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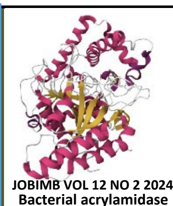

Inhibitory Effects of Oil Palm Leaf Extract on Osteoclastogenesis in RAW 264.7 Macrophages

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ABSTRACT

Osteoporosis is a bone disorder caused by an imbalance in the bone remodelling process, specifically between osteoblastogenesis and osteoclastogenesis, and is associated with increased oxidative stress. This study aims to investigate the antioxidant activity of oil palm leaf extracts (OPLEs) and their effects on osteoclastogenesis in murine macrophages (RAW 264.7), comparing the results with those of vitamin C (VC). Methanol extract of oil palm leaves (MEOPL) demonstrated the highest total phenolic content (TPC) at 284.26 mg GAE/g dry weight. The antioxidant activity, assessed via DPPH scavenging and FRAP assays, showed that MEOPL had a DPPH inhibition rate of 89.41% and a FRAP value of 105.67%. In cytotoxicity assays, MEOPL-treated cells exhibited significantly higher viability compared to VC-treated cells, with viability percentages exceeding 60% at concentrations up to 0.625 mg/mL. MEOPL also significantly reduced osteoclastogenesis, as indicated by a dose-dependent decrease in TRAP-positive multinucleated cells and a notable reduction in RANKL gene expression. These findings suggest that MEOPL possesses superior anti-osteoclastogenic properties compared to VC and holds promise as a potential therapeutic agent for the prevention and treatment of osteoporosis. However, as this study utilised an in vitro model, the direct translation of these results to clinical scenarios is limited, and further in vivo studies are necessary to confirm the clinical relevance of MEOPL.

INTRODUCTION

Osteoporosis, a systemic skeletal disorder characterised by reduced bone mass and microarchitectural deterioration, leads to increased bone fragility and fracture risk. Central to its pathophysiology is the imbalance in bone remodelling, particularly the heightened activity of osteoclasts, which are specialised cells responsible for bone resorption. Osteoclastogenesis, the process of osteoclast formation, is primarily regulated by two key cytokines: Macrophage Colony-Stimulating Factor (M-CSF) and Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL). Excessive osteoclast activity, often due to hormonal imbalances (like oestrogen deficiency), genetic factors, or lifestyle aspects, results in more bone being resorbed than formed, thus weakening the bones. Given this context, our study delves into the potential of Methanol Extract of Oil Palm Leaves (MEOPL) as a natural intervention, focusing on its impact on osteoclastogenesis and offering a potentially safer alternative to conventional osteoporosis treatments [1].

Numerous researchers have endeavoured to develop novel treatments for bone metabolic disorders by harnessing natural plant-derived compounds, aiming to mitigate the adverse effects associated with current osteoporosis drugs. The utilisation of plants in the treatment of various ailments has a rich historical background [2, 3]. Extensive research has been conducted on numerous plant extracts to explore their potential in combating osteoporosis. Some of these extracts have demonstrated significant anti-osteoporosis activity and have been recognised for their potent effects [4, 5]. Furthermore, the process of

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fermentation using plant products has the potential to augment the functional characteristics of medicinal plants or mitigate their toxicity and adverse effects [6, 7]. During the fermentation process, microorganisms break down complex substances into smaller molecules, resulting in the production of stable products. This transformative process enhances the pharmacological efficacy of these substances, making them more effective for disease prevention purposes [8].

Osteoclasts are multinucleated cells derived from the monocyte/macrophage lineage and are responsible for resorbing mineralised matrices in bone. Their maturation process is regulated by M-CSF and RANKL, which are recruited by hematopoietic stem cells to specific bone regions [9, 10]. Osteoclastogenesis factors are activated in the early stages of osteoclast development, initiating bone resorption. During this process, the expression of tartrate-resistant acid phosphatase (TRAP) is specific to the differentiation of immature osteoclasts into multinucleated osteoclasts, serving as a marker for osteoclast proliferation [11]. Plants are a valuable source of bioactive compounds, including various phenolics such as phenolic acids, flavonoids, hydroxycinnamic acid derivatives, and anthocyanins [12, 13]. These compounds possess significant physiological properties, such as free radical scavenging, antimutagenic, anti-inflammatory, and anti-carcinogenic effects, making them of great scientific interest [14].

