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Nutritional profiles of Moringa pods (*Moringa oleifera*) and their extract activities on SaOS-2 osteoblast cells

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ABSTRACT

Background: *Moringa oleifera* has long been valued in traditional Asian medicine and cuisine for its numerous health benefits. It is rich in nutrients and bioactive compounds and exhibits powerful antioxidants and anti-inflammatory properties.

Objectives: This study investigated the nutritional profile and potential health benefits of Moringa pods, focusing on their anti-inflammatory properties and ability to promote bone formation at the cellular level. Furthermore, it explores the in vitro anti-inflammatory potential of Moringa pod extract by evaluating its ability to reduce inflammatory cytokine secretion and promote bone formation in osteoblast cells.

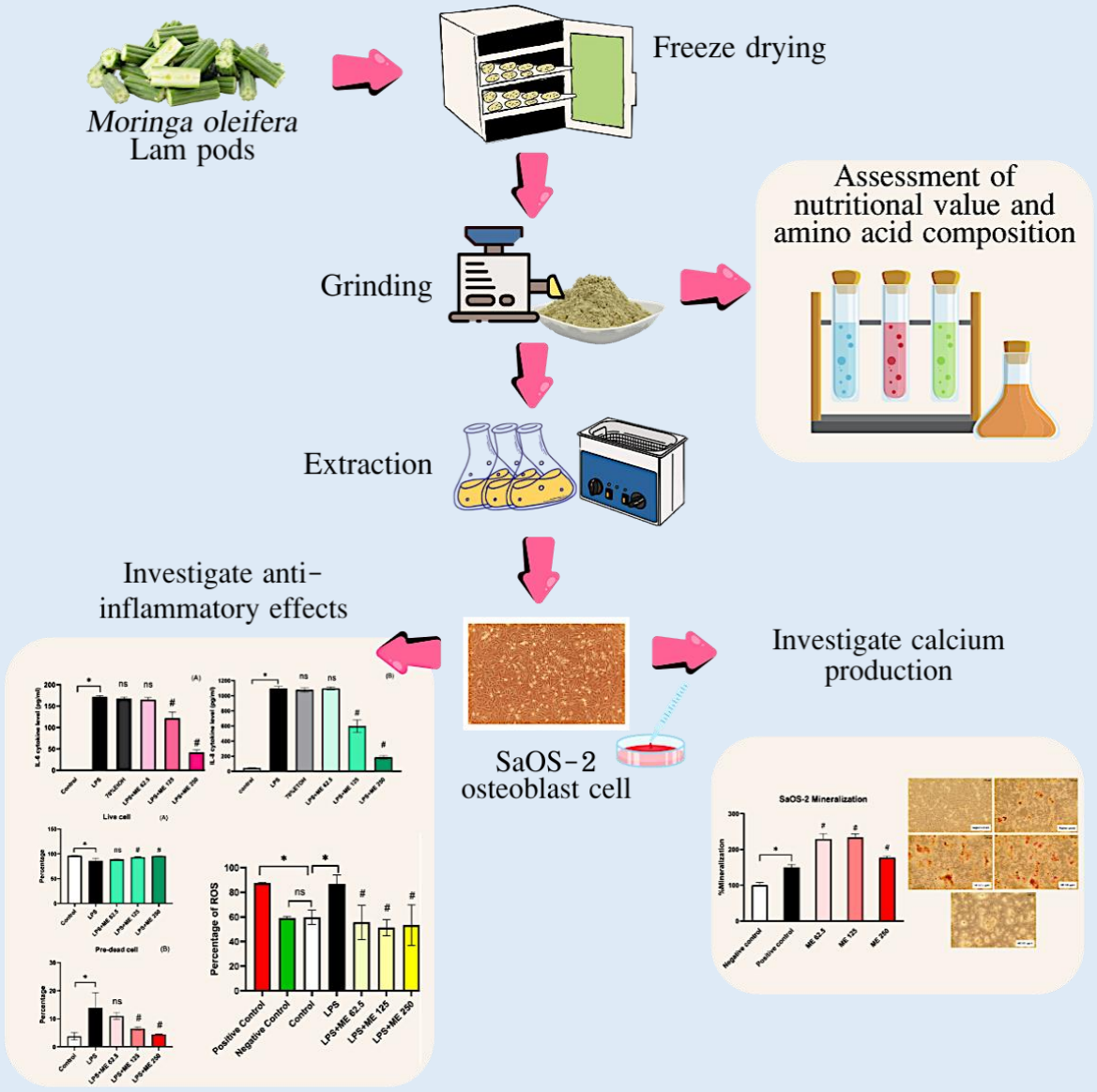
Methods: Freeze-dried Moringa pod powder was analyzed for its caloric content, macronutrients, vitamins, minerals, and amino acid composition.

Results: The pods contain 29 calories per 100 g (dry weight), with a high protein content of 19.69 g and low-fat levels (3.2 g). Notably, they are rich in dietary fiber (48.66 g) and essential minerals such as potassium (2251 mg) and magnesium (151.4 mg). The highlighted key amino acids are glycine (2167.88 mg) and L-arginine (1923.08 mg). In vitro experiments have demonstrated that Moringa pod extract at concentrations of 62.5 to 250 µg/mL significantly reduced inflammation in LPS-stimulated SaOS-2 osteoblast cells, leading to decreased levels of inflammatory cytokines (IL-6 and IL-8) and reactive oxygen species (ROS). Additionally, the extract enhanced cell viability and calcium production, suggesting its potential to promote bone health.

Novelty: This study provides a comprehensive nutritional profile of Moringa pods, including detailed amino acid and mineral content. It also uniquely demonstrates Moringa pod extract's in vitro anti-inflammatory and bone-forming potential in SaOS-2 osteoblast cells, highlighting its rich nutritional composition and bioactivity.

Conclusion: Moringa pods exhibit significant potential to modulate inflammation and promote bone formation in SaOS-2 cells. The extract effectively reduced inflammatory cytokines and reactive oxygen species, enhancing cell viability and decreasing apoptosis. It also significantly increased calcium production, a key indicator of bone health. These findings suggest Moringa pod extract is a promising therapeutic agent for maintaining bone homeostasis and preventing inflammatory bone disorders, warranting further research for clinical applications. These findings highlight the nutritional value of Moringa pods and their therapeutic potential in attenuating inflammation, inhibiting bone formation.

Keywords: Anti-inflammation, Bone formation, Calcium production, Moringa oleifera, Moringa pod, Nutritional profile Osteoblast cell and SaOS-2.



Graphical Abstract: Nutritional profiles of Moringa pods (*Moringa oleifera*) and their extract activities on SaOS-2 osteoblast cells.

