogenesis Diagnosis dan Penanganan Terkini. *J. Peny Dalam,* 2009, 10(2): 166.

Manolagas S C. 2000. Birth and death of Bone Cells: Basic Regulatory Mechanisms and Implications for the Pathogenesis and Treatment of Osteoporosis. *Endocrine Reviews* 21(2): 115-37.

Mazziotti, G., Cimino, V., De Menis, E., Bonadonna, S., Bugari, G., De Marinis, L., Veldhuis, J., D., Giustina, A. 2006. *Active acromegaly enhances spontaneous parathyroid hormone pulsatility. Metabolism*. 55:736–740.

Meeta. 2013. *Postmenopause Osteoporosis Basic and Clinical Consepts.* Jaypee Brothers Medical Publishers, New Delhi, p. 2, 20-22.

Meira NA, Klein LC Jr, Rocha LW, Quintal ZM, Monache FD, Cechinel Filho V and Quintao NL. 2014. Anti-inflammatory and anti-hypersensitive effects ofthe crude extract, fractions and triterpenes obtained from *Chrysophyllum cainito* leaves in mice. *J Ethnopharmacol*. 151: 975-983.

Misnadiarly, 2013.Osteoporosis: Pengenalan, Faktor Risiko, Pencegahan, dan Pengobatan. 1 st edn. Jakarta Barat: Akademia Permata.

Mithal, A., Ebeling, P., & Kyer, C. S. (2013). *The Asia-Pacific Regional Audit: Epidemiology, costs & burden of*

osteoporosis in 2013. International Osteoporosis Foundation. https://doi.org/10.1002/yd.20044
Morton. 1987. Star Apple Fruits of Warm Climates. *Miami Florida*. 408-410.
Mustofa, A.S. 2018. Aktivitas Ekstrak Etil Asetat Daun Kenitu (*Chrysophyllum cainito* L.) Terhadap Peningkatan Kepadatan Tulang Trabekular Vertebra Mencit Betina Yang Diinduksi Deksametason [skripsi]. Malang: Jurusan Farmasi Fakultas Kedokteran dan Ilmu-ilmu Kesehatan, Universitas Islam Negeri Maulana Malik Ibrahim.
Nurrochmad, A., Leviana, F. Wulancarsari, C. G., Lukitaningsih, E. 2010. Phytoestrogens of Pachyrhyzus erosus prevent Bone Loss in and Ovariectomized Rat Model of Osteoporosis. *International Journal of Phytomedicine* 2, 363-372.
Pawitan, J. A. 2002. *Phytoestrogens-Protection Against a Wide Range of Diseases*. Medical Progress. Volume 1, halaman 9-13
Pertawarman, A. & Hestiantoro, A. 2002. Manfaat Isoflavon pada Wanita Menopause. *Majalah Obstet Ginekol Indonesia*, Vol 26 1, 49-55.
Reid, Ian R. 2000. Glucocorticoid-Induced Osteoporosis. *Bailliere's Best Practice and Research in Clinical Endocrinology and Metabolism* 14 (2): 279-98. https://doi.org/10.1053/beem.2000.0074.
Riskesdas. (2013). Riset Kesehatan Dasar tahun 2013. Kementerian Kesehatan Republik Indonesia. https://doi.org/10.1517/13543784.7.5 .803
Rizalah, Suci I, Muhammad H., Septa S. W. 2016. Pengaruh Pemberian Kitosan Cangkang Udang Putih (*Penaeus merguiensis*) terhadap Ketebalan Trabekular Femur Tikus Wistar Betina Pasca Ovariektomi. *eJurnal Pustaka Kesehatan*. Volume 4, Nomor 1.
Schwinghammer, T.L. 2015. *Chapter 3: Osteoporosis*. Dalam: J.T. Dipiro penyunt. *Pharmacotherapy Handbook 9th ed*. United State of America : McGraw- HillCompanies, Inc. P.16.
Urasopon, N., Hamada, Y., Asaoka, K., & Poungmali, U. 2008. Isoflavon Content of rodent Diets amd Its Estrogenic Effect on Vaginal Cornification in Pueraria mirifica Treated Rats. Science Asia. 34: 371-376.
Vauzour, D., Rodriguez-Mateos, A., Corona, G., Oruna-Concha, M. J., & Spencer, J.P E. 2010. *Polyphenols and Human Health: Preventionn of Disease and Mechanisms of Action . Nutrients.* Volume 2, halaman : 1106-1131
Villiers, T. J. 2009. Bone health and osteoporosis in postmenopausal women. Elsevier : *Best Practice & Research Clinical Obstetrics and Gynaecology.* Volume 23 halaman: 73- 85.
Wardhana, W. 2012. Faktor-faktor Risiko Osteoporosis Pada Pasien dengan Usia di Atas 50 Tahun. *Karya Tulis Ilmiah.* Semarang: Universitas Diponegoro.
Wardhana, W. 2012. Faktor-faktor Risiko Osteoporosis Pada Pasien dengan Usia di Atas 50 Tahun. *Karya Tulis Ilmiah.* Semarang: Universitas Diponegoro.
Yang, T. S. Wang, S. Y. Yang, Y. C. Su, C. H. Lee, F. K. Chen, S. C. Tseng, C. Y. Jou, H. J. Huang, J. P. Huang, K. E. (2012) 'Effects of Standardized Phytoestrogen on Taiwanese Menopausal Women', *Taiwanese Journal of Obstetrics and Gynecology*, Vol 51(2): 229- 235.