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Original Article

Effects of a Hydroalcoholic Extract of *Qualea grandiflora*, a Brazilian Cerrado Plant, on the Function of MC3T3-E1 Preosteoblasts

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Abstract

Background and objectives: *Qualea grandiflora* Mart. is a Brazilian Cerrado plant known for its antimicrobial and anti-inflammatory properties. MC3T3-E1 cells are a murine preosteoblast model used to study osteoblast function. However, the effects of this extract on preosteoblast function and matrix metalloproteinase-related outcomes remain unclear. This study aimed to evaluate the effects of a hydroalcoholic *Q. grandiflora* leaf extract on MC3T3-E1 cell function.

Methods: We assessed metabolic activity using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, alkaline phosphatase activity, and mineralization by alizarin red staining, together with matrix metalloproteinase (MMP)-2 (*Mmp2*) and MMP-9 (*Mmp9*) gene expression, protein expression, and activity by quantitative polymerase chain reaction, western blotting, and zymography, respectively, using *Q. grandiflora* extract at 0.1, 1.0, and 10.0 μg/mL.

Results: At 24 h, MTT metabolic activity was lower at 0.1 μg/mL than in the control group ($P < 0.05$), whereas no significant differences were observed at 48 or 72 h. No significant differences were observed in alkaline phosphatase activity, mineralization, or MMP-2/MMP-9 expression or activity at the tested concentrations.

Conclusions: Except for the lower MTT metabolic activity at 0.1 μg/mL after 24 h, no significant changes in the measured MC3T3-E1 functional outcomes are evident at the selected concentrations; these findings do not establish osteogenic efficacy or clinical safety.

Introduction

Osteoblasts are bone cells that originate from mesenchymal stem cells. They are cuboidal and located along the bone surface. The expression of specific genes, such as bone morphogenetic proteins, Wnt, runt-related transcription factor 2, osterix, alkaline phosphatase (ALP), and others, is key to osteoblast differentiation.[1,2] Osteoblasts synthesize the bone matrix, which is important for responding to gravitational forces and for intercellular communication.[3] Bone also has a high capacity for remodeling, a complex cycle of cellular events controlled by local and systemic factors.[1] Matrix metalloproteinases (MMPs) also play a significant role in embryonic growth and development, contributing to tissue morphogenesis and organogenesis by remodeling the extracellular matrix.[4] They are extensively involved in both physiological and pathological processes and regulate crucial biological mechanisms, including angiogenesis, embryogenesis, dynamic tissue remodeling, growth, and aging.[5] Physiologically, MMPs play a vital role in bone homeostasis.[6] MMP-2 contributes

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to bone remodeling. Additionally, MMP-9 is involved in various biological processes, including cell-surface protein cleavage, extracellular matrix degradation, cleavage of proteins and peptides in the extracellular environment, and modification of cell-cell and cell-extracellular matrix interactions.[7]

Fractures are an important health concern among older adults in the United States.[8] As life expectancy increases, the incidence of bone fractures among older adults is expected to rise, representing an important and costly public health problem.[9,10]

Given this, natural products have been studied as therapeutic approaches and as alternatives in tissue engineering to minimize side effects in bone repair.[9] Many studies have shown that plant-derived substances combined with scaffolds may improve the bioactivity of biomaterials for osteogenic differentiation.[11] Previous studies suggest that some plant-derived biomaterials have osteoconductive properties and act in repair mechanisms. For example, acemannan, a fraction of *Aloe vera* gel, demonstrated upregulation of osteogenesis-related gene expression when primary periodontal ligament cells were treated with it.[12]