INTRODUCTION

Osteoporosis is a multifactorial condition characterized by an imbalance between bone formation and bone resorption, leading to a loss of bone mass and increased fracture risk. Normal bone remodeling involves the continuous process of resorption, where old bone is removed, and new bone is generated to maintain bone strength. However, osteoporosis enhances bone resorption and reduces bone formation [1]. Contributing factors include hormonal changes, particularly the decline in estrogen levels often experienced by postmenopausal women, which increases osteoclast activity and bone resorption. Aging is also a factor that slows bone turnover and skews the balance toward resorption. Nutritional deficiencies, such as inadequate calcium and vitamin D intake, impair bone formation, while lifestyle factors, including sedentary behavior, smoking, excessive alcohol consumption, and certain medications, further accelerate bone loss. These factors collectively disrupt bone homeostasis, weakening bone structure and increasing the risk of fractures [2-4].

Inflammation plays a critical role in many chronic diseases, including osteoporosis. The interplay between osteoblasts, responsible for bone formation, and osteoclasts, which break down bone tissue, is essential for maintaining bone health. Elevated levels of inflammatory cytokines can disrupt this balance, leading to increased bone resorption and decreased formation [5]. Therefore, reducing inflammation will likely decrease bone resorption and promote bone formation.

Moringa oleifera, commonly known as Moringa, has gained significant attention for its potential health benefits [6-8]. Moringa contains essential nutrients, including vitamins, minerals, proteins, and bioactive compounds. With a history of use in traditional medicine across various cultures, Moringa is renowned for its antioxidant, anti-inflammatory, and nutrient-dense properties [9-11].

Although the effects of Moringa leaf extract on bone health have been studied extensively, Moringa pods, another commonly used food ingredient in Asia, have not been researched with the same level of attention. Therefore, this study aims to investigate the nutritional profile of Moringa pods, focusing on their amino acid composition, mineral content, and other essential nutrients. Additionally, this study explores the anti-inflammatory effects of Moringa pod extract in vitro, evaluating its potential to reduce inflammatory cytokine secretion and promote bone formation in osteoblast cells.

MATERIALS AND METHODS

Materials and Chemicals: Fully mature Moringa (*Moringa oleifera* Lam.) pods were purchased from a local market in Ayutthaya, Thailand. Various chemicals, including dexamethasone, Lipopolysaccharide (*E. coli* 011B4), formaldehyde solution, cetylpyridinium chloride, and Alizarin Red S, a calcium-binding dye, were sourced from Sigma-Aldrich (St. Louis, MO, USA). CellTiter 96® Aqueous One Solution reagent (MTS) was sourced from Promega Corporation (Madison, WI, USA).

The Human SaOS-2 cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cell culture components, such as Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS), were purchased from GIBCO (Grand Island, NY, USA). An ELISA kit, Fxcycle™ PI/RNase Staining Solution, CellROX® flow cytometry kits (ROS), and Alexa Fluor 488 annexin V/Dead cell apoptosis kit were purchased from Thermo Fisher Scientific (Waltham, MA, USA). All chemicals and reagents used were of analytical or biological grade.

Sample Preparation: Following washing and chopping, Moringa pods underwent freezing. Frozen pods were processed via freeze-drying and then pulverized into a fine powder. Before analysis, all prepared samples were kept in a controlled environment with regulated humidity

at ambient temperature. The freeze-dried Moringa pod powder was treated with 70% ethanol (1:10 w/v) and subjected to ultrasonic-assisted extraction at room temperature (25 ± 2°C) for 30 minutes using an ultrasonic cleaner operating at a frequency of 45 kHz. The resulting mixture was centrifuged at 5000 rpm for 10 minutes, and the supernatant was filtered and stored at −20°C for further analysis.

Nutrition composition and amino acid profiles: The nutritional composition of Moringa was conducted using in-house methods certified under ISO/IEC 17025 by SGS (Thailand) Limited (Bangkok, Thailand), a standard laboratory service in Thailand. Calorie and carbohydrate content were analyzed following the analysis method for nutrition labeling (1993): 106. Other nutrients, including protein, fat, cholesterol, dietary fiber, vitamins, and minerals, were analyzed using in-house methods based on the official AOAC method (2019).

Amino acid analysis was performed by using the LC-MS/MS technique. For total amino acid analysis, 0.1 g of powder was weighed and mixed with 10 mL of 6 M hydrochloric acid (HCl). The mixture was digested in a heating bath at 110°C for 24 hours. After digestion, the sample volume was adjusted to 10 mL with DI water, and a portion of the solution was diluted with 0.1 M ammonium formate and then filtered through a 0.2 µm syringe filter. The digestion step used 4.2 M sodium hydroxide (NaOH) instead of HCl for tryptophan analysis. The final prepared samples were placed in vials for LC-MS/MS analysis.

The LC-MS/MS analysis was conducted using an LCMS-8060 system (Shimadzu, Japan), equipped with an Intrada amino acid column (50 x 3 mm, 3 µm). A 1 µL sample was injected into the system, and a gradient elution program was applied with two mobile phase solvents: Solvent A (acetonitrile with 0.1% Formic Acid) and Solvent B (0.1 M ammonium formate). The gradient profiles were 0-3 minutes (14% B), 3-10 minutes (100% B), and 10-15 minutes (14% B) at a flow rate of 0.6 mL/min. The amino acid concentrations were calculated by comparing the chromatogram of the sample with standard curves for each compound.

Cell Culture Study: SaOS-2 osteoblast cells were grown in Dulbecco's Modified Eagle Medium (DMEM) that included 10% fetal bovine serum (FBS). [1] Culturing occurred at 37°C under a 5% CO_2 environment. Once the cells achieved 80% confluence, they were utilized. Cell viability was evaluated via the MTT assay, employing a density of 1×10^4 cells per well in 96-well plates. Treatment involved exposing the cells to Moringa pod extract at concentrations spanning 31.25 to 1000 µg/mL. Following a 24-hour treatment period, 20 µL of MTS reagent was introduced, and absorbance was quantified at 490 nm using a microplate reader (Omega, BMG Labtech, Ortenberg, Germany) [12].

Anti-inflammatory Assessment: For this evaluation, cells were seeded into 24-well plates at a density of 1×10^5 cells per well. After 24 hours, the growth medium was replaced with serum-free medium. Lipopolysaccharide (LPS) was added to each well at a final concentration of 100 ng/mL, along with Moringa pod extract at 62.5 to 250 µg/mL concentrations. The cells were then incubated for 24 hours. Following incubation, the cell culture medium was harvested and centrifuged at 3000 rpm for 10 minutes to isolate the supernatant. The levels of anti-inflammatory cytokines (IL-6 and IL-8) in these supernatants were measured using an enzyme-linked immunosorbent assay (ELISA) kit sourced from Invitrogen (Frederick, MD, USA). Cytokine concentrations were determined based on the generated standard curve [13].

Cell Cycle Assessment, ROS Detection, and Apoptosis Analysis: Cells were prepared at a concentration of 5×10^5 cells per well in a 6-well plate and incubated with the extract for 24 hours. After incubation, the cells were washed with PBS and treated with 0.5% Trypsin-EDTA. After removing Trypsin-EDTA, the cells were collected,

fixed with 70% ethanol, and stored at −20°C. Before testing, the cells were washed with PBS and centrifuged at 3000 rpm to extract the PBS.

