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Review Paper

Phytoestrogens Therapy for Osteoporosis Treatment Using Indonesian Medicinal Plants: A Brief Review

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Abstract
A problematic bone remodeling cycle is known to produce more osteoclasts than osteoblasts, making bones more fragile and this condition shows osteoporosis. In this context, estrogen deficiency in the human body is associated with the regulation of osteoporosis. Therefore, this study aimed to investigate the knowledge about basic concepts of bone, osteoporosis, phytoestrogens, and Indonesian medicinal plants for osteoporosis treatment. Data were obtained from literature on various databases including Science Direct, Wiley Online Library, Scopus, Pubmed, and Google Scholar. Adequate therapy is needed to increase estrogen content and an effective approach is to consume medicinal plants that contain phytoestrogens, which have identical structure and activity to human estrogen (17β-estradiol). The results showed that there were observations comprising *in vitro*, *in vivo*, and *in silico* studies on 18 Indonesian medicinal plants as antiosteoporosis treatments. The Leguminosae or Fabaceae family, which has a significant amount of isoflavones (the primary group of phytoestrogens) was found to dominate as an antiosteoporosis agent. Therefore, the development of phytoestrogens therapy from Indonesian medicinal plants must be implemented for the future treatment of osteoporosis.

Keywords
Indonesia, Isoflavones, Natural Resources, Osteoporosis, Phytoestrogens

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1. INTRODUCTION

Approximately 20 million individuals throughout the globe are reported to suffer from osteoporosis, making it a serious global health issue. Osteoporosis affects one-third of women and one-fifth of men over the age of 50 globally (Liu et al., 2024; Xu et al., 2024). The bones become porous more quickly in women than in men because estrogen hormone, known to influence bone development is reduced in women. Estrogen deficiency is supported by the lack of follicular activity in the ovary. Therefore, a major factor leading to increased rates of osteoporosis and fractures is the decrease of bone mineral content that occurs during menopause (Baral and Kaphle, 2023; Hernawati and Soesilawati, 2020; Li et al., 2024).

Osteoporosis is currently at a concerning level in Indonesia, affecting 19.7% of the population. Following the International Osteoporosis Foundation (IOF), approximately one out of four women between the ages of fifty and eighty are at risk of developing osteoporosis. Therefore, Indonesian women have a four-fold increased risk of osteoporosis (Peycheva et al., 2022). Given that over 90% of the greatest bone density is reached between the ages of 20-30 years old, maintaining bone health is most effective while a person is still productive (He et al., 2024; Sietsema, 2020; Yue et al., 2024).

The management procedures for treating osteoporosis are currently divided into two categories namely pharmacological and non-pharmacological therapy. Pharmacological therapy comprises antiresorptive agents (bisphosphonates, receptor activator of nuclear factor kappa-β ligand (RANKL) antibody); hormonal agents (Selective estrogen receptor modulators or SERMs; parathyroid hormone); as well as intake of calcium, vitamin D, and vitamin K. Meanwhile, maintaining a healthy lifestyle and consuming a nutritious diet is part of non-pharmacological therapy (Adejuyigbe et al., 2023; Karimi et al., 2024; Cheng et al., 2022).

The use of natural ingredients containing active compounds has the potential to be an anti-resorption agent due to the fewer side effects compared to anabolic drugs which can cause cancer when consumed continuously. These organic substances directly promote bone growth, serving as an effective method for treating osteoporosis (Karimi et al., 2024; Poorirani et al., 2022). Previous studies state that phytoestrogens are biomarkers in the treatment of osteoporosis. This is because phytoestrogens have the ability to function similarly to human estrogen, specifically estradiol, which attaches to estrogen receptors and affects bone health. Phytoestrogens also control pro-inflammatory, oxidative stress, and bone matrix protein regulatory pathways can increase estrogenic activity in the body (Chen et al., 2024; Faienza et al., 2024; Li et al., 2023; Liu et al., 2024; Tomczyk-Warunek et al., 2024).

Keywords such as osteoporosis, Leguminosae, phytoestrogens, isoflavones, proinflammation cytokines, antioxidants, and alkaline phosphatase were used to collect 1,200 Scopus-indexed published articles (Year 2013-2024) through Publish or Perish software. Subsequently, analysis was conducted using Network (Figure 1), Overlay (Figure 2), and Density Visualization (Figure 3) through VOS Viewer software. Figure 1 shows that Leguminosae, medicinal plants, and phytoestrogens were highly correlated. Leguminosae and isoflavone items were correlated, osteoporosis and pro-inflammatory cytokines formed one cluster, while estrogen receptors and phytoestrogens formed another cluster for bone regeneration. There are various studies on legumes as shown in Figure 2. However, Indonesian medicinal plants for treatment of osteoporosis have not been found in many publications indexed by Scopus (Figure 3). Therefore, this study aimed to investigate some Indonesian medicinal plants for treatment of osteoporosis based on phytoestrogens which have been proven *in vitro*, *in silico*, and *in vivo*. The results are expected to provide a novel insight into finding osteoporosis treatment based on Indonesian medicinal plants.

2. BASIC CONCEPTS OF BONE

The human skeleton is made up of 206 bones, which are connective tissue offering various supports to the body, including movement, protection of organs, and storage of minerals (Dishad Ahmed and Hawezi, 2023; Su et al., 2023). In general, there are two primary structural types of bones in the skeleton namely cortical (compact bone) and the trabecular (spongy or cancellous bone). Approximately 80% of the total bone mass is produced by cortical bone, with the other 20% coming from trabecular bone. The marrow cavity is surrounded by dense cortical bone and constructed of Haversian systems, composed of bone tissue lamellae that cover a central canal filled with blood vessels. Trabecular bone contains a larger surface area and a reduced density than cortical bone. The centers of the long and flat bones, alongside vertebrae, are covered with trabecular bone (Osterhoff et al., 2017; Ralston, 2017; Rique et al., 2019).

Bone consists of an extracellular matrix composed of an inorganic component (70-90%) and an organic component (10-30%). The inorganic components are hydroxyapatite, calcium carbonate, and phosphate. These inorganic components enhance compression stability. Collagen type I constitutes 90% of the organic components and non-collagenous proteins constitute 10% of the organic components, such as alkaline phosphatase, osteocalcin, proteoglycans, and osteopontin. These organic components contribute to the material's flexibility, resistance to twisting forces, and strength (Carvalho et al., 2021; Le et al., 2017; Setiawati and Rahardjo, 2019). Bone also contains cell components of osteoblasts, osteoclasts, and osteocytes. Osteoblasts, produced through mesenchymal stem cells, are in charge of bone generation. Resorbing cells are osteoclasts, which degenerate on hematopoietic stem cells. A significant proportion of bone cells in the skeleton, namely osteoclasts, function as mechanical sensors (Setiawati and Rahardjo, 2019; Thomas and Jaganathan, 2022).