Qualea grandiflora Mart. (QG) is commonly known as "pau-terra," "pau-terra-do-campo," "pau-terra-do-cerrado," "pau-terra-da-folha-larga," "ariavá," among other names.[13,14] It is among the most widespread tree species in the Cerrado.[15] The literature indicates that extracts from QG leaves have depressant effects on the central nervous system and exhibit analgesic and anticonvulsant properties.[16] Cordeiro *et al.*[17] reported that QG has antiprotozoal activity and no cytotoxicity against L-6 cells (IC_{50} > 100 µg/mL). Furthermore, Pires *et al.*[18] reported antimicrobial activity against *Streptococcus mutans* at a concentration of 5 mg/mL. The ethanolic extract of QG bark shows antiulcer activity,[14] and studies of its methanolic bark extract have shown antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, and *Pseudomonas aeruginosa*.[19] However, research on the application of QG in bone cells remains limited, although the potential healing effects of crude ethanolic QG extract were evaluated in the alveoli of rats with induced dental alveolitis. QG was associated with bone formation comparable to that observed with the commercially used drug.[20]

Therefore, this study aimed to investigate the effects of a hydroalcoholic QG leaf extract on preosteoblast function in the MC3T3-E1 cell line.[21] Given the substantial global burden of fractures among older adults,[22] an alternative with bone-forming properties could be promising.

Materials and methods

Preosteoblasts

The MC3T3-E1 (subclone 4) preosteoblast cell line derived from mouse calvaria was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). It was maintained at 37°C in Minimum Essential Medium alpha (α-MEM; Gibco, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, NY, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin in a humidified atmosphere with 5% CO_2.[23] The medium was changed every 3 days. The cells were subcultured when the flask became subconfluent using 0.25% trypsin-EDTA (Sigma-Aldrich, USA). The cells were then plated for the assays, and the experiments were performed in triplicate, except for the MTT assay, which was performed in quintuplicate; independently repeated three times.

Qualea grandiflora

QG leaves were collected at the Bauru Municipal Botanic Garden (S 22° 18′ 54″, W 49° 3′ 39″) in November 2018. The voucher specimen was deposited in the Herbarium of the Department of Biological Sciences at UNESP (UNBA6034), and the collection was approved by SISBIO under permit No. 39825-1. The extraction procedures were previously described by Oliveira *et al.*[24] The plant name was verified on the website http://www.theplantlist.org/tpl1.1/record/kew-2868182 on August 28, 2021.

Cell viability assay

Cells were seeded in 96-well plates at 3 × 10^3 cells per well in α-MEM supplemented with 10% FBS. After adhesion, the cells were treated with QG extract (0.1, 1.0, or 10.0 µg/mL) or control medium (α-MEM with 10% FBS). Cell metabolic activity was measured at 24, 48, and 72 h using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were washed with phosphate-buffered saline (PBS) and then incubated with MTT solution (0.5 mg/mL) for 4 h at 37°C. Dimethyl sulfoxide was added afterward, and absorbance was measured at 550 nm using a spectrophotometer (Fluostar OPTIMA, BMG Labtech, Offenburg, Germany).[25,26]

Alkaline phosphatase activity

A total of 2 × 10^4 cells were plated in 24-well plates in triplicate. After 24 h of adhesion, the medium was replaced with osteogenic medium containing α-MEM, 10% FBS, 50 µg/mL ascorbic acid, and 10 mM β-glycerophosphate; QG extract (0.1, 1.0, or 10.0 µg/mL) or control medium was then added. On days 7, 10, and 14, the cells were washed with PBS, and lysis buffer (10 mM Tris, pH 7.5, 0.5 mM $MgCl_2$, and 0.1% Triton X-100) was added. The cell lysate was collected and centrifuged at 14,000 rpm for 15 min at 4°C. The supernatants were used to determine ALP activity.[27]

Calcium nodule formation

Mineralization was assessed using alizarin red staining (Sigma). Cells at a density of 4 × 10^5 cells per well were plated in 12-well plates in triplicate and cultured in osteogenic medium with QG extract (0.1, 1.0, or 10.0 µg/mL) or control medium; the medium was replaced every 3 days. After 21 days, the supernatants were removed, and the cells were washed with PBS and fixed with 4% formaldehyde for 5 min at room temperature. The cells were then incubated with 2% alizarin red solution at pH 4.2 for 10 min, followed by three washes with ultrapure water. Quantitative analysis was performed using a colorimetric method. In the dried, stained microplate, 10% acetic acid was added for 30 min with agitation at room temperature. The contents of each well were heated at 85°C for 10 min and then kept on ice for 5 min before centrifugation at 20,000 × g for 15 min. Then, 100 µL of the supernatant was transferred to 96-well plates, and 10% ammonium hydroxide was added to neutralize the acid. Absorbance was measured at 405 nm.[25,28]