For cell cycle assessment, FxCycle PI/RNase (500 µL) was added to the cell pellet and incubated in the dark for 15 minutes. The results were analyzed using an Attune NxT Flow Cytometer (Thermo Fisher Scientific, Waltham, MA, USA).

For ROS detection, after the initial steps, CellROX Reagent was added to achieve a final concentration of 500-1000 nM, and the cells were incubated at 37°C for 30-60 minutes in the dark. Dead cells were stained with 5 µM SYTOX Red Dead Staining Solution, and results were analyzed using the Attune NxT Flow Cytometer.

A 1X annexin-binding buffer was prepared for apoptosis analysis by diluting a 5X stock with DI water. A working 100 µg/mL propidium iodide (PI) solution was diluted 5 µL of a 1 mg/mL PI solution with 45 µL of the 1X annexin-binding buffer and stored on ice. After fixation, 100 µL of annexin-binding buffer was added to the cells, followed by 5 µL of Alexa Fluor 488 Annexin V and 1 µL of the PI working solution. This mixture was incubated in the dark for 15 minutes, after which 400 µL of annexin-binding buffer was added, mixed well, and kept on ice until analysis. Finally, all results were analyzed using the Attune NxT Flow Cytometer. Live cells were identified by Annexin V-FITC−/PI/PI-staining. Early apoptotic cells were defined as Annexin V-FITC+/PI−, while late apoptotic cells were classified as Annexin V-FITC+/PI+. In this study, the term "pre-dead cell" population refers to the combined percentage of early and late apoptotic cells. This group represents cells undergoing apoptosis before progressing to complete cell death. Necrotic cells were identified by the percentage of Annexin V-FITC−/PI+ cells. [12].

Percentage of Mineralization: The culture medium was replaced to induce cell differentiation. SaOS-2 cells were plated at a density of 5×10^4 cells per well in a 6-well plate and incubated with DMEM supplemented with 10 nM dexamethasone and 1% antibiotics for 14 days. The culture medium was changed every 2-3 days, and changes in cell morphology were observed under a microscope. The cells were rinsed with deionized water and stained with 1% Alizarin Red S in 2% ethanol at pH 4.0 for 30 minutes. After the cells were washed twice, the staining characteristics were observed and photographed under a microscope. Subsequently, the cells were treated with 100 mM cetylpyridinium chloride for 1 hour. A 200 µL aliquot of the solution was transferred to a 96-well plate for absorbance measurement at a wavelength of 570 nm [13].

Statistical analysis: This study used a completely randomized design (CRD), with each treatment replicated three times. Treatment comparisons were conducted across various parameters, including cell viability, % mineralization, cytokine expression, cell cycle analysis, % ROS, and apoptosis, using one-way ANOVA followed by Tukey's multiple comparisons test, with a significance level set at $p < 0.05$ for a 95% confidence interval. All statistical analyses were performed using GraphPad Prism software (version 10.2.2, California, USA).

RESULTS AND DISCUSSION

Nutrition composition and amino acid profiles: As shown in Table 1, Moringa pods are a nutritious food source, providing 29 calories per 100 g powder, with a significant contribution from protein at 19.69 g and a low-fat content of 3.2 g, of which 0.66 g are saturated fats with no cholesterol. They are predominantly composed of carbohydrates (65.9 g), including total sugars such as fructose (2.79 g), glucose (3.72 g), and sucrose (2.87 g), with an impressive 48.66 g of dietary fiber. Mineral content is notable, featuring potassium (2251 mg), sodium (274 mg), calcium (101 mg), iron (3.49 mg), and magnesium (151.4 mg). In addition, the pods are rich in vitamins, providing 338.12 µg of beta-carotene (vitamin A), along with vitamin B1 (0.23 mg) and vitamin B2 (0.63 mg) per 100 g.

Table 1. Nutritional composition of freeze-dried Moringa pod powder

Nutrition	Moringa Pod
Calories (kcal/100g)	29
Calories from fat (kcal/100g)	371
Protein (g/100g)	19.69
Fat (g/100g)	3.2
Saturated fat (g/100g)	0.66
Cholesterol (mg/100g)	ND
Carbohydrate (g/100g)	65.9
Total sugar (g/100g)	9.38
Fructose (g/100g)	2.79
Glucose (g/100g)	3.72
Sucrose (g/100g)	2.87
Maltose (g/100g)	ND
Lactose (g/100g)	ND
Dietary fiber (g/100g)	48.66
K (mg/100g)	2251
Na (mg/100g)	274
Ca (mg/100g)	101
Fe (mg/100g)	3.49
Mg (mg/100g)	151.4
V.A (b-carotene (µg/100g)	338.12
V.B1 (mg/100g)	0.23
V.B2 (mg/100g)	0.63

The amino acid profile of Moringa pod powder is shown in Table 2, with glycine being the most abundant at 2167.88 mg, followed by L-arginine (1923.08 mg), L-aspartic acid (969.23 mg), L-serine (586.15 mg), and L-glutamic acid (582.88 mg). Other notable amino acids include L-threonine (457.69 mg), L-lysine (423.08 mg), L-leucine (331.15 mg), L-tryptophan (240.86 mg), taurine (345.38 mg), and L-alanine (319.62 mg). Additionally, smaller amounts of L-valine (250.58 mg), L-proline (205.38 mg), L-histidine (204.81 mg), and L-phenylalanine (196.54 mg) were detected. However, L-asparagine, L-glutamine, and beta-alanine were not detected.

Table 2. Amino acid profile of freeze-dried Moringa pod powder

Amino acid profile	Total Amino acid (mg/100g)
Glycine	2167.88
L-Arginine	1923.08
L-Aspartic acid	969.23
L-Serine	586.15
L-Glutamic acid	582.88
L-Threonine	457.69
L-Lysine	423.08
Taurine	345.38
L-Leucine	331.15
L-Alanine	319.62
L-Valine	250.58

Amino acid profile	Total Amino acid (mg/100g)
L-Tryptophan	240.86
L-Proline	205.38
L-Histidine	204.81
L-Phenylalanine	196.54
4-Aminobutyric acid	158.85
L-Isoleucine	158.08
L-Tyrosine	155.58
L-Hydroxyproline	74.81
L-Cystine	40.19
Sarcosine	11.15
L-Ornithine	15.19
L-Citrulline	6.35
L-Asparagine	ND
L-Glutamine	ND
L-Methionine	ND
Beta-Alanine	ND

Effect of Moringa pod extracts on SaOS-2 cell line: This study assessed the optimal concentrations and anti-inflammatory effects of Moringa pod extract on bone cells, along with its potential to promote bone formation at the cellular level. A model was utilized to induce inflammation in the cells while co-treating with the extract to evaluate its inhibitory effects on inflammatory cytokine secretion. The experiment began with toxicity assessments of Moringa extract at various concentrations. The results indicated that concentrations ranging from 31.5 to 250 µg/mL did not induce toxicity in bone cells stimulated with LPS, as shown in Figure 1. However, the survival rate of the cells dropped below 80% at concentrations of 500 and 1000 µg/mL, indicating cytotoxic effects. Consequently, the suitable concentration range for further experimental studies was 62.5 to 250 µg/mL.