Skeletal bone production frequently occurs throughout development and growth without preceding bone resorption, a process called bone modeling. On the other hand, bone remodeling is an approach to renewing old with new bone cells by combining resorption and formation. The purpose of remodeling is to allow the bones to respond to and accommodate the mechanical stress caused by physical activity. Every ten years, the adult skeleton is renewed by bone remodeling, which comprises four stages as shown in Figure 4 (Delaisse et al., 2020; Kim et al., 2020; Beck-Nielsen et al., 2021):

1. **Resorption Phase:** Osteoclast progenitors are activated on the bone surface and differentiate into osteoclasts. During resorption, osteoclasts resorb damage or old bone and the extracellular matrix. Minerals such as calcium and phosphorus are lost, causing the bones to weaken and become fragile. The resorption phase extends for about 2-4 weeks.
2. **Reversal Phase:** Osteoclasts die and osteoblasts progenitors are recruited on the bone surface to stimulate osteoblasts.
3. **Formation Phase:** The formation phase includes the proliferation of mesenchymal cells, maturation of osteoblasts to form a new bone matrix (osteoid), and mineralization. This phase needs 4-6 months to produce the new bone.
4. **Resting Phase:** This phase occurs when osteoblast activity terminates due to apoptosis induced by tumor necrosis factor (TNF) secreted in the surrounding marrow cells. Dormancy occurs in bone tissue, which is covered by inactive osteoblasts until the next remodeling cycle occurs.

Bone resorption is immediately followed by growth in normal remodeling. Abnormal remodeling occurs when the quantity of bone resorption rises above the quantity of synthesis. This condition leads to primary osteoporosis type I (postmenopausal osteoporosis) and secondary osteoporosis (Hioki et al., 2021; Kim et al., 2020; Wu et al., 2020).

Estrogen is a steroid hormone that plays an active role in the activity of osteoblast and osteoclast cells. It inhibits bone remodeling while regulating the ratio between resorption and formation. In osteoclasts, estrogen can suppress bone resorption activity and trigger death by apoptosis. Furthermore, estrogen stimulates osteoblast differentiation to produce an organic matrix that activates migration towards areas of bone suffering resorption (Cauley, 2015; Emmanuelle et al., 2021).

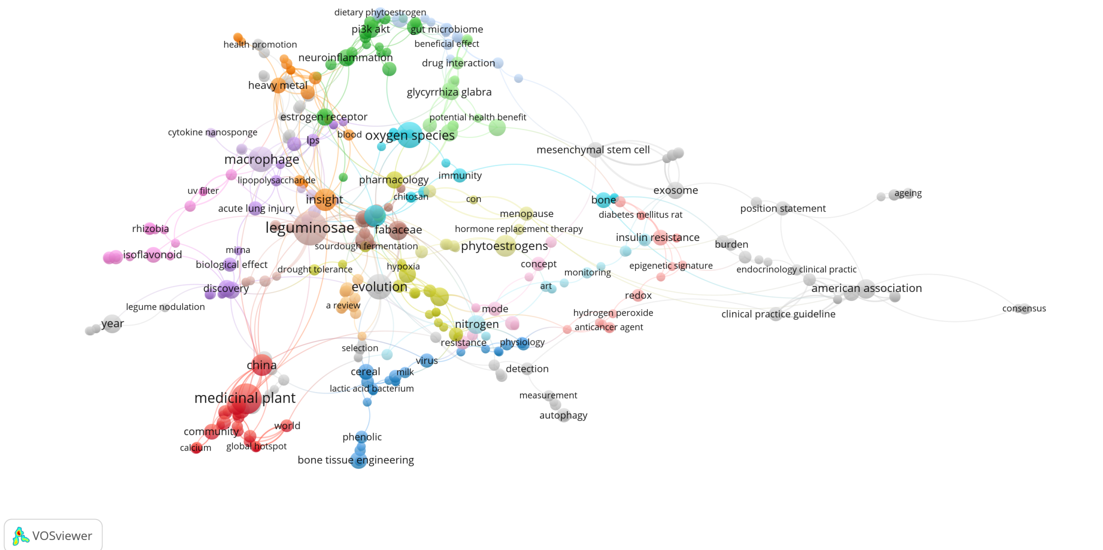

Figure 1. Network Visualization

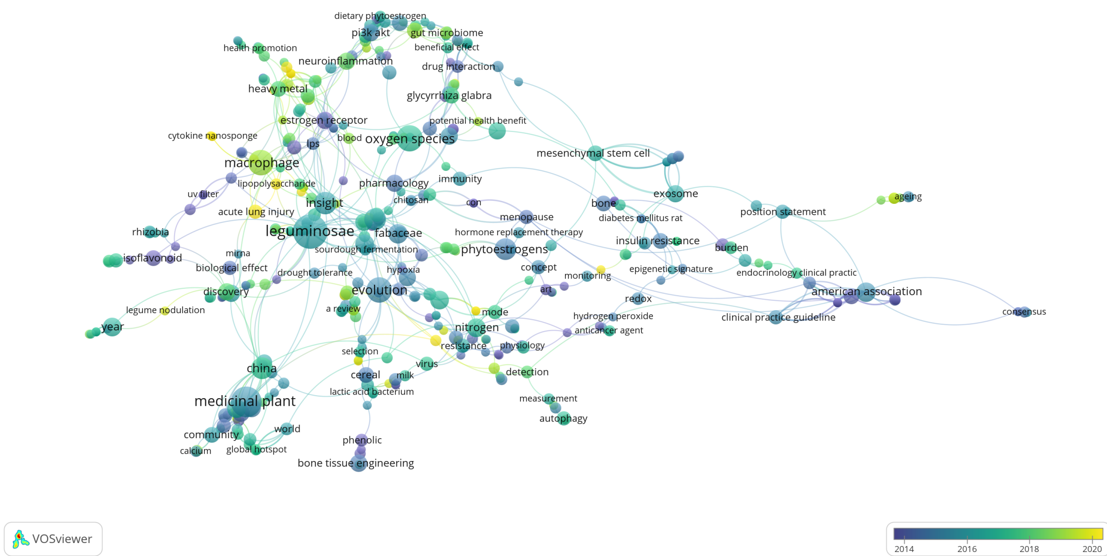

Figure 2. Overlay Visualization

3. OSTEOPOROSIS

The World Health Organization (WHO) defines osteoporosis as a progressive systemic bone disease marked by decreased mass and damage to the microarchitecture of tissue, rendering it fragile and susceptible to breaking down. The T-score is used to define the WHO measurement of bone mineral density (BMD), which is calculated as the difference between observed BMD and BMD in healthy young women to obtain the standard deviation (SD). Osteoporosis occurs when the T-score $\leq$ -2.5 SD. The condition is recognized as a "silent epidemic disease" because it attacks without warning until the sufferer breaks a bone (Baim and Leslie, 2012). According to WHO, osteoporosis is predicted to be associated with the primary risk factors influencing human health and the national economy