The food industry annually produces a substantial amount of waste and by-products, leading to environmental challenges and significant disposal costs. However, there is increasing interest in reassessing this biomass as it holds potential as a valuable source of bioactive molecules with health benefits. Oil palm (*Elaeis guineensis*) leaves, which are abundant by-products of the palm oil industry in tropical countries such as Indonesia, Thailand, Malaysia, Africa, and South America, have traditionally been used as ruminant feed without any reported toxicity. Among the bioactive components found in these leaves, the methanol extract stands out due to its high content of flavonoids and catechins [15]. Catechins, which belong to the class of polyphenolic compounds, exhibit antioxidant activity surpassing that of vitamins C and E by a significant margin. The extract derived from oil palm leaves, known as oil palm leaf extract (OPLE), is rich in these compounds and has been extensively studied for its exceptional antioxidant properties [16,17]. Therefore, this study aimed to ascertain the antioxidant activity of OPLEs. Additionally, we examined the inhibitory effects of the extract and VC on osteoclasts in RAW 264.7 macrophage cells, which serve as precursors to osteoclasts. Through this investigation, we sought to explore the potential of these natural plant-based substances as an alternative therapeutic approach for the prevention and treatment of osteoporosis.

MATERIALS AND METHODS

Sample collections

The mature oil palm (*Elaeis guineensis*) leaves used in this study were obtained from palm trees that were six years old and grown at the University Putra Malaysia (UPM) farm. The leaves were collected from the central area of the farm and from the middle section of the leaves on 21 March 2018. Importantly, these palm trees were not subjected to pesticide application, unlike many farmed palm oils, to avoid potential disruptions of signalling pathways crucial for cell differentiation.

Preparation of Oil Palm leaves extracts (OPLEs)

The collected oil palm leaves were thoroughly washed with tap water and then finely chopped. The chopped leaves were dried in an oven at 40°C for 24 h. The dried leaves were then ground into a fine powder using a mechanical blender. The powdered leaves were soaked in different solvents, including 100% ethanol, methanol, hexane, ethyl acetate, and water, with a ratio of 1:10 (weight/volume). The mixtures were agitated for two h using a gyro-rocker shaker (SSL3 Stuart). After shaking, the mixtures were filtered through Whatman No. 1 filter paper. The solvents were then evaporated using a rotary evaporator (Premium7 Heidolph) at 40°C until a dark green waxy material was obtained [18].

Determination of Total phenolic content

The total phenolic content in OPLEs was determined using the Folin-Ciocalteu (F-C) method [19]. A 0.1 mL sample of OPLE was mixed with 0.5 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, USA) and 7 mL of distilled water. The mixture was incubated in the dark at room temperature for five min. Subsequently, 1.5 mL of carbonate solution (Hach, USA) was added, and the mixture was further incubated for two h at room temperature. The absorbance was measured at 765 nm using a UV spectrophotometer (Infinite M200 TECAN). Gallic acid (Sigma-Aldrich, USA) was used as a standard, and results were expressed as micrograms of gallic acid equivalent per gram of sample extract (µg GAE/g).

Determination of Antioxidant Capacity of OPLEs

DPPH Radical Scavenging Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was conducted following the method described by [20, 21] with slight modifications. A stock solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared by dissolving 24 mg of DPPH in 100 mL of methanol. A working solution was made by mixing 10 mL of the stock solution with 45 mL of methanol. Then, 30 µL of OPLEs at a concentration of 5 mg/mL was added to 170 µL of the DPPH working solution. The mixture was kept in the dark for 0, 25, 50, 75, and 100 min. The absorbance was measured at 515 nm. The percentage of DPPH radical scavenging activity was calculated using the formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 is the absorbance of the DPPH solution with methanol (negative control), and A_1 is the absorbance of the sample.

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric-reducing antioxidant power (FRAP) assay was conducted as described by Benzie and Strain [22]. The FRAP reagent was freshly prepared by mixing 2.5 mL of a 10 mmol/L solution of 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mmol/L hydrochloric acid, 2.5 mL of 20 mmol/L $FeCl_3$, and 25 mL of 0.3 mol/L acetate buffer (pH 3.6). The reagent was maintained at 37°C. A 50 µL aliquot of OPLEs was mixed with 0.2 mL of distilled water and 1.8 mL of FRAP reagent. The reaction mixture was incubated at 37°C for 14 min, and absorbance was measured at 593 nm. Iron sulphate ($FeSO_4$) was used as a standard, and results were expressed as mmol/L $FeSO_4$ equivalent.

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Cell Culture
The RAW 264.7 murine macrophage cell line (ATCC, Manassas, VA, USA) was cultured in DMEM medium supplemented with 10% heat-inactivated foetal bovine serum (FBS), 2 mM L-glutamine, and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin). Cells were maintained at 37°C in a 5% CO_2 atmosphere. For experiments, RAW 264.7 cells were seeded at a density of 1×10^4 cells/cm^2 and incubated overnight before treatments.