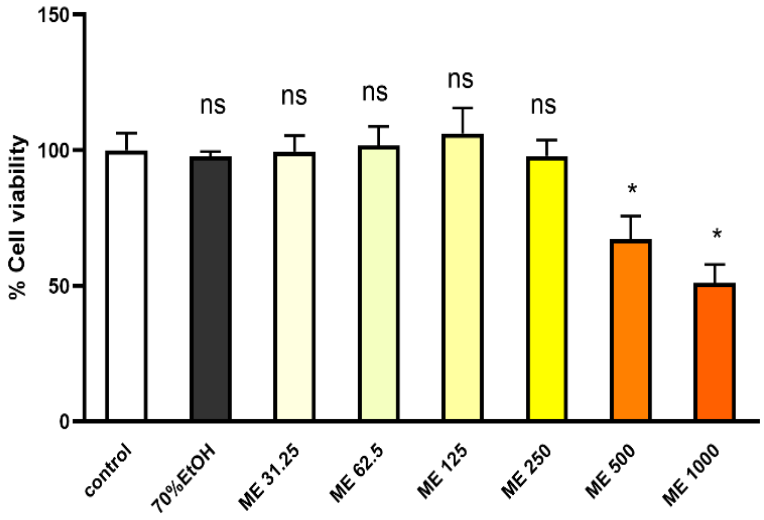


Figure 1. Cell viability of SaOS-2 osteoblasts stimulated with Moringa pod extract for 24 hours. Values are expressed as mean ± SD (n=3). 'ns' above each bar indicates no significant difference, while '*' indicates a statistically significant difference ($p < 0.05$) compared to the control.

An anti-inflammatory assay is a critical method for evaluating the properties of extracts or active compounds that affect bone system function. When bone cells experience heightened inflammation, they release inflammatory cytokines that can diminish bone mass formation or prevent it entirely, while enhancing bone-resorbing cells' activity (osteoclasts). This imbalance between osteoblasts and osteoclasts can lead to osteoporosis or low bone mass in the future. Therefore, reducing inflammation in bone cells is attenuated for bone homeostasis. For the experiments in this study, Moringa extract at concentrations of 62.5, 125, and 250 µg/mL significantly reduced inflammation in osteoblast cells stimulated with LPS by decreasing the inflammatory cytokines IL-6 and IL-8, as shown in Figure 2.

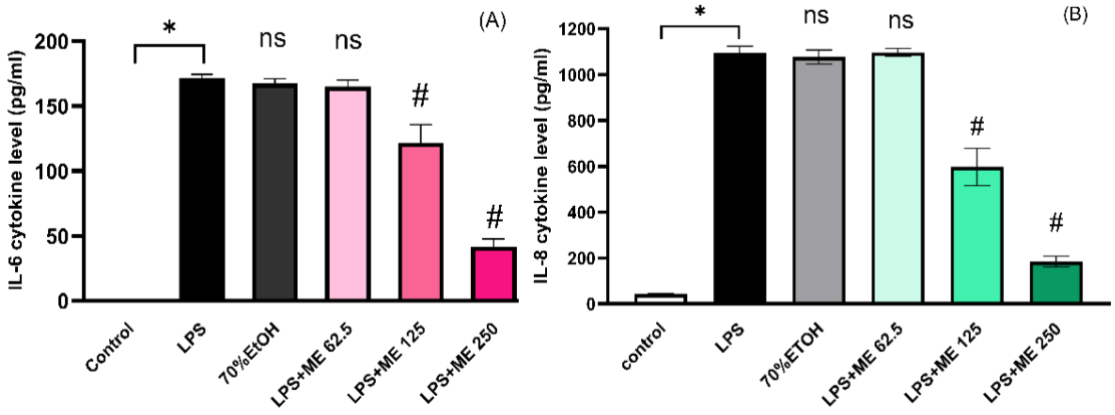


Figure 2. The anti-inflammatory cytokine levels (A: IL-6, B: IL-8) of LPS (100ng/ml) stimulated SaOS-2 osteoblasts and were co-incubated with Moringa pod extract for 24 hours. Values are expressed as mean ± SD (n=3). '*' above a horizontal line indicates significantly ($p < 0.05$) different compared to the control; 'ns' above each bar indicates no significant difference, while '#' indicates a statistically significant difference ($p < 0.05$) compared to the control.

The assessment of the effects of Moringa extract on cell cycle changes indicated that, at concentrations of 62.5-250 µg/mL, there were no alterations in the cell cycle phases after 24 hours (Figure 3). Specifically, the extract did not affect the G0/G1 phase, where cells are either quiescent or preparing for DNA synthesis and the necessary components for division. It did not impact the S phase, which is characterized by DNA replication and the synthesis of materials required for division. Additionally, there were no significant effects on the G2/M phase, during which cells verified DNA duplication and ensured that replicated chromosomes were evenly distributed to the two daughter cells during division.

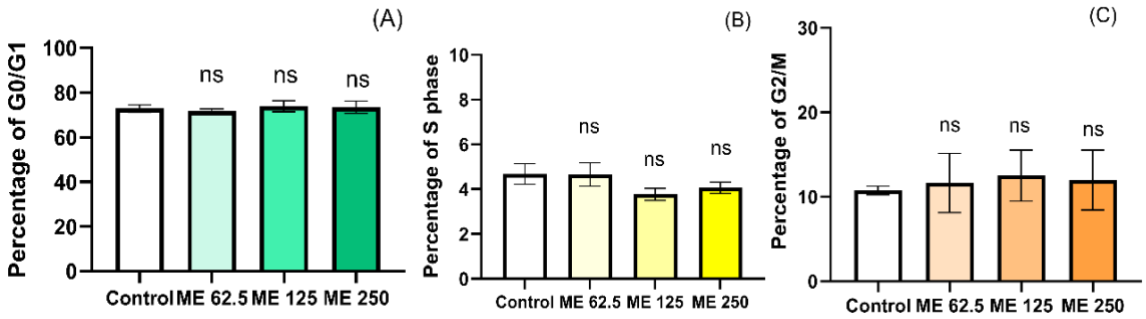


Figure 3. Cell cycle of SaOS-2 incubated with Moringa pod extracts (A: G0/G1 phase, B: S phase, C: G2 phase). Values are expressed as mean ± SD (n=3). 'ns' above each bar indicates no significant difference, while '*' indicates a statistically significant difference ($p < 0.05$) compared to the control.