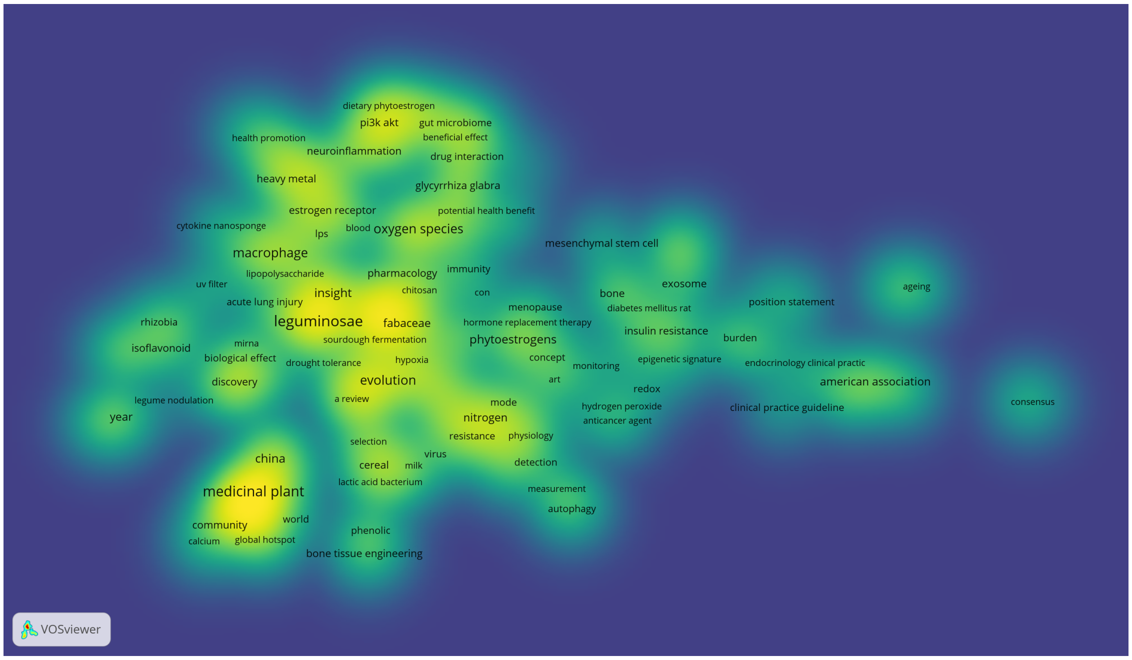

Figure 3. Density Visualization

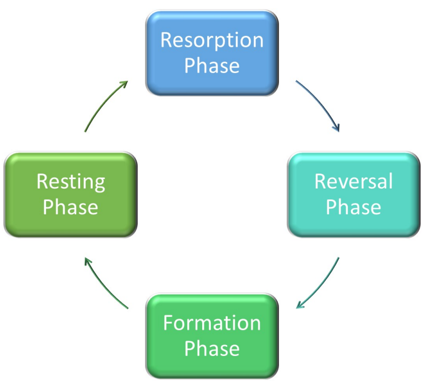

Figure 4. Bone Remodeling Cycle (Modified from Beck-Nielsen et al. (2021))

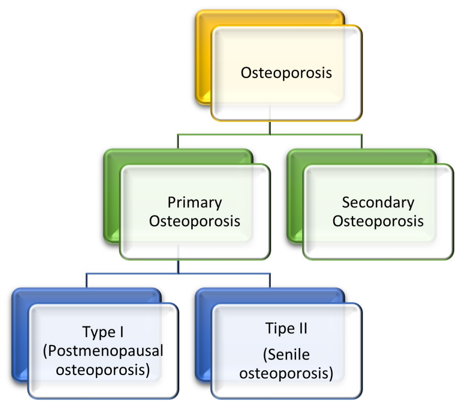

Figure 5. Classification of Osteoporosis

worldwide in the coming years (de Wit et al., 2019).

The term osteoporosis comes from two Greek words, namely "osteon" meaning bone, and "porous" referring to a pore or porous, hence, osteoporosis implies porous bones (Al-Ani et al., 2019; de Villiers and Goldstein, 2021; Schapira and Schapira, 1992). The ability to maintain bone mass with age and reach maximum peak bone mass throughout growth are both risk factors for osteoporosis. Smoking, alcohol consumption, glucocorticoid use, way of life, and genetic factors also have significant impact on the disorder (de Villiers and Goldstein, 2021; Lenzi and Migliaccio, 2018; Sozen et al., 2017).

Primary (type I and II) and secondary osteoporosis are the two main categories used in classification (Figure 5). Postmenopausal osteoporosis, also known as type 1 primary osteoporosis, is caused by the menopause phase, which leads to a decrease in estrogen levels and bone loss. The decrease in estrogen levels starts about two to three years before menopause and lasts for three to four years after. Patients with primary osteoporosis type 1 lose 1-3% of bone mass in the early stages, and this loss continues until 35–50% has been lost. On the other hand, primary osteoporosis type 2 (senile osteoporosis) can affect both men and women around the age of 70 and is carried on by low levels of calcium and vitamin D-producing cells. Secondary osteoporosis is carried on by long-term illness, vitamin D insufficiency, and the use of chemicals or medications or bone degradation (Chitra and Sharon, 2021; Dobbs et al., 1999; Sozen et al., 2017).

At the biological level, osteoporosis occurs due to differences in the number and activity of osteoclast cells which are more abundant than osteoblast. Increased osteoclast cell devel-

Table 1. Indonesian Medicinal Plants That Contain Phytoestrogens and Are Used to Treat Osteoporosis