Gene expression by quantitative polymerase chain reaction (qPCR)

A total of 5 × 10^5 cells were seeded in 6-well plates in triplicate. After adhesion, QG extract (0.1, 1.0, or 10.0 µg/mL) or control medium was added to the wells. After 24 and 48 h of treatment,

messenger RNA was extracted using the RNeasy Mini Kit (spin column) with DNase (Qiagen) and reverse-transcribed into cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's recommendations. The cDNA samples were incubated with SensiMix Hi-Rox SYBR Green (Bioline) and analyzed on the ViiA 7 Real-Time PCR System (Applied Biosystems). qPCR amplification was performed using the following primer sets, with GAPDH used to normalize the results: *Mmp2* (ACAGGACATTGTCTTTGATG/TACACAGCGTCAATCTTTTC); *Mmp9* (CTTCCAGTACCAAGACAAAG/ACCTTGTTCACCTCATTTTG); and GAPDH (TGGCAAAGTGGAGATTGTTGC/AAGATGGTGATGGGCTTCCCG).[26,29]

MMP-2 and MMP-9 expression by western blotting

Cells were plated in 100 × 20 mm Petri dishes at a density of 1 × 10^6 cells in triplicate. After treatment with QG extract (0.1, 1.0, or 10.0 µg/mL) or control medium for 24 or 48 h, the cells were lysed with a buffer solution containing 50 mM Tris-HCl (pH 7.4), 25 mM KCl, 5 mM $MgCl_2$, and 0.2% Nonidet P-40 supplemented with protease inhibitors (Sigma-Aldrich) and phosphatase inhibitor (0.2 M sodium orthovanadate; Calbiochem). The lysate was pooled, sonicated, and centrifuged at 14,000 rpm for 10 min at 4°C. Protein samples (50 µg) were subjected to electrophoresis on a 10% Tris-HCl polyacrylamide gel and subsequently transferred to a polyvinylidene difluoride membrane. The membrane was immunolabeled with goat polyclonal anti-MMP-2 and anti-MMP-9 primary antibodies (sc-6838; sc-6840, Santa Cruz Biotechnology), followed by secondary anti-goat IgG conjugated to horseradish peroxidase (sc-2354, Santa Cruz Biotechnology). Rabbit polyclonal anti-β-actin (sc-130657; Santa Cruz Biotechnology) was used as an endogenous control, followed by secondary anti-rabbit IgG conjugated to horseradish peroxidase (sc-2357, Santa Cruz Biotechnology). Enhanced chemiluminescence reagent (Amersham Biosciences) was used for detection, and the band densities were determined by densitometric analysis using Image Studio Lite software (LI–COR, Lincoln, Nebraska, USA) and ImageJ software (National Institutes of Health, NIH Image). The density values obtained were corrected by subtracting the background values.[25,30]

MMP-2 and MMP-9 activity

A total of 2 × 10^6 cells were plated in a 100 × 20 mm Petri dish. After 24 h of adhesion, QG extract (0.1, 1.0, or 10.0 µg/mL) was added, along with a control group. After 24 and 48 h of incubation, the supernatant was collected, concentrated using Amicon tubes, and centrifuged at 14,000 rpm for approximately 30 min or until the volume reached 150 µL. Then, 50 µg of protein was loaded onto the zymography gel. Molecular-weight standards and recombinant hrMMP-2 and hrMMP-9 proteins (Calbiochem; Merck, Darmstadt, Germany) were used as controls. The gels were stained with Coomassie Blue G-250 (0.5%) and scanned using the Loccus Biotecnologia DS-6000, followed by analysis in Lab Image 1D software. Gel degradation was determined by densitometric analysis using ImageJ software (National Institutes of Health, NIH Image). Only active MMP bands were quantified, and densitometric values were normalized to the corresponding control group.[25]

Statistical analysis

The results were analyzed using GraphPad Prism 5 software (La Jolla, USA). The groups were subjected to one-way analysis of variance with Tukey's test, and normality was assessed using the Kolmogorov-Smirnov test. Statistical significance was set at $P < 0.05$. A sample size of $n = 5$ was used for the MTT assay, and $n = 3$ for ALP activity, alizarin red staining, qPCR, western blotting, and zymography.