Testing of reactive oxygen species (ROS) production in osteoblast cells stimulated with 100 ng/mL LPS and treated with Moringa pod extract at concentrations from 62.5 to 250 µg/mL for 24 hours revealed that cells exposed to LPS alone exhibited ROS levels exceeding 80%, similar to the positive control using 200 µM tert-butyl hydroperoxide (TBHP). In contrast, cells stimulated with LPS and treated with Moringa extract at concentrations from 62.5 to 250 µg/mL showed no significant difference in ROS level compared to the control group, at a 95% confidence, as shown in Figure 4.

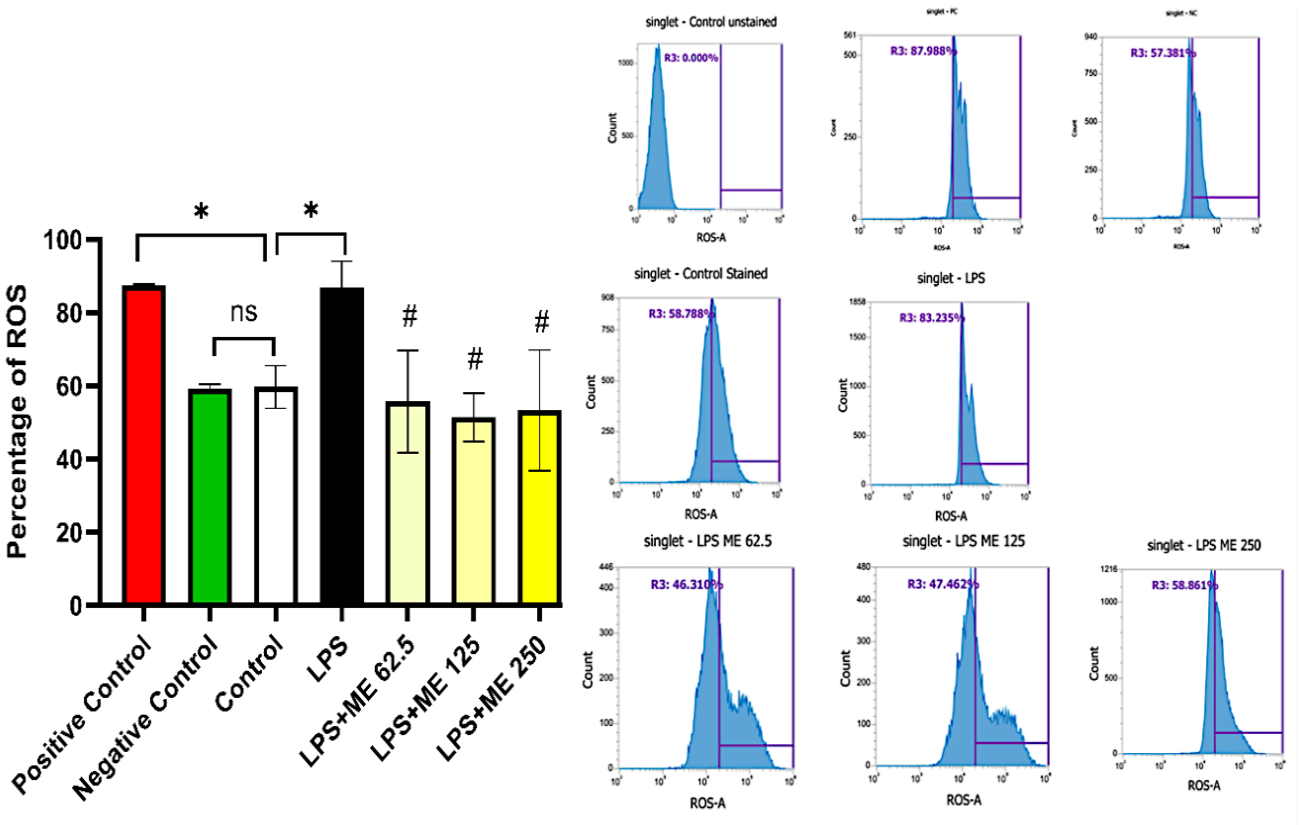


Figure 4. ROS of SaOS-2 stimulated with 100 ng/ml of LPS and Moringa extracts. Values are expressed as mean ± SD (n=3). 'ns' above each bar indicates no significant difference, while '*' indicates a statistically significant difference ($p < 0.05$) compared to the control; '#' above each bar indicates significantly ($p < 0.05$) different compared to LPS.

Apoptosis detection in osteoblast cells stimulated with 100 ng/mL LPS and treated with Moringa extract at concentrations from 62.5 to 250 µg/mL for 24 hours showed significant differences in cell viability (Figure 5). Cells exposed solely to LPS showed a marked reduction in live cells and an increase in dying cells compared to the unstimulated control group. However, in cells treated with Moringa extract at concentrations from 125 to 250 µg/mL alongside LPS, there was a significant increase in live cells and a decrease in dying cells compared to the LPS-only group. These findings indicate that Moringa extract at concentrations from 125 to 250 µg/mL can effectively reduce cell death and enhance the viability of osteoblasts subjected to LPS stimulation.