Medical Plant Local name	Medical Plant Scientific name	Family	Plant Part Used	Method	Extraction Solvent	Antiosteoporosis Compounds	Pharmacological Activity	Reference
Bengkoang	*Pachyrhizus erosus*	Leguminosae or Fabaceae	Fruits	*In vivo*	Ethyl acetate	Phytoestrogens: daidzein, (8,9)-Furanyl-pterocarpan-3-ol; 5-Hydroxy-daidzein-7-O-β-glucopyranose; daidzein-7-O-β-glucopyranose	The administration of the extract 200,400, and 800 mg/kg BW greatly reduced bone loss in ovariectomized rats and this effect was similar to the use of estradiol.	(Rochmad et al., 2010)
Kacang panjang	*Vigna unguiculata* (L.) Walp.	Leguminosae	Fruits	*In vivo*	NA	Phytoestrogens: isoflavones (genistein)	The extract at a dose 2.5 and 5.0 mg/kg reduced superoxide dismutase levels in the vaginal tissue of rats and at a dose 1.25 mg/kg had equivalent of malondialdehyde levels to the control rats. The extract also increases proliferation fibroblast in ovariectomized rats	(Setyarini et al., 2019)
Buncis	*Glycine max* (L) Merill	Leguminosae	Leaves	*In vivo*	Water	Isoflavones (aglycones daidzein and genistein)	The water extract of isoflavone-enriched soybean leaves inhibited osteoclastogenesis in ovariectomized rats.	(Xie et al., 2020)
Gedi	Abelmoschus *manihot* L.	Malvaceae	Leaves	*In vitro*	96% Ethanol	Phytoestrogens (Isoflavones), triterpenoids (betulinic acid, ursolic acid, oleanolic acid)	96% ethanol extract of gedi leaves can increase the activity of forming alkaline phosphatase with MC3T3-E1 preosteoblast cells.	(Aditama et al., 2016)
Labu kuning	*Cucurbita pepo* L.	Cucurbitaceae	Seed	*In vitro*	Water	Phytoestrogens	Labu kuning seeds extract can increase the activity of ALP, the bone mineral density of rats in the group of ovariectomized rats and those consuming labu kuning seed extract.	(Oh et al., 2024)
Semanggi	*Marsilea crenata* Presl.	Marsileaceae	Leaves	*In vitro*	N-hexane	Phytoestrogens	The n-hexane extract in concentration of 400 ppm can increase ALP activity (1282.512) in MC3T3-E1 osteoblast cell.	(Ma'arif et al., 2018)
Bunga matahari	*Helianthus annuus* L.	Asteraceae	Seeds	*In vitro*	70% Ethanol	Phytoestrogens	The ethanol extract of bunga matahari seeds has estrogenic activity with increased ALP activity and reduced lipopolysaccharide-induced production of TNF-α; IL-6, and NO in osteoblast cells at a dose of 50 μg/mL	(Choi, 2012)
Kenitu	*Chrysophyllum cainito* L.	Sapotaceae	Leaves	*In silico,*	96% Ethanol,	11-Aminoundecanoic acid, megalanthonine, cetylamine, N-[4-Ethoxy-3-(1-pyrrolidinylsulfonyl)phenyl]-2-[4-(2-pyrimidinyl)-1-piperazinyl]acetamide, Lauryldiethanolamine, N-[(2-Isopropoxyethyl) sulfonyl]glycyl-O,2-dimethylserine, safingol, Megalanthonine; epi-jasmonic acid; 11-Aminoundecanoic acid; 1-Amino-3-(hexadecyloxy)-2-propanol	Based on pharmacokinetic and pharmacodynamic analysis, the seven compounds are suspected of being phytoestrogens with ER-β agonist properties against the 3OLS protein.	(Ma'arif et al., 2021)
				In vivo	70% Ethanol	Phytoestrogens	Optimal dose of 48.6 mg/200 g from *C. caimito* can increase the osteoblast cell number and bone mass density in wistar rats.	(Ma'arif et al., 2024)
				In vivo *In silico*	96% Ethanol	Terephthalohydrazide; 1-[(6,7-Dimethyl-2-oxo-1,2-dihydro-3-quinolinyl)methyl]-1,3-bis[3-(4-morpholinyl)propyl] thiourea; IsoSildenafil; N-(3R,4S)-9-[(5-Chloro-1,3-dimethyl-1H-pyrazol-4-yl)methyl]-3-hydroxy-4-methyl-1-oxa-9-azaspiro[5.5] undec-4-yl-2-methoxyacetamide	The male mouse trabecular vertebrae's bone density was raised by the 96% ethanol extract of kenitu leaves, and the optimal dose was 0.1 mg/g/day (bone density value: 266.65±1.38 μm; ED50 value: 95.4 mg/g/day). It was predicted that eight compounds might function similarly to 17β-estradiol.	(Riwanti et al., 2021)
Jintan Hitam	*Nigella sativa* L.	Ranunculaceae	Seeds	*In vivo*	NA	Phytoestrogens	Jinten Hitam extract suppressed tumor necrosis factor associated factor 6 (TRAF6) and nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) in ovariectomized rats at an optimum doses of 5 mg/kg BW ($p = 0.001$).	(Syamsul et al., 2021)
Sipatah-patah	*Cissus quadrangularis* Salisb.	Vitaceae	Stems	*In vitro*	Ethyl acetate	Phytoestrogens (flavonoids, resveratrol, piceatannol)	The ethyl acetate extract of Sipatah-patah can increase the maturation of bone marrow mesenchymal stem cells obtained from Sprague Dawley rats.	(Eriani et al., 2023)

Medical Plant Local name	Scientific name	Family	Plant Part Used	Method	Extraction Solvent	Antiosteoporosis Compounds	Pharmacological Activity	Reference
Jahe	*Zingiber officinale* Rosc.	Zingiberaceae	Rhizome	*In vivo*, *In vitro*	Water	Flavonoids and phenolics	Jahe extract has a strong inhibition on the post stimulation osteoclastogenesis rat models. The combination of jahe extract (GE) and Difructose anhydride III (DFA III) can reduce the osteoclastogenesis confluency in RAW 264.7 cells.	(Ikawati et al., 2022)
Kelapa Sawit	*Elaeis guineensis Jacq.*	Arecaceae	Leaves	*In vivo*	50% Methanol	Catechins (Flavonoids)	The extract increased the ALP activity, reduced COX-2 and IL-6 activity at a dose of 300 mg/kg BW/day on ovariectomized female rats.	(Bakhsh et al., 2013)
Landik	*Barleria lupulina Lindl.*	Acanthaceae	Herbs	*In vitro*	70% Ethanol, fractionation with ethyl acetate, n-hexane, n-butanol, and water	Iridoid glycosides: 8-O-acetylipolamiidic acid; 8-O-acetyl-6-O-(p-methoxy-cis-cinnamoyl)shanzhiside, and 8-O-acetyl-6-O-(p-methoxy-trans-cinnamoyl)shanzhiside	The isolated compounds increased ALP activity in MC3T3-E1 cells.	(Widyowati et al., 2010)
Secang	*Caesalpinia sappan* L.	Caesalpiniaceae	Woods	*In vivo*	70% Ethanol	Phytoestrogens	70% ethanol extract has the highest bone density of 0.60 gr/cm^3 at an optimum dose of 500 mg/kg BW in ovariectomized rats.	(Shabrina et al., 2013)
Pegagan	*Centella asiatica*	Apiaceae	Leaves	*In vivo*	Ethanol	Triterpenoids (asiaticoside)	Increase in the femur mass in ovariectomized rats at a dose of 60 mg/kg BW.	(Bong et al., 2019)
Delima	*Punica granatum*	Lythraceae	Fruits	*In vivo*	Ethanol	Flavonoids	Treatment groups with doses of 100, 300, and 500 mg/kg BW for 90 days had significantly higher femoral length, weight, volume, density ($p < 0.001$), and fourth lumbar hardness ($p < 0.001$) compared to the ovariectomized rats control group.	(Yogesh et al., 2020)