Results

Effects of QG on MC3T3-E1 metabolic activity, differentiation, and mineralization

We assessed MTT metabolic activity (Fig. 1a). At 24 h, metabolic activity at 0.1 µg/mL was lower than in the control group ($P < 0.05$); no significant differences were observed at 48 or 72 h (Fig. 1a). No significant differences among groups were observed in ALP activity at days 7, 10, or 14 (Fig. 1b). Likewise, no significant differences in mineralization were observed by alizarin red staining at day 21 (Fig. 1c–d).

QG did not modulate matrix metalloproteinase expression or activity

No significant differences were observed in *M*mp*2* or *M*mp*9* gene expression at 24 or 48 h (Fig. 2a–b). Western blot densitometry and zymography likewise showed no significant differences in MMP-2 or MMP-9 protein expression or activity after 24 or 48 h of QG exposure (Fig. 2c–e and 2f–i, respectively).

Discussion

The use of plants for therapeutic purposes has attracted considerable research attention, particularly in Brazil because of its extensive biodiversity. In our study, the tested concentrations did not produce a sustained reduction in MTT metabolic activity, although a lower value was observed at 0.1 µg/mL after 24 h. We used the total extract rather than an isolated fraction because the literature reports antibacterial activity of QG.[19] Regarding the expression or activity of specific molecules, including MMP-2 and MMP-9, no modulation was observed in MC3T3-E1 cells. Under physiological conditions, MMPs are normally expressed at lower levels to maintain homeostasis, whereas higher MMP expression has been associated with several cancer types and tumor development.[31] Taken together, these findings do not provide evidence of osteogenic efficacy for QG extract in MC3T3-E1 cells. The absence of sustained metabolic suppression at the tested concentrations supports only preliminary *in vitro* compatibility and should not be interpreted as evidence of clinical safety.

MMP-2 and MMP-9 are expressed during osteoblast differentiation, and MMP-2 contributes to extracellular-matrix remodeling; its absence has been associated with impaired fracture repair.[32,33] Conversely, MMP-9 appears to participate in osteoclast-based bone remodeling.[5] An imbalance of these MMPs may contribute to pathological bone remodeling. Oliveira *et al.*[24] reported increased MMP-14 and HIF1α expression in fibroblast and preosteoblast cells treated with 10.0 µg/mL of QG extract.