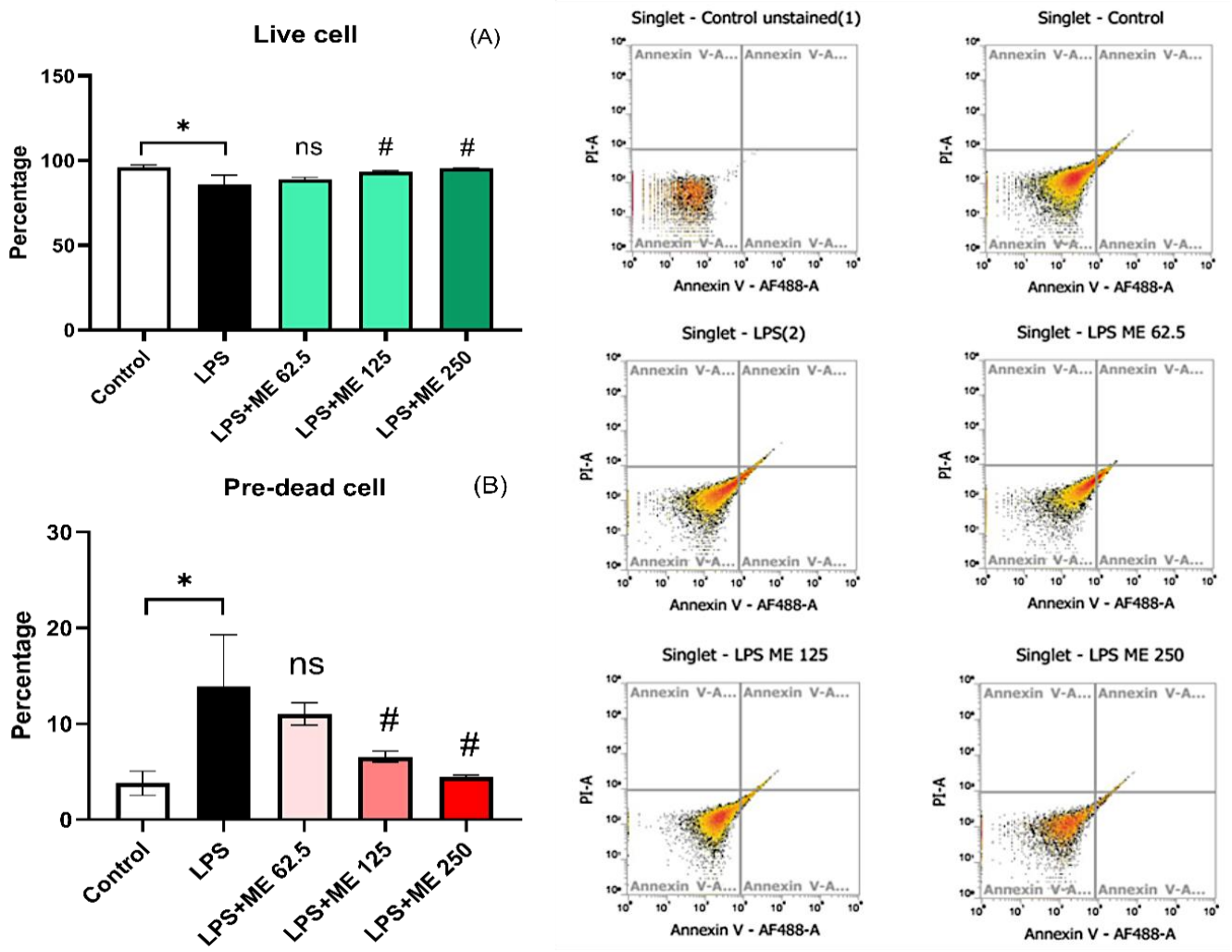


Figure 5. Apoptosis of SaOS-2 stimulated with 100 ng/ml LPS and Moringa extracts. Values are expressed as mean ± SD (n=3). 'ns' above each bar indicates no significant difference, while '#' indicates a statistically significant difference ($p < 0.05$) compared to the control.

The effectiveness of Moringa pod extract in promoting bone formation was evaluated by measuring calcium production, since calcium is a key component of bone mass. Enhanced calcium production in cells indicates a potential increase in bone mass formation. In previous research, dexamethasone has been shown to promote calcium deposition in SaOS-2 cells, as indicated by visualization of calcium staining after 7, 14, and 21 days of treatment [14]. In our preliminary study, we tested calcium deposition at 7, 14, and 21 days, and found that 14 days provided clearer calcium accumulation compared to 7 days, with no significant difference observed between 14 and 21 days. Therefore, we selected 14 days as the optimal treatment duration. The results showed that cells treated with Dexamethasone 10 nM (positive control) produced significantly more calcium than the control group, as shown in Figure 6. Furthermore, when testing the effects of Moringa extract on calcium production in bone cells, concentrations of 62.5 and 125 µg/mL significantly increased calcium levels compared to the positive control. At the same time, the 250 µg/mL concentration exhibited cytotoxic effects. Therefore, the optimal concentrations of Moringa extract for stimulating calcium production in bone cells are 62.5 and 125 µg/mL.

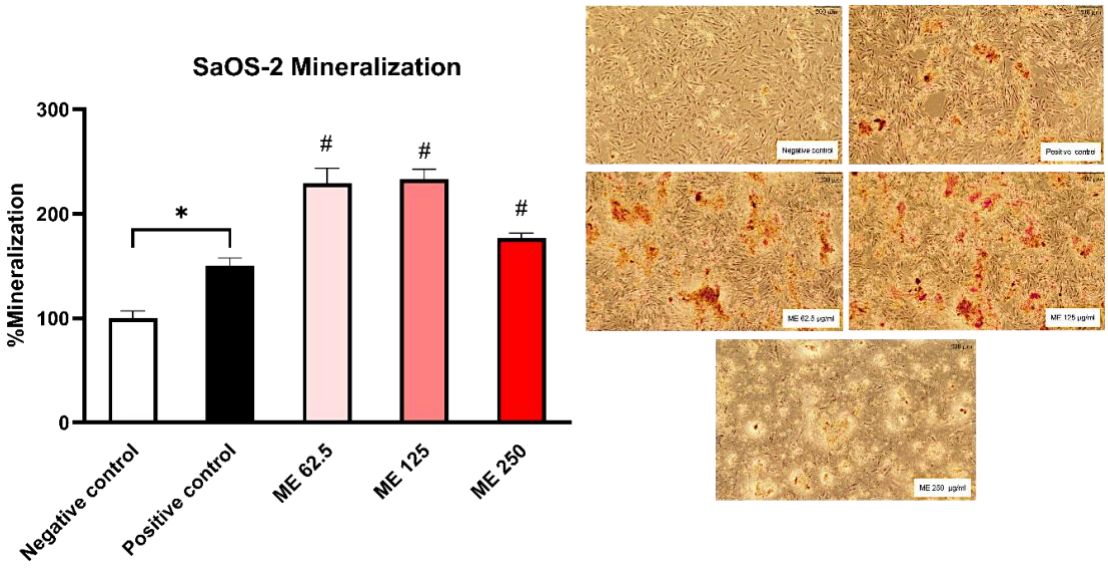


Figure 6. %Mineralization of SaOS-2 incubated with Moringa pod extract for 14 days. Values are expressed as mean ± SD (n=3). '*' above a horizontal line indicates significantly ($p < 0.05$) different compared to the negative control; '#' above each bar indicates significantly ($p < 0.05$) different compared to the positive control.

As demonstrated in this study, Moringa pods possess a rich nutritional and bioactive profile that contributes to anti-inflammatory and bone-forming effects in osteoblast cells. Their composition includes high levels of dietary fiber, proteins, essential vitamins (including beta-carotene, vitamin A, and vitamin B complex), and minerals like potassium, calcium, and magnesium, positioning Moringa pods as a functional component in the food industry [15-16].

Among the amino acid profiles of Moringa pods, the high concentration of glycine (2167.88 mg) and L-arginine (1923.08 mg) is particularly noteworthy. Glycine is crucial in synthesizing bone matrix proteins and supports osteoblast differentiation, a key bone formation process [17]. Additionally, glycine has anti-inflammatory properties, helps mitigate oxidative stress, and modulates inflammatory pathways critical for bone metabolism [18]. On the other hand, L-arginine is a precursor for synthesizing nitric oxide (NO), which has complex effects on bone health. Nitric oxide can promote osteoblast differentiation, support bone remodeling, and play an anti-inflammatory role by reducing excessive immune cell activation and regulating the activity of osteoclasts (bone-resorbing cells). Thus, arginine could help maintain bone homeostasis by balancing bone resorption and formation [19]. Other amino acids, such as L-aspartic acid, L-glutamic acid, and L-serine, also synthesize collagen and bone matrix components. Additionally, amino acids like L-threonine, L-leucine, and L-lysine contribute to osteoblast function and bone mineralization, which are critical to maintaining bone integrity [20]. However, the hypothesis regarding the effects of amino acids in Moringa pods on bone health requires further research to be substantiated.