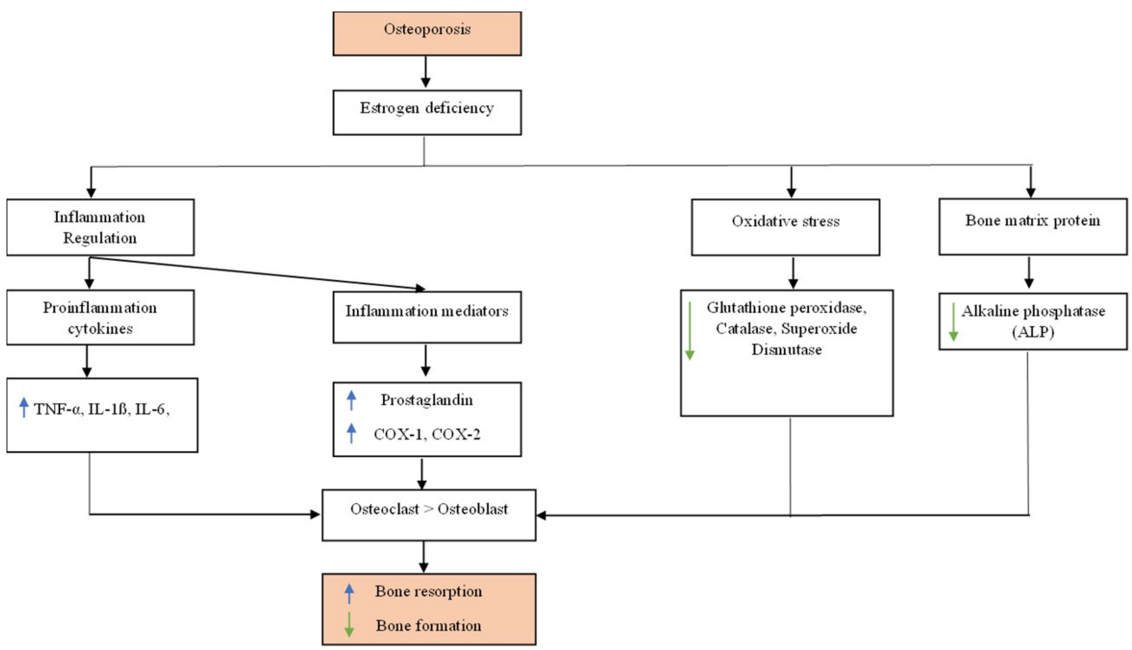

Figure 6. Regulation of Osteoporosis (Modified from Harmer et al. (2019); He and Wang (2015); Hua et al. (2018))

opment is based on four factors namely estrogen insufficiency, oxidative stress, chronic inflammation, and age (Li et al., 2023). Osteoblast and osteoclast activity is significantly influenced by the sex steroid hormone estrogen, which further plays a significant role in bone metabolism and the formation of bone matrix proteins, including alkaline phosphatase (ALP). Estrone (E1), generated after menopause, 17β-estradiol (E2), the most potent and well-known estrogen, estriol (E3), released by the placenta during pregnancy, and estetrol (E4) produced by the fetal liver are the four recognized estrogens (Emmanuelle et al., 2021). The effects of therapy include increased calcium absorption in the bones, decreased production of proinflammatory

Table 2. Description of the Location and Conditions for Growing 18 Indonesian Medicinal Plants as Anti-Osteoporosis Agents

Medicinal Plants	Location	Growing Conditions		Reference
		Soil pH	Temperature (°)	
Bengkoang	Sumatera, Java, Kalimantan, East Nusa Tenggara, Sulawesi, Bali	4.5–8	25–28	(Kumalasari et al., 2014) (Rahmaddiansyah et al., 2024)
Kacang Panjang	East Java, West Java, DI Yogyakarta, Central Java	5.5–6.5	20–30	(Ramadhanti et al., 2024)
Buncis	Sumatera, Java, Sulawesi	4.5–7.5	25-27	(Nasruddin et al., 2021) (Pismarović et al., 2022)
Kedelai	North Sumatera, South Sumatera, South Kalimantan, West Kalimantan, Java, Southeast Sulawesi, Maluku	6–6.8	21–34	(Budianta et al., 2019) (Rinaldi et al., 2023)
Bawang Dayak	Central Kalimantan, East Kalimantan	5.6–6.5	25–32	(Ahmad et al., 2016) (Bahtiar and Dewi, 2019)
Gedi	Eastern Indonesia, Papua	5.5–7.0	25–30	(Luan et al., 2020)
Labu Kuning	East Java, South Kalimantan	5.5–7.0	25–30	(Hosen et al., 2021) (Supriyono et al., 2023)
Semanggi	East Java, West Java	6.5–8.5	27–29	(Ma'arif et al., 2018) (Agil et al., 2021) (Tripatmasari et al., 2021)
Bunga matahari	Sumatera, Java, Kalimantan, Sulawesi, Papua	6.5–7.5	15–30	(Herwati et al., 2021) (Martinsyah et al., 2023) (Puttha et al., 2023)
Kenitu	Low mountain and downstream parts of Java	6,5–7,8	22–38	(Ma'arif et al., 2021) Ningsih et al. (2016) (Ningsih et al., 2020)
Jintan Hitam	Central Java	5–6	20–25	(Alshammari, 2017) (Sutrisna et al., 2022) (Jufri et al., 2022)
Sipatah-patah	Aceh	6.5–7.5	25–30	(Eriani et al., 2023)
Jahe	Riau, Java, Bali, West Nusa Tenggara, Central Sulawesi	6.8–7.4	25–30	(Riptanti et al., 2018) (Supriyono et al., 2023)
Kelapa Sawit	Sumatera, Kalimantan	4–6.5	29–30	(Astari et al., 2024) (Nabila et al., 2023) (Satriawan et al., 2021)
Landik	Sumatera, Kalimantan, Java, Bali, Sulawesi, Ambon	6.0–7.0	25–32	(Ismail-Suhaimy et al., 2021) (Gangaram et al., 2022) (Widyowati et al., 2010)
Secang	South Sulawesi	5–7.5	24–7.5	(Hasanah et al., 2021) (Meiyanto et al., 2018)
Pegagan	Sumatera, Kalimantan, Java, Bali, Sulawesi, Ambon	6–7	25–30	(Maruzy and Susandarini, 2024) (Mumtazah et al., 2020)
Delima	Sumatera, Java, Bima	6–6,5	20–30	(Hernawati and Soesilawati, 2020) (Mo et al., 2022; Moga et al., 2021) (Nirwana et al., 2017)

cytokines like interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), and inhibition of inflammatory mediators including cyclooxygenase-2 (COX-2). Furthermore, estrogen has two main receptor types: estrogen receptor-alpha (ER-α) and estrogen receptor-beta (ER- β), which have an agonizing and antagonistic effect on bone growth (Figure 6). ER-α is more abundant in cortical bone, whereas ER-β is more present in trabecular bone (Harmer et al., 2019; He and Wang, 2015; Hua et al., 2018; Steppe et al., 2022).

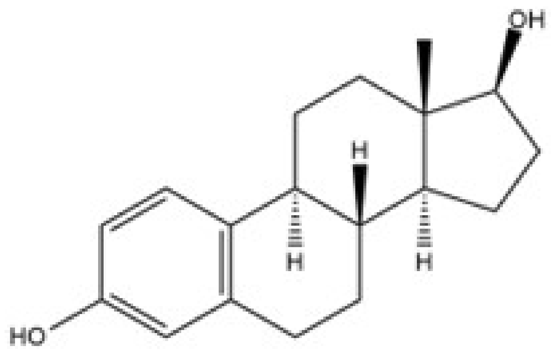

Figure 7. The Structure of 17-β Estradiol (PubChem ID: 5757)

4. PHYTOESTROGENS

Phytoestrogens were introduced in 1926 and the term was derived from the Greek words phyto, meaning "plant," and estrogen, referring to "sex hormone" (Gupta et al., 2016; Nikolić et al., 2017). These compounds have the same properties and efficacy as the hormone estrogen or can interact with the receptors. Phytoestrogens, often present in fruits, vegetables, and whole grains frequently consumed by people are also non-steroidal diphenolic substances that are structurally and functionally similar to human estrogens, specifically estradiol (17β-estradiol) (Figure 7). The four main groups include isoflavones, lignans, coumestans, and stilbens (Figure 8) (Desmawati and Sulastri, 2019; Desta and Abd El-Aty, 2022; Domínguez-López et al., 2020).