Cytotoxicity Assay (MTT Assay)
Cell viability was assessed using the MTT assay [23]. RAW 264.7 cells were seeded in 96-well plates at a density of 1 × 10^4 cells per well. After treatment with OPLEs and vitamin C (VC) at various concentrations (0.156, 0.312, 0.625, and 0.8 mg/mL) for 24, 48, and 72 h, MTT reagent (5 mg/mL) was added to each well and incubated for 4 h. Formazan crystals formed were dissolved in DMSO, and absorbance was measured at 570 nm. Cell viability was calculated as a percentage of the control group.

Osteoclast Differentiation Assay
The murine macrophage cell line (RAW 264.7) with passage numbers between 3 to 6 (ATCC, USA) was cultured in T25 flasks using Dulbecco's Modified Eagle Medium (DMEM) (Nacalai Tesque, Japan) containing L-glutamine, sodium pyruvate, 10% Fetal Bovine Serum (FBS), and 100 µL/mL penicillin/streptomycin (Nacalai Tesque, Japan / Sigma Aldrich, USA). Cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2 [24]. For the osteoclastic differentiation assay, RAW 264.7 cells were seeded at a density of 1x10^4 cells/well in a 24-well plate with complete DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. After 24 h of incubation, cells were stimulated with 100 ng/mL RANKL and treated with MEOPL (0.625, 0.312, 0.156 mg/mL) and vitamin C (0.156 mg/mL). The experiment was conducted for 6 days, with the medium changed every three days [25].

After 6 days, multinucleated osteoclasts were observed under a microscope (CKX41, Olympus). TRAP staining was performed by washing cells with PBS three times, fixing them in 4% formaldehyde for 10 min, and staining at 37°C for 60 min away from light. Cells were washed with deionised water, and TRAP-positive multinucleated cells containing more than three nuclei were counted under an inverted microscope at 20x magnification (Olympus, Tokyo, Japan). Control groups included untreated cells (negative control) and cells treated only with RANKL (positive control). Each condition was conducted in triplicate to ensure statistical robustness.

Gene Expression Analysis (RT-PCR)
The total RNA from RAW-OC was extracted using the RNeasy Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. To ensure the removal of genomic DNA contamination, DNase treatment was performed using the QuantiNova Internal control RNA Assay kit (Qiagen, Germany) according to the provided protocol. Subsequently, reverse transcription was conducted to synthesise complementary DNA (cDNA) from the DNase-treated RNA. For the synthesis of the first strand cDNA, a reaction mixture containing all the necessary components was prepared. The components of the reverse transcription reaction from the Quant Nova Internal control RNA Assay kit for RT-qPCR (Qiagen, Germany) were added sequentially to a sterile and RNase-free tube: 1 µL of reverse transcription enzyme, 4 µL of reverse transcription mix, and 15 µL of template RNA, resulting in a total reaction volume of 20 µL.

The obtained cDNA template (previously synthesised) was then mixed with the green qPCR master mix from the SYBR Green PCR Kit (Qiagen, Germany) and subjected to real-time quantitative PCR analysis. The primers used for RANKL amplification were as follows: forward primer 5'-TCCTAACAGAATATCAGAAGACAG-3' and reverse primer 5'-AGGCTTGCCTCGCTGGGCCACA-3'. GAPDH was selected as the housekeeping gene, and the primers used for its amplification were as follows: forward primer GGTGAAGGTCGGTGTGAACG and reverse primer CTCGCTCCTGGAAGATGGTG. The gene expression values were calculated as the ratio of the specific gene expression to GAPDH expression using the relative quantification method (ΔΔCT). The results are presented as means ± standard deviation (SD) based on triplicate determinations.

RESULTS

Total Phenolic Content of Oil Palm Leaves Extracts (OPLEs)
The methanol extract of oil palm leaves (MEOPL) exhibited the highest total phenolic content (TPC) among the five tested extracts, measuring 284.26 mg GAE/g dry weight. Hexane, ethyl acetate, ethanolic, and water extracts showed TPC values of 232%, 180.23%, 158.4%, and 45.89%, respectively. The differences in TPC among all samples were statistically significant compared to the methanolic extract of OPL ($p<0.05$) (**Fig. 1**).

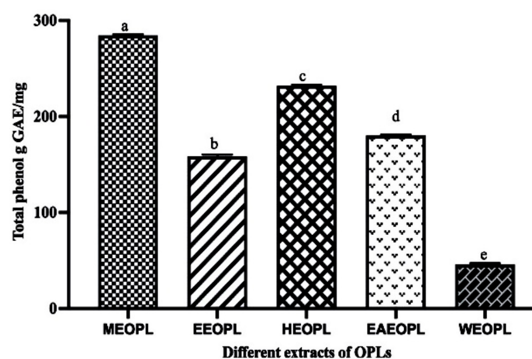

Fig. 1. Total phenolic content of oil palm leaf extract (OPLE) extracted by different methods. Results are expressed as mean ± SEM (n=5). The comparison between the methanolic extract of OPL and other types of OPL extracts was analysed using one-way ANOVA Tukey (a-p<0.05).