In addition to its nutrient and amino acid content, it has been reported that Moringa pod extract also contains other bioactive compounds, such as phenolic acids and flavonoids, which possess antioxidant properties [21]. Previous studies have shown that freeze-dried Moringa pod extract contains genistein (39.70 mg/100 g), trans-ferulic acid (19.50 mg/ 100g), p-coumaric acid (9.41 mg/ 100g), myricetin (8.57 mg/ 100g), gallic acid (7.16 mg/ 100g), and syringic acid (4.89 mg/ 100g). These compounds have demonstrated significant antioxidant and anti-inflammatory activities in Raw 264.7 macrophage cells [9].

Chronic inflammation leads to an imbalance in bone remodeling, where excessive osteoclast activity and reduced osteoblast function contribute to bone loss [5]. In addition, a correlation has been reported between inflammation and reactive oxygen species (ROS), which are generated as proinflammatory mediators during inflammatory processes. This factor can influence the activity of bone cells, thereby affecting bone homeostasis by promoting bone resorption and inhibiting bone formation [22]. Therefore, reducing inflammation will likely help decrease bone resorption and promote bone formation.

Our findings revealed that Moringa pod extract significantly reduced the secretion of IL-6 and IL-8, proinflammatory cytokines closely associated with osteoclast activation and impaired osteoblast function. Elevated levels of these cytokines are commonly observed in bone-related inflammatory conditions. Similarly, studies on other herbs have reported that they can reduce inflammation, helping to alleviate the inflammatory response that may contribute to bone mass loss [23-24]. These findings highlight the importance of managing inflammation to maintain bone homeostasis and prevent osteoporosis. Additionally, oxidative stress is a key contributor to bone loss, as ROS overproduction can damage osteoblasts and enhance osteoclastogenesis. The Moringa extract notably reduced ROS production in LPS-stimulated SaOS-2 cells, confirming its antioxidative activity. Another critical component examined in this study was the effect of Moringa extract on apoptosis and the cell cycle. Moringa extract preserved cell viability and reduced apoptosis in inflammatory conditions, suggesting its protective effect on osteoblasts. Cell cycle analysis further revealed that the extract did not disrupt the normal progression of osteoblast proliferation, reinforcing its biosafety for therapeutic applications.

For the evaluation of calcium production, a critical factor in bone mass formation, it was indicated that Moringa extracts of 62.5 and 125 µg/mL significantly enhanced calcium levels compared to controls. In contrast, the 250 µg/mL concentration resulted in cytotoxicity. Previous studies have reported that *Moringa oleifera* pods exhibited a dose-dependent effect, showing a 6-fold increase in alkaline phosphatase (ALP) activity at 200 µg/mL. However, no further increase was observed at 400 µg/mL, stimulated bone formation, enhanced hydroxyproline content, and promoted bone mineral formation in the SaOS-2 osteoblast cell line. [25]. Additionally, it has been reported that supplementation with *Moringa oleifera* leaves, seeds, and their combinations significantly increased calcium and phosphorus serum levels in osteoporotic rats. Moreover, the femur bone mineral density (BMD) was also considerably enhanced [26]. Similarly, studies on Moringa seed extract in broiler chickens showed that administering the extract through drinking water improved bone health by increasing bone density and strength, enhancing calcium metabolism, and reducing the risk of osteoporosis. The extract promoted healthier bone development, highlighting its potential to improve bone parameters in poultry production [27]. In summary, the results of this study demonstrated that the potential optimal Moringa pod extract concentrations for promoting bone health are 62.5 and 125 µg/mL for both anti-inflammatory and bone formation-stimulating effects. However, the definite mechanisms of Moringa pod extract on bone health need further investigation.

Scientific Innovation and Practical Implications:

Scientific Innovation: This research combines a detailed nutritional analysis of Moringa pods with an in vitro investigation of their bioactivity on osteoblast cells. The comprehensive characterization of the pods' macronutrient, vitamin, mineral, and amino acid composition, particularly highlighting the high protein and fiber content alongside key amino acids like glycine and L-arginine, provides a novel understanding of their nutritional richness. Furthermore, the study's demonstration of the Moringa pod extract's ability to significantly reduce inflammatory markers (IL-6, IL-8,

ROS) in LPS-stimulated osteoblasts while simultaneously enhancing cell viability and calcium production offers a novel insight into its potential as a dual-action agent for bone health. This integrated approach provides a holistic view of Moringa pods' value beyond basic nutrition.

Practical Implications: The findings suggest that Moringa pods represent a valuable nutritional resource and that their extracts hold promise as a natural agent for mitigating inflammation and promoting bone health. This supports the potential development of Moringa pod-based dietary supplements or functional food ingredients aimed at bone health and inflammatory conditions."

CONCLUSIONS

In conclusion, this study demonstrates that Moringa pods hold significant potential for modulating anti-inflammatory responses and bone formation in SaOS-2 cells with their rich nutritional and bioactive profile. The Moringa pod extract showed marked anti-inflammatory effects and promoted bone formation in the osteoblast SaOS-2 cell line, particularly at optimal concentrations of 62.5 and 125 µg/mL. The extract effectively reduces the secretion of inflammatory cytokines, such as IL-6 and IL-8. It decreases reactive oxygen species levels in LPS-stimulated bone cells, contributing to enhanced cell viability and reduced apoptosis. Furthermore, Moringa extract significantly increases calcium production, an essential indicator of bone health. These findings suggest that Moringa pod extract has the potential to serve as a therapeutic agent in maintaining bone homeostasis and preventing inflammatory-related bone disorders, making it a promising candidate for future research and clinical applications in bone health management.