The flavonoid group is also considered a class of phytoestrogens, and the identified functions include anti-inflammatory, anti-colon cancer, anti-obesity, anti-diabetes, antiosteoporotic, anti-thrombotic, antioxidants, anti-tumoral, anti-allergic, and regulator in reproduction (Alexander V., 2014; Hsiao et al., 2020; Torrens-Mas and Roca, 2020). Phytoestrogens are also referred to as 'natural' selective estrogen receptor modulators (SERMs) in mammals and have specific characteristics for binding to estrogen receptors (ER-α and ER-β) to increase the activity (Lagari and Levis, 2014; Schilling et al., 2014; Wyse et al., 2022).

5. INDONESIAN MEDICAL PLANTS FOR TREATMENT OF OSTEOPOROSIS

Indonesia is a tropical archipelago country that spans two continents, Asia and Australia, alongside two oceans, the Pacific and Indian. According to data from the Ministry of National Development Planning, the Ministry of Environment and Forestry, and The Indonesian Institute of Sciences' 2015-2020 Indonesian Biodiversity Strategy and Action Plan (IBSAP), the country has more than 15% of the total global botany. In 2014 (26,473 plant species) and 2017 (31,750 plant species), there was an increase of 5,277 plant species. This ranks Indonesia as the country with the second biggest megabiodiversity behind Brazil in terms of flora and fauna. Therefore, it is necessary to acknowledge the significance of biodiversity management, documentation, and investigation (Cahyaningsih et al., 2021; Maskun et al., 2021; Saputera et al., 2008; Sianipar et al., 2022).

Table 1 presents Indonesian medicinal plants used to treat osteoporosis and contain phytoestrogens. Based on the results, a total of 18 plant species were discovered within 15 families. The Leguminosae or Fabaceae family is the most widely used for antiosteoporosis (22%) with four plant species namely bengkoang, kacang panjang, buncis, and kedelai (Figure 9). This family has been extensively reported to contain isoflavone compounds, the main group of phytoestrogens (Desta and Abd El-Aty, 2022; Hsiao et al., 2020; Popa and Rusu, 2017), evidenced in the review results presented in Table 1. Isoflavone compounds that play the most significant roles in the four plants are daidzein, glycitein, and genistein (Figure 10). Iridaceae, Malvaceae, Cucurbitaceae, Marsileaceae, Asteraceae, Sapotaceae, Ranunculaceae, Vitaceae, Zingiberaceae, Arecaceae, Acanthaceae, Caesalpiniaceae, and Lythraceae are 13 other families containing phytoestrogens. Additionally, the Apiaceae is the famili has anti-osteoporosis properties.

The majority of these 18 plants can thrive in regions of Indonesia that have tropical conditions with sunlight exposure such as in Sumatra, Java, Kalimantan, Bali, Sulawesi, and Papua (Cahyaningsih et al., 2021). Four plants are characteristic of certain regions, such as Bawang Dayak in Kalimantan Island, Gedi in North Sulawesi, Sipatah-patah in Aceh, and Secang in South Sulawesi. However, these typical plants can also grow in other locations when the ideal growing conditions are adjusted to the soil pH and temperature required (Table 2).

Table 1 shows that phytoestrogens have six roles in the *in vivo* management of osteoporosis, namely 1) reducing IL-6 expression in vertebral bones in menopausal animal models; 2) thickening of the trabecular bone layer in the vertebral bone marrow in postmenopausal model animals; 3) increasing the number of osteoblasts in terms of morphology; 4) decreasing the number of osteoclasts on the bone growth plates of ovariectomized rats; 5) increasing plasma ALP levels in female Wistar rats; and 6) increasing bone calcium and bone density. Meanwhile, three roles are carried out by phytoestrogens in the *in vitro* approach, namely 1) increasing the proliferation of osteoblast cells; 2) increasing the activity of forming alkaline phosphatase with MC3T3-E1 preosteoblast cells; and 3) reducing the activity of the osteoclasts. Using in silico method, phytoestrogens bind ER-β through hydrogen bonds. These data proved that phytoestrogens can enhance bone mineral density by increasing osteoblast production and decreasing resorption.

Table 1 also serves as a graphical abstract (Figure 11), showing that 18 medicinal plants from Indonesia, mostly from the Leguminosae or Fabaceae families, can prevent osteoporosis. According to this study, the primary class of phytoestrogens, isoflavones, are marker molecules for osteoporosis treatment. The proposed mechanism of action from the extract is shown in Figure 12. Indonesian medicinal plants dominated by the Legu-

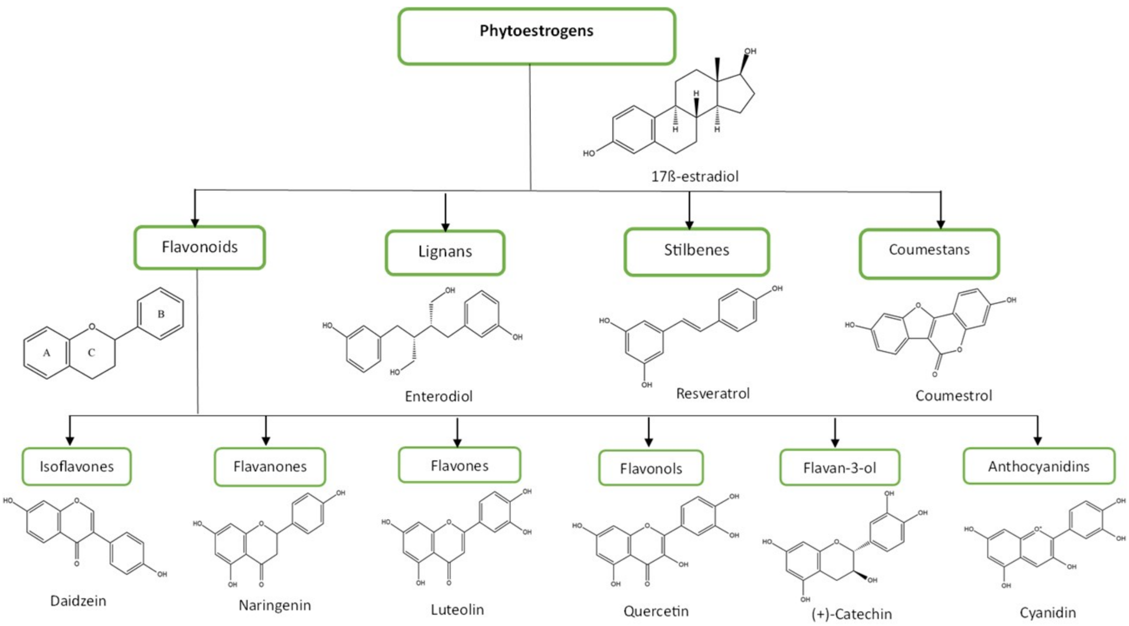

Figure 8. Classification of Phytoestrogens (Modified from Domínguez-López et al. (2020); Torrens-Mas and Roca (2020))