Antioxidant Activity of Oil Palm Leaves Extracts (OPLEs)
The antioxidant properties of different extracts obtained from Oil palm Leaves (OPLEs) were assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay and FRAP (ferric reducing antioxidant power) assay. The DPPH scavenging activity of MEOPL and EEOPL exceeded 80%, while VC showed more than 70% activity. The other extracts exhibited activities below 50% (**Fig. 2A**).

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Statistical analysis indicated a significant increase in DPPH scavenging over time for all extracts (p<0.05), with MEOPL and EEOPL outperforming VC at 100 min. The FRAP assay results showed that MEOPL had the highest FRAP value (105.67%), followed by the ethanolic extract (94.00%), VC (94.00%), hexane extract (65.59%), water extract (57.67%), and ethyl acetate extract (17.88%) (**Fig. 2B**). Statistical differences (p<0.05) were observed between VC and the methanolic, hexane, water, and ethyl acetate extracts.

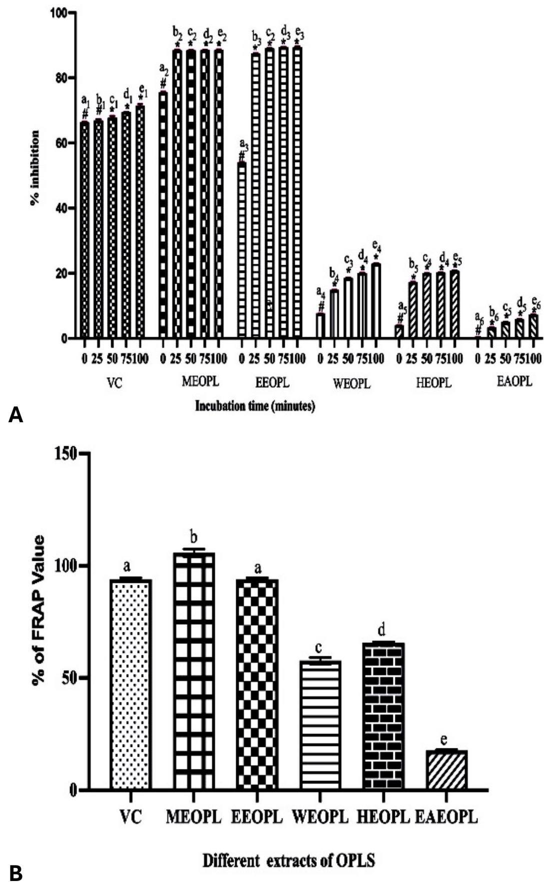

Fig. 2. A) DPPH radical scavenging activity and B) FRAP values of OPLEs and VC. Results are expressed as mean ± SEM (n=5). One-way ANOVA Tukey was used for significance analysis (a-p<0.05).

Cytotoxicity of MEOPL, EEOPL, and VC

Cytotoxicity was evaluated using the MTT assay in RAW 264.7 (**Fig. 3**) and RAW-OC (**Fig. 4**) cells. MEOPL-treated cells exhibited significantly higher viability than those treated with EEOPL and VC across all concentrations (0.156-0.625 mg/mL) and time points (24, 48, 72 h). After 24 h of treatment, the viability of RAW 264.7 and RAW-OC cells decreased in a concentration-dependent manner following treatment with EEOPL and VC, with significant reductions observed at all concentrations (p<0.05) compared to the control. MEOPL treatment, on the other hand, led to a significant increase in cell viability (p<0.05) across all concentrations. Following 48 h of treatment, the number of cells exhibited a concentration-dependent decrease. The viability of cells treated with VC and EEOPL was significantly reduced (p<0.05) at all concentrations, while MEOPL treatment led to a viability reduction at only the two highest concentrations. Notably, VC at the highest concentrations resulted in viability reduction below 50%, unlike MEOPL and EEOPL.

At 72 h, the pattern of viability reduction remained consistent, with MEOPL-treated cells showing higher viability compared to VC and EEOPL. VC and EEOPL significantly reduced viability at all concentrations, with reductions over 50% at higher concentrations, whereas MEOPL did not cause such reductions at any tested concentration.