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REFERENCES

1. Gao Y, Patil S, Jia J. The development of molecular biology of osteoporosis. International journal of molecular sciences. 2021;22(15):8182. DOI: https://doi.org/10.3390/ijms22158182.
2. Liang B, Burley G, Lin S, Shi Y-C. Osteoporosis pathogenesis and treatment: existing and emerging avenues. *Cellular and Molecular Biology Letters*. 2022;27(1):72. DOI: https://doi.org/10.1186/s11658-022-00371-3.
3. Bolamperti S, Villa I, Rubinacci A. Bone remodeling: an operational process ensuring survival and bone mechanical competence. *Bone Research.* 2022;10(1):48. DOI: https://doi.org/10.1038/s41413-022-00219-8.
4. Rowe P, Koller A, Sharma S. Physiology, bone remodeling, 2018.
5. Iantomasi T, Romagnoli C, Palmini G, Donati S, Falsetti I, Miglietta F, et al. Oxidative stress and inflammation in osteoporosis: molecular mechanisms involved and the relationship with microRNAs. *International journal of molecular sciences*. 2023;24(4):3772. DOI: https://doi.org/10.3390/ijms24043772.
6. Abe, A., Kapoor, M. P., Morishima, S., Timm, D., Nakajima, A., Ozeki, M., and Sato, N. Alleviating the physical discomfort in healthy individuals with Moringa seed extract: a randomized, double-blind, placebo-controlled, parallel-group trial. *Functional Foods in Health and Disease*, 2023. 13(10), 459-475. DOI: https://doi.org/10.31989/ffhd.v13i10.1150
7. Calabrese EJ, Dhawan G, Kapoor R, Agathokleous E, Calabrese V. Moringa induces its beneficial effect via hormesis. *Nutrition Research Reviews*. 2023:1-37. DOI: https://doi.org/10.1017/S0954422423000161.
8. Tadevosyan, A., Hakobjanyan, A., Tovmasyan, A., Asatryan, A., Roosta, H. R., and Daryadar, M. Hypoglycemic and hypolipidemic activity of moringa grown in hydroponics and soil in Ararat Valley. *Functional Foods in Health and Disease*, 2023, *13*(8), 398-408. DOI: https://doi.org/10.31989/ffhd.v13i8.1121
9. Hunthayung K, Bhawamai S. Polyphenol compounds of freeze-dried Moringa oleifera Lam pods and their anti-inflammatory effects on RAW 264.7 macrophages stimulated with lipopolysaccharide. Bioactive Compounds in Health and Disease-Online ISSN: 2574-0334; Print ISSN: 2769-2426. 2024;7(4):185-98. DOI: https://doi.org/10.31989/bchd.v7i4.1356.

10. Lin M, Zhang J, Chen X. Bioactive flavonoids in Moringa oleifera and their health-promoting properties. *Journal of functional foods. 2018; 47:469-79.* DOI: https://doi.org/10.1016/j.jff.2018.06.011.
11. Tranquilino-Rodríguez, E., and Martínez-Flores, H. E. (2023). Ultrasound-assisted extraction of phenolic compounds from Moringa oleifera leaves by response surface methodology. *Bioactive Compounds in Health and Disease-Online*, 2023; *6*(11), 325-337. DOI: https://doi.org/10.31989/bchd.v6i11.1270
12. Ma B, Guan G, Lv Q, Yang L. Curcumin ameliorates palmitic acid-induced Saos-2 cell apoptosis via inhibiting oxidative stress and autophagy. *Evidence-Based Complementary and Alternative Medicine*. 2021;2021(1):5563660. DOI: https://doi.org/10.1155/2021/5563660.
13. Zakłos-Szyda M, Nowak A, Pietrzyk N, Podsędek A. Viburnum opulus L. juice phenolic compounds influence osteogenic differentiation in human osteosarcoma Saos-2 cells. *International Journal of Molecular Sciences*. 2020;21(14):4909. DOI: https://doi.org/10.3390/ijms21144909.
14. Vieira S, Vial S, Maia FR, Carvalho M, Reis RL, Granja PL, et al. Gellan gum-coated gold nanorods: an intracellular nanosystem for bone tissue engineering. *RSC advances*. 2015;5(95):77996-8005. DOI: https://doi.org/10.1039/C5RA13556G.
15. Hairi HA, Sadikan MZ. The Therapeutic Effects of Moringa Oleifera on Osteoporosis and Diabetes 2024. 23 p.
16. Sharma K, Kumar M, Waghmare R, Suhag R, Gupta OP, Lorenzo JM, et al. Moringa (Moringa oleifera Lam.) polysaccharides: Extraction, characterization, bioactivities, and industrial application. *International Journal of Biological Macromolecules.* 2022; 209:763-78. DOI: https://doi.org/10.1016/j.ijbiomac.2022.04.047.
17. Kim M-H, Kim H-M, Jeong H-J. Estrogen-like osteoprotective effects of glycine in in vitro and in vivo models of menopause. *Amino acids*. 2016; 48:791-800. DOI: https://doi.org/10.1007/s00726-015-2127-6.
18. Aguayo-Cerón KA, Sánchez-Muñoz F, Gutierrez-Rojas RA, Acevedo-Villavicencio LN, Flores-Zarate AV, Huang F, et al. Glycine: the smallest anti-inflammatory micronutrient. *International Journal of Molecular Sciences.* 2023;24(14):11236. DOI: https://doi.org/10.3390/ijms241411236.
19. Van'T Hof RJ, Ralston SH. Nitric oxide and bone. *Immunology*. 2001;103(3):255-61. DOI: https://doi.org/10.1046/j.1365-2567.2001.01261.x.
20. MacDonell R, Hamrick MW, Isales CM. Protein/amino-acid modulation of bone cell function. *BoneKEy reports*. 2016;5. DOI: https://doi.org/10.1038/bonekey.2016.58.
21. Arawande JO, Adeleke AR, Orimoloye OR, Adebisi SA, Amuho EU, Ijitona OO. Extractive values and antioxidant properties of leaves, seeds, pods and coats Moringa Plant. *Biomedical Journal of Scientific and Technical Research*. 2021;39(4):006334. DOI: https://doi.org/10.26717/BJSTR.2021.39.006334.
22. Zhou X, Yuan W, Xiong X, Zhang Z, Liu J, Zheng Y, et al. HO-1 in bone biology: potential therapeutic strategies for osteoporosis. *Frontiers in Cell and Developmental Biology*. 2021; 9:791585. DOI: https://doi.org/10.3389/fcell.2021.791585.
23. Li Q, Tian C, Liu X, Li D, Liu H. Anti-inflammatory and antioxidant traditional Chinese Medicine in treatment and prevention of osteoporosis. *Frontiers in Pharmacology*. 2023; 14:1203767. DOI: https://doi.org/10.3389/fphar.2023.1203767.
24. Oh H, Masuno K, Okusa N, Makita Y, Fujiwara S-i, Imamura Y. Effect of orento, a traditional Japanese medicine, on IL-6, IL-8 secretion, type 1 collagen production and alkaline phosphatase secretion in the human osteosarcoma cell line Saos-2. *Medicines*. 2020;7(10):61. DOI: https://doi.org/10.3390/medicines7100061.
25. Patel C, Rangrez A, Parikh P. The anti-osteoporotic effect of *Moringa oleifera* on osteoblastic cells: SaOS 2. *IOSR Journal of Pharmacy and Biological Sciences.* 2013;5(2):10-7. DOI: DOI: https://doi.org/10.9790/3008-0521017
26. Habib M, Al-Moalem MH. Effect of Moringa Leaves and Seeds on Osteoporosis in Rats. *Journal of Food and Dairy Sciences*. 2018;3(3):129-35. DOI: https://doi.org/10.21608/ifds.2018.77771.
27. Egbu CF, Motsei LE, Yusuf AO, Mnisi CM. Effect of Moringa oleifera seed extract administered through drinking water on physiological responses, carcass and meat quality traits, and bone parameters in broiler chickens. *Applied Sciences.* 2022;12(20):10330. DOI: https://doi.org/10.3390/app122010330.