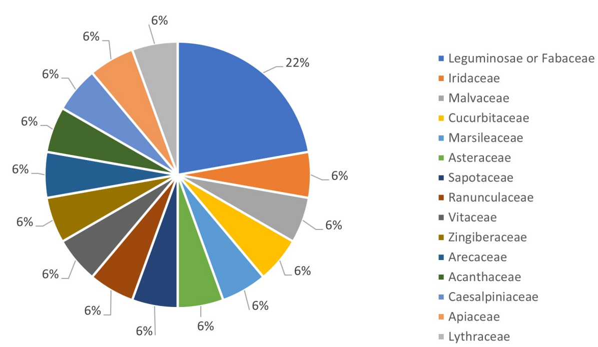

Figure 9. The Ratio of Families and Plant Species to Treat Osteoporosis

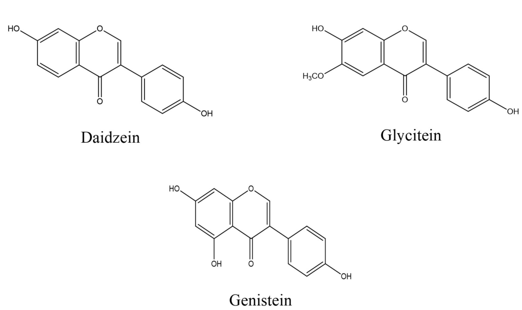

Figure 10. The Structure of Daidzein, Glycitein, and Genistein

minosae or Fabaceae family are prepared into simplicia until an extract is obtained. This extract contains many of the four main groups of phytoestrogens including such as isoflavones, lignans, stilbenes, and coumestans. The extract has been investigated *in vitro*, *in vivo*, and *in silico*. Based on the results, the activity of inflammatory pathways such as TNF-α, IL-1β, IL-6, and COX-2 was reduced, while endogenous antioxidants and bone matrix protein (alkaline phosphatase) increased. This prevents estrogen deficiency, increasing the number of osteoblasts while decreasing osteoclasts. The condition inhibits osteoporosis and enhances bone density.

6. PREPARATION OF ANTIOSTEOPOROSIS EXTRACTS FROM INDONESIA MEDICAL PLANTS

The extract studied as antiosteoporosis was produced from methods such as maceration, reflux, digestion, percolation, and super-critical fluid extraction. Based on the results, maceration was used in making antiosteoporosis extracts. The method is commonly used for fresh, dry, or powdered simplicia whose active ingredients cannot tolerate heating up and the solvent used is water or organic solvent. One benefit of maceration is that it requires little effort and minimal equipment. Meanwhile, the downsides revolve around the extended extraction time and huge quantities of solvent needed (Zhang et al., 2018).

During maceration, the coarse powder from a plant sample is immersed in a solvent in a closed container for a specific time interval and stirred occasionally to dissolve the sample components. Using filtration or decantation, the solvent and dissolved substance mixture are extracted and separated from the coarse powder sample. The filtrate is separated from the solvent through vacuum evaporation in an oven or water bath. This method is suitable for the extraction of thermolabile chemical compounds (Bitwell et al., 2023; Okselni et al., 2023; Rosalina et al., 2022). Factors to consider in selecting a solvent are selectivity, safety, solubility, cost, and reactivity. The principle

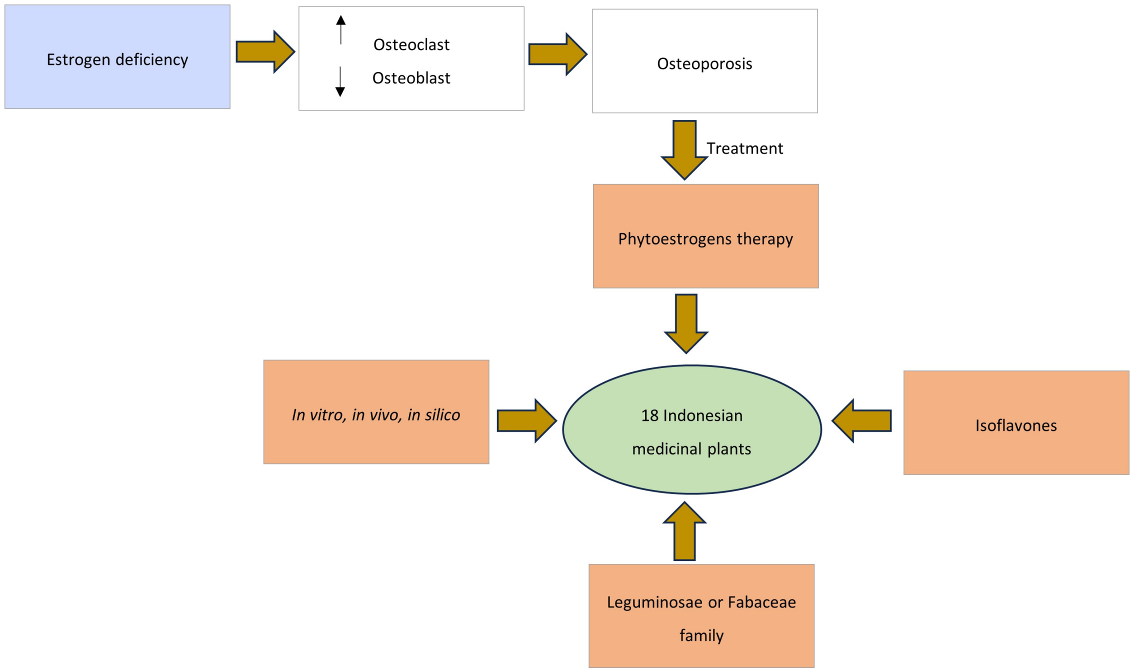

Figure 11. Graphical Abstract of Phytoestrogens Therapy for Osteoporosis Treatment Using Indonesian Medicinal Plants