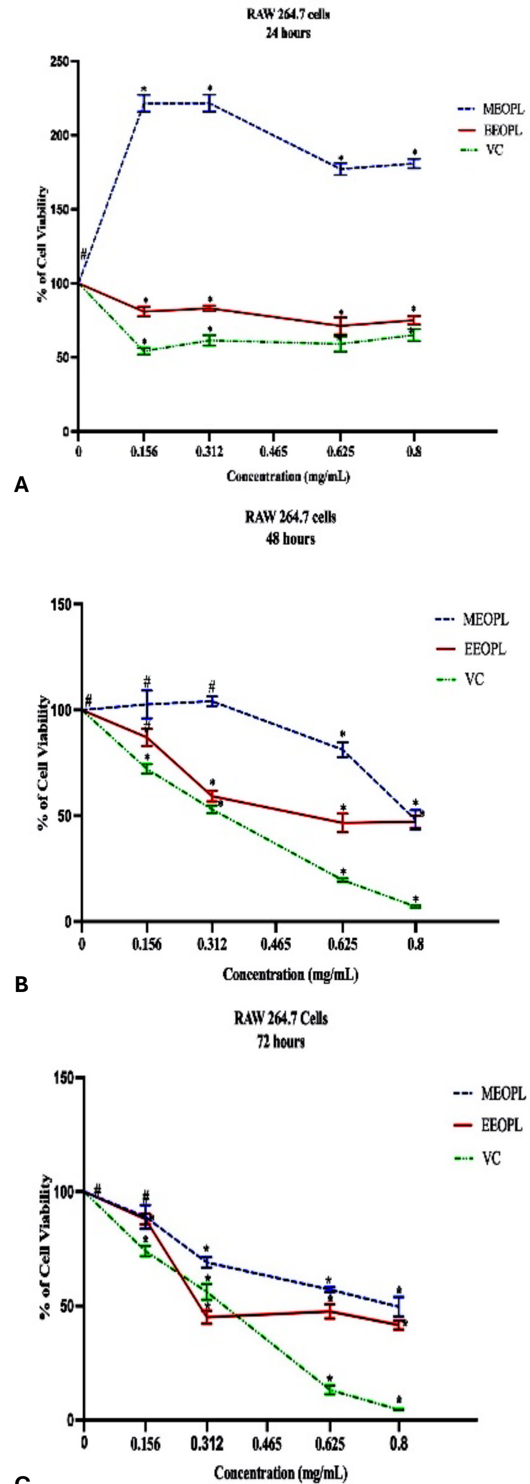

Fig. 3. Effect of various concentrations of MEOPL, EEOPL, and VC on RAW 264.7 cell viability at A) 24 h, B) 48 h, and C) 72 h. Results are expressed as mean ± SEM (n=5). One-way ANOVA Dunnett's was used for comparison (a-p<0.05).

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Inhibition of Osteoclast Formation by MEOPL and VC

To assess the effects of MEOPL and VC on osteoclast differentiation, a tartrate-resistant acid phosphatase (TRAP) staining assay was conducted as a marker for osteoclasts. RAW 264.7 cells were cultured in osteoclast differentiation media with or without RANKL (50 ng/mL) for 6 days in 24-well plates. The presence of RANKL alone induced the formation of numerous multinucleated TRAP-positive cells, indicating the differentiation of osteoclast-like cells (**Fig. 5A**).

However, the addition of MEOPL resulted in a concentration-dependent decrease in RANKL-stimulated osteoclast-like cell formation in RAW 264.7 cells. Treatment with both MEOPL and VC significantly reduced osteoclastogenesis compared to the negative control (RAW 264.7 cells induced with RANKL, referred to as RAW-OC). Quantification of TRAP-positive multinucleated cells (>3 nuclei) revealed a significant decrease in osteoclastogenesis with all concentrations of MEOPL tested (0.156, 0.312, and 0.625 mg/mL) compared to VC-treated cells (**Fig. 5B**). Importantly, no significant differences (p>0.05) in osteoclastogenesis were observed between the groups treated with 0.625 mg/mL and 0.312 mg/mL of MEOPL.

Gene Expression Analysis of RANKL

RT-PCR analysis demonstrated a dose-dependent downregulation of RANKL expression in MEOPL-treated groups compared to VC. The results displayed in **Fig. 6** demonstrated the presence of RANKL expression in all experimental groups; however, the groups treated with VC and MEOPL exhibited a significant reduction (p<0.05) in RANKL expression compared to the negative control group. Notably, the MEOPL-treated groups demonstrated a greater reduction in RANKL gene expression compared to the VC-treated group. Additionally, the MEOPL-treated groups at concentrations of 0.312 and 0.625 mg/mL exhibited a significantly lower expression of the RANKL gene compared to the group treated with 0.156 mg/mL of MEOPL (p<0.05).

Effects of MEOPL and VC on RANKL Gene Expression in RAW 264.7 Cells

To further understand the molecular mechanisms involved in osteoclastogenesis inhibition, we examined the effects of MEOPL and VC on the expression of RANKL, a key transcription factor in osteoclast differentiation. The RT-PCR analysis showed that MEOPL significantly downregulated RANKL expression in a dose-dependent manner compared to the control and VC-treated groups. The highest reduction in RANKL expression was observed at 0.625 mg/mL MEOPL (p<0.05), (**Fig. 6**) demonstrating a more pronounced inhibitory effect than VC at the same concentration.

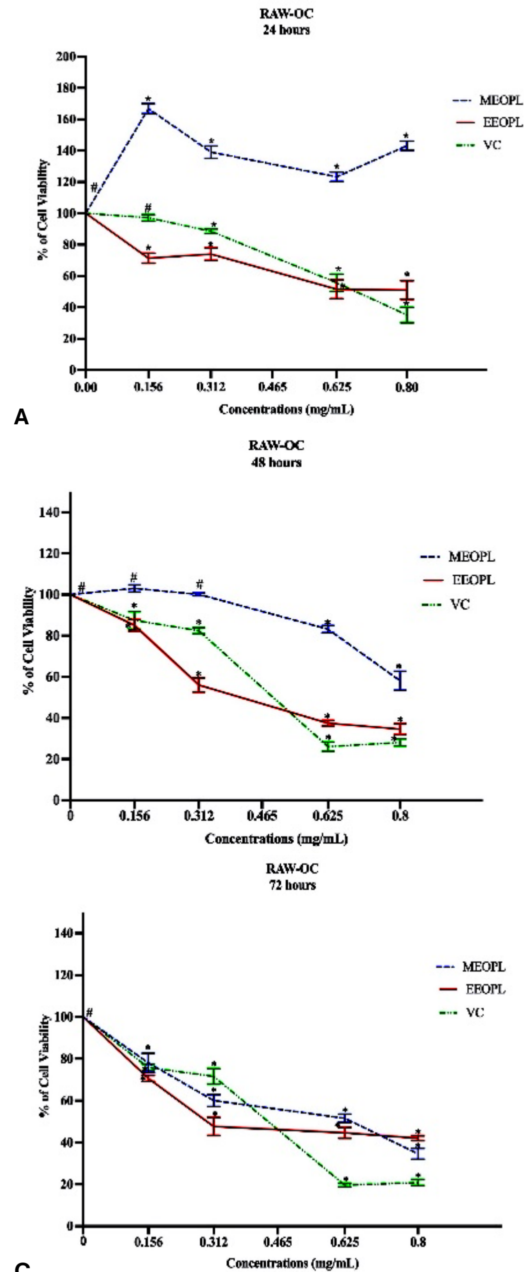

Fig. 4. Effect of various concentrations of MEOPL, EEOPL, and VC on RAW-OC cell viability at A) 24 h, B) 48 h, and C) 72 h. Results are expressed as mean ± SEM (n=5). One-way ANOVA Dunnett's was used for comparison (a-p<0.05).

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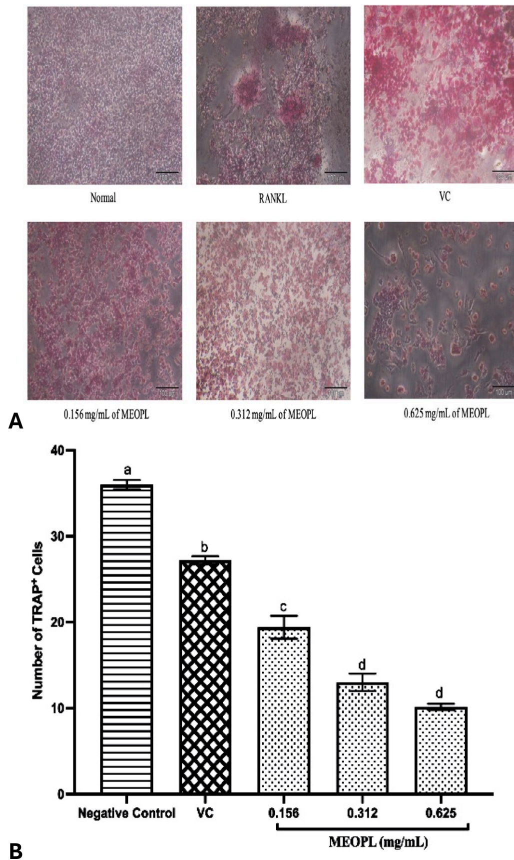

Fig. 5. Effect of MEOPL and VC on DMEM RANKL-induced osteoclast differentiation of RAW 264.7 cells. RAW 264.7 cells were cultured in DMEM medium with RANKL (100 ng) for 6 days, along with VC (0.156 mg/mL) and MEOPL at varying concentrations (0.625, 0.312, 0.156 mg/mL). **A)** TRAP staining of cells under a microscope. Bar: 100 µm. **B)** Quantification of TRAP-positive multinucleated osteoclasts (>3 nuclei). Data are presented as mean ± SEM; comparisons were analysed by one-way ANOVA, Tukey's test (ap<0.05).