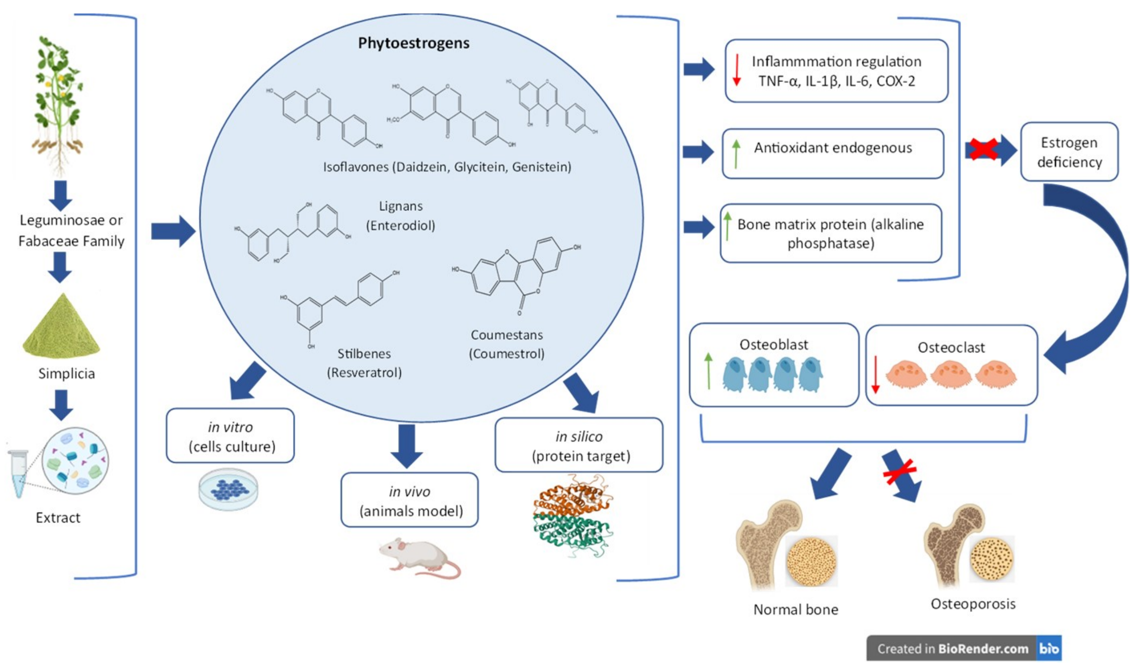

Figure 12. Proposed Mechanism of Action from Extract for Osteoporosis Treatment

of "like dissolves like" determines the solubility of the solute in a solvent that has a near polarity index (Patel et al., 2019; Zhang et al., 2018). The majority of studies frequently use 70% ethanol solvent (Figure 13). Four primary solvents were used namely ethyl acetate, methanol, water, and ethanol, with polarity index of 1,000, 0.762, 0.654, and 0.228 respectively. The polarity index of the extraction solvent decreases in line with the polarity of the compounds. The group of phytoestrogens classified as semipolar, including flavonoids, lignans, stilbenes, and coumestans can be used more easily in the solvents methanol, ethanol, 70% ethanol, 96% ethanol but slightly soluble in water solvents. Less polar steroid compounds will dissolve more easily in ethyl acetate solvent (Domínguez-López et al., 2020; Pavlopoulos et al., 2023; Torrens-Mas and Roca,

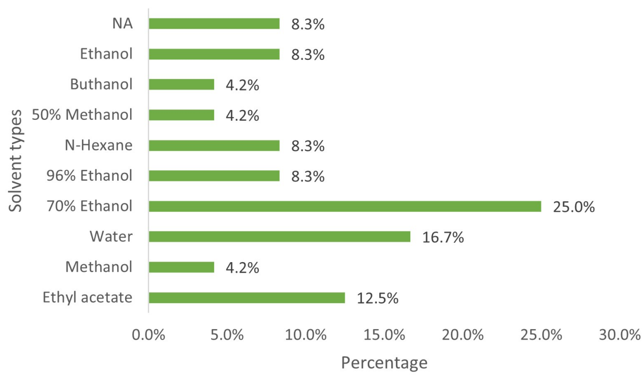

Figure 13. Percentage of Use of Extraction Solvent Types

2020).

7. CHARACTERIZATION OF EXTRACTS

Phytochemical screening is used for a preliminary examination to identify the presence of compounds, such as alkaloids, flavonoids, tannins, saponins, quinones, monoterpenes, sesquiterpenes, steroids, and terpenoids in extracts (Rahminiwati et al., 2023; Shaikh and Patil, 2020). Xie et al. (2020) worked on determining isoflavone profiles in soybean extracts using high-performance liquid chromatography (HPLC). The study used a C18 reversed-phase chromatography column, with a flow rate of 1 mL/min and the measurement at 254 nm. Daidzin, genistin, genistein, daidzein, malonyldaidzin, and malonylgenistin are the isoflavone profiles identified in soybean extract. Pharmaceutical analysis can be facilitated by the use of HPLC. A mobile phase, pump, injector, column, detector, computer/data system, and solvent supply system are among the parts. Each analyte in the sample is represented as a sharp peak at a certain time in a chromatogram, which displays the separation process (Chew et al., 2021; Saini et al., 2024). In liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), the sample is separated in an LC with a stationary phase consisting of a separating column, and a mobile phase consisting of an eluent with a specific composition. In addition, the distinct sample species are introduced into a pressurized ion source to undergo gas phase ionization. Ions are sorted by mass per charge (m/z) using a mass analyzer, and each ion's signal is amplified and counted using a detector. The MS2 acquired will be compared to the m/z value in multiple existing data sets. MS1 generates a collection of ions in the gas phase based on m/z (Calabrese, 2018; Tarhan and Jafari, 2020).

8. ASSAY OF PHARMACOLOGICAL ACTIVITY AS ANTIOSTEOPOROSIS

Aditama et al. (2016) studied the effects of a 96% ethanol extract of A. manihot on alkaline phosphatase (ALP) activity. Preosteoblasts from mouse calvaria 3T3-E1 (MC3T3-E1) were the cells used. The capacity of these cells to track bone formation by upregulating ALP synthesis led to the selection. A combination of Confocal Laser Scanning Microscopy (CLSM) and Immunocytochemistry (ICC) was used. This method is comparatively quick to perform and very sensitive in detecting ALP activity in osteoblast cells. According to the findings, 3.49% more ALP activity could be produced by using a 96% ethanol extract of A. manihot at a concentration of 25 ppm. The phytoestrogens in the extract were thought to be responsible for this increase. The test animal used in the study by Ma'arif et al. (2024) was female ovariectomized Wistar rats administered a daily dose of 0.18 mg/200 g BW rats/day of alendronate to induce osteoporosis. Rats' femur trabecular bone mass density (428.91±10.11; negative control: 232.53±15.15) and osteoblast cell number (101.15±4.12; negative control: 31.58±2.37) increased at the optimal dose of 48.6 mg/200 g BW rats/day.

The principle used in silico-molecular docking is to predict the interaction between ligand compounds and the active site of the receptor (Wahyudi et al., 2023). The protein used by Ma'arif et al. (2021) was ER-β with PDB code ID: 3OLL downloaded from the protein data bank (`https://www.rcsb.org`), while the native ligand was 17β-estradiol. Re-docking between 17β-estradiol and 3OLL protein which had been separated from native ligand was carried out first using AutoDock Vina. The root mean square deviation (RMSD) value of < 2 Åshows that AutoDock Vina is valid for molecular docking toward ER-ß. Subsequently, phytoestrogens were molecularly docked against 3OLL using AutoDock Vina, and the results were analyzed using Biovia Discovery Studio Visualizer software. The Lipinski's Rule test was continued on each phytoestrogens compound to determine the physico-chemical properties. In addition, toxicity tests were carried out using the admetSAR2 web tool. Catechin and epicatechin are non-toxic phytoestrogens that can bind 3OLL with a binding affinity of -5.6 kcal/mol and -5.9 kcal/mol respectively.

9. CONCLUSION

In conclusion, four plants from the Leguminosae or Fabaceae families dominate as antiosteoporosis agents in 18 Indonesian medicinal plants. The content of isoflavones as the main group of phytoestrogens in this family can suppress osteoclast activity, thereby increasing the amount of bone formation. Future studies should examine synergistic mechanisms such as *in vitro*, *in silico*, *in vivo*, and clinical combinations to prove that phytoestrogens, specifically isoflavones have a very strong role in the treatment of osteoporosis.

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