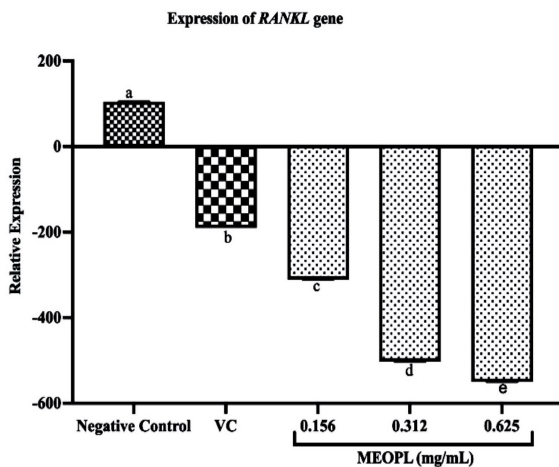

Fig. 6. Effect of MEOPL and VC on RANKL gene expression in RAW 264.7 cells. Cells were treated with MEOPL (0.156-0.625 mg/mL) and VC for 6 days. Data are expressed as mean ± SEM (n=5). The comparison between all treated groups and the negative control group was analysed using one-way ANOVA Tukey's (a-p<0.05).

DISCUSSION

Osteoporosis is characterised by excessive bone resorption caused by osteoclasts, leading to reduced bone mass and increased fracture risk [26]. Oxidative stress and pro-inflammatory cytokines are critical in osteoclast differentiation and osteoporosis development [27], [28]. Polyphenolic antioxidants, including genistein, quercitrin, ferulic acid, piceatannol, and taxifolin, have been shown to inhibit bone matrix degradation and promote osteoblast activity, thereby maintaining bone health [26–33]. Increased consumption of fruits and vegetables, which are rich in antioxidants and anti-inflammatory compounds, correlates with a reduced risk of bone fractures [34]. Flavonoids, commonly found in the human diet and traditional herbal medicine, are recognised for their therapeutic effects in various conditions, including cancer, heart disease, neurodegenerative disorders, and metabolic diseases [35]. Recent studies highlight flavonoids' positive impact on bone remodelling by regulating osteoblast and osteoclast functions, underscoring their role in promoting bone health [36–38].

Oil palm leaves (OPL), a by-product of the palm oil industry, are rich in bioactive phenolic compounds such as epigallocatechin, catechin, epicatechin, EGCG, and their glycosides. Traditionally used in rice-cooking bags, OPLs have potential applications in wound healing and other medicinal uses [39]. Our study evaluated the antioxidant activity of OPL extracts using DPPH and FRAP assays, revealing a strong correlation between high phenolic content and antioxidant properties, consistent with previous research [18], [40].

Importantly, the oil palm leaves used in this study were obtained from pesticide-free sources. This consideration is crucial as several pesticides, such as glyphosate and organophosphate compounds, have been identified as disruptors of signalling pathways critical for cell differentiation. Glyphosate, for instance, interferes with the RAS/RAF/MEK and PI3K/AKT pathways, which are vital for osteoblast and osteoclast differentiation, potentially altering the cellular processes underlying bone remodelling [41]. Similarly, organophosphate pesticides have been linked to disruptions in transcription factors and DNA repair mechanisms that impact osteoclastogenesis [42]. By ensuring the absence of pesticide contamination, our findings provide a clearer assessment of MEOPL's inherent bioactive properties without confounding environmental factors.

The DPPH and FRAP assays showed that MEOPL had significant antioxidant activity, attributed to its polyphenolic compounds. The TPC assay confirmed high phenolic content in MEOPL, followed by hexane, ethyl acetate, ethanol, and water extracts. Methanol proved to be the most effective solvent for extracting polyphenols and flavonoids [43], [44]. Cytotoxicity assays using MTT tests demonstrated that MEOPL, at concentrations of 0.156–0.625 mg/mL, did not reduce cell viability by more than 50% in RAW 264.7 and RAW-OC cells. Lower concentrations of MEOPL showed higher cell viability, consistent with previous studies [45], [46]. In contrast, higher concentrations of vitamin C (VC) exhibited cytotoxic effects, highlighting the potential safety of MEOPL as an alternative antioxidant [47], [48].

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The current study revealed that MEOPL significantly inhibited osteoclastogenesis in a dose-dependent manner, as evidenced by reduced TRAP-positive multinucleated cells and downregulation of RANKL and NFATc1 gene expression. These findings align with studies demonstrating the inhibitory effects of natural flavonoids on osteoclast formation and activity [49]. Flavonoids like ferulic acid suppress osteoclast differentiation by targeting the NF-κB and MAPK signalling pathways, while piceatannol inhibits RANKL-induced osteoclastogenesis through MAPK, NF-κB, and AKT pathways, along with promoting apoptosis in mature osteoclasts [32], [50], [51]. Taxifolin has similarly shown anti-osteoclastogenic effects by regulating oxidative stress and NFATc1 expression [52]. These findings suggest that MEOPL may function through comparable mechanisms, given its flavonoid-rich composition.

Contrasting studies, such as Jia et al. (2019) [29] on garcinol, provide further insights into the variability of flavonoid-mediated osteoclast inhibition. While garcinol effectively suppresses RANKL-induced NFATc1 expression and reduces osteoclast formation, its effects vary depending on experimental conditions and concentrations. This variability emphasizes the need for more mechanistic studies on MEOPL to elucidate its specific bioactive components and their molecular targets. Findings of the current study suggest that the inhibitory effect of MEOPL on RANKL expression may be attributed to its flavonoid and catechin content, offering a safer and more effective alternative or complementary therapy to oestrogen replacement therapy for osteoporosis [53–59]. Furthermore, the antioxidant activity of MEOPL, as demonstrated by its high DPPH scavenging and FRAP values, likely mitigates oxidative stress—a key driver of osteoclastogenesis.

Future research should include in vivo studies to validate the efficacy and safety of MEOPL in more complex biological systems. Additionally, clinical trials are needed to evaluate its therapeutic potential in human populations. Identifying the specific bioactive compounds within MEOPL responsible for its effects on osteoclastogenesis will be crucial for developing targeted therapies. Recent studies have also explored synergistic effects of flavonoids with conventional anti-resorptive agents, such as bisphosphonates, suggesting that MEOPL could be developed as part of combination therapies to enhance treatment efficacy while minimising side effects [60].

Given the global prevalence of osteoporosis and its diverse aetiology, subsequent research should focus on the differential efficacy of MEOPL across various osteoporosis subtypes and populations. This approach could pave the way for more personalised and effective treatment strategies, catering to the specific needs of different patient groups. The promising anti-osteoclastogenic and antioxidant properties of MEOPL highlight its potential as a natural therapeutic agent, contributing to safer and more sustainable management of osteoporosis.

CONCLUSION

The Methanol Extract of Oil Palm Leaves (MEOPL) demonstrated significant anti-osteoclastogenic effects, suggesting its potential as a plant-based therapeutic for osteoporosis. MEOPL effectively inhibited osteoclastogenesis in RAW 264.7 cells, evidenced by the dose-dependent reduction in TRAP-positive multinucleated cells and the downregulation of key osteoclastogenic markers, including RANKL and NFATc1. Furthermore, MEOPL exhibited favourable antioxidant properties, which may contribute to its observed inhibitory effects on osteoclast differentiation.

These findings underscore the potential of MEOPL as an alternative or complementary treatment to conventional osteoporosis therapies. However, considering the in vitro nature of this study, further research is necessary to determine the translational applicability of these findings. In vivo studies are crucial to evaluate MEOPL's pharmacokinetics, bioavailability, and overall safety in a more complex biological context. Additionally, clinical trials are needed to assess the efficacy of MEOPL in human populations and to establish the optimal dosing regimen.

Future studies should also aim to isolate and characterise the specific bioactive compounds responsible for the observed anti-osteoclastogenic effects. This could facilitate the development of more targeted therapeutic agents derived from MEOPL. Investigating the synergistic potential of MEOPL in combination with current osteoporosis treatments could further enhance its clinical utility.

ABBREVIATIONS

OPLE: Oil Palm Leaf Extract
MEOPL: Methanol Extract of Oil Palm Leaves
TPC: Total Phenolic Content
DPPH: 2,2-Diphenyl-1-picrylhydrazyl
FRAP: Ferric Reducing Antioxidant Power
VC: Vitamin C
TRAP: Tartrate-Resistant Acid Phosphatase
RANKL: Receptor Activator of Nuclear Factor Kappa-B Ligand
RAW 264.7: Murine Macrophage Cell Line

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AUTHORS ROLE

The study's design and oversight were the result of the collaborative efforts of N.A.M., P.S., S.M.N., and P.I. taking a leading role in conducting the experiments, data analysis, and the discussion of outcomes. The manuscript is a product of joint authorship, with each contributor having approved the final manuscript for publication

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