In our previous work,[24] we identified gallic acid and flavonoids

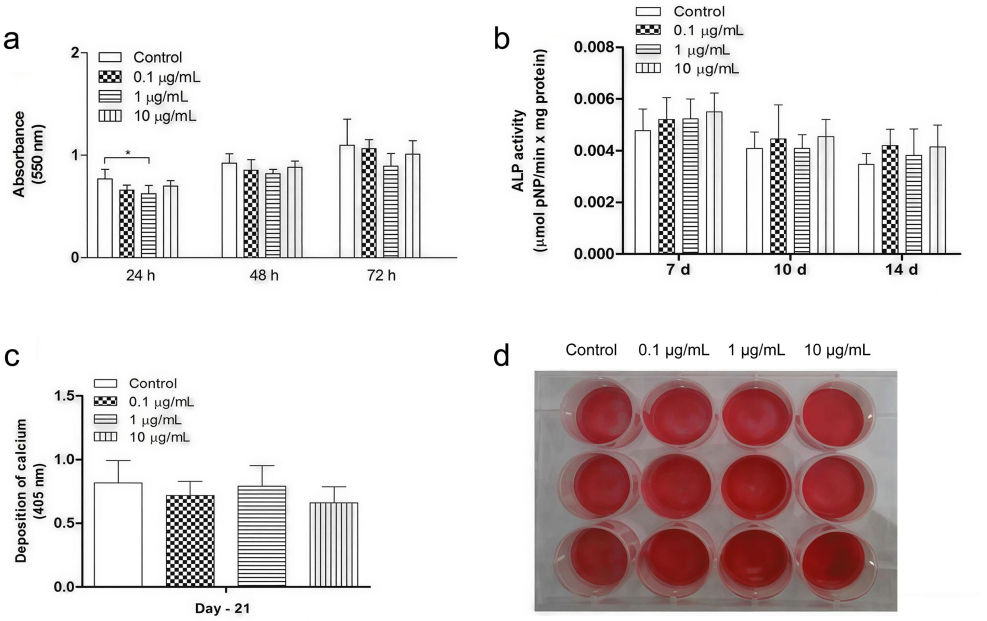


Fig. 1. Viability and osteogenic differentiation of MC3T3-E1 cells treated with different concentrations of QG extract. MC3T3-E1 cells were treated with QG extract (0.1, 1.0, and 10.0 µg/mL) or control medium. The MTT assay was performed after 24, 48, and 72 h of treatment (n = 5) (a). ALP activity (n = 3) (b) and alizarin red staining (n = 3) (c–d) were assessed at three and one time points, respectively. No significant differences were observed in ALP activity or alizarin red staining between treated and control groups. *P < 0.05 versus the control group within the same time point. Values are represented as mean ± SD, and all experiments were performed independently three times. ALP, alkaline phosphatase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; pNP, p-nitrophenol; QG, *Qualea grandiflora*; SD, standard deviation.

in the leaf extract of QG. BenSaad *et al.*[34] suggested that gallic acid, ellagic acid, and punicalagin isolated from *Punica granatum* might be responsible for its potential anti-inflammatory effects. Additionally, bioactive plant molecules are known to exhibit various properties, such as anti-inflammatory, antiseptic, and antioxidant effects. Furthermore, these compounds may act through multiple cellular and molecular signaling pathways.[35]

ALP activity is an important early marker of cell differentiation, followed by the alizarin red mineralization assay. Our data showed no modulation of these processes by the extract (Fig. 1b–d). The literature reports that certain plants can modulate osteogenic processes; a review summarized that berberine, an ingredient in traditional Chinese medicine, showed increased osteogenic gene expression in a periodontitis experiment.[36] A similar effect was not observed in this study. Recent reviews similarly indicate that plant-derived bioactives can influence osteoblastogenesis and bone regeneration through multiple signaling pathways, while emphasizing the need for mechanistic validation and cautious translation.[37-40]

Limitations of the study

This study was limited to *in vitro* experiments and specifically investigated the effect of QG on preosteoblast cell function, focusing on MMP-2 and MMP-9. Only one murine preosteoblast cell line was examined, and the functional assays were restricted to three low extract concentrations selected after preliminary screening indicated reduced metabolic activity at higher concentrations. Primary osteoblasts, osteoclast co-culture, inflammatory models, other osteogenic signaling pathways, and *in vivo* validation were not assessed. Accordingly, the findings cannot be generalized to other cell types or used to establish a complete dose-response relationship. We also note that the MTT assay measures metabolic activity and is not a direct measure of cell viability; therefore, other assays should be evaluated to support the safe use of QG.

Future directions

This study evaluated the effect of hydroalcoholic QG extract on osteoblast function, with a focus on MMPs. Future studies should evaluate a broader concentration range, additional primary or co-culture bone-cell models, and osteogenic signaling pathways, and should characterize standardized extract composition before translational applications are considered. Our previous work identified gallic acid and flavonoids in the extract.[24]

Conclusions

Except for the transient reduction in MTT metabolic activity at 0.1 µg/mL after 24 h, QG extract does not significantly alter the measured MC3T3-E1 functional outcomes at the tested concentrations. These findings do not demonstrate osteogenic efficacy or establish clinical safety; further dose-ranging and multi-model studies are needed.

Acknowledgments

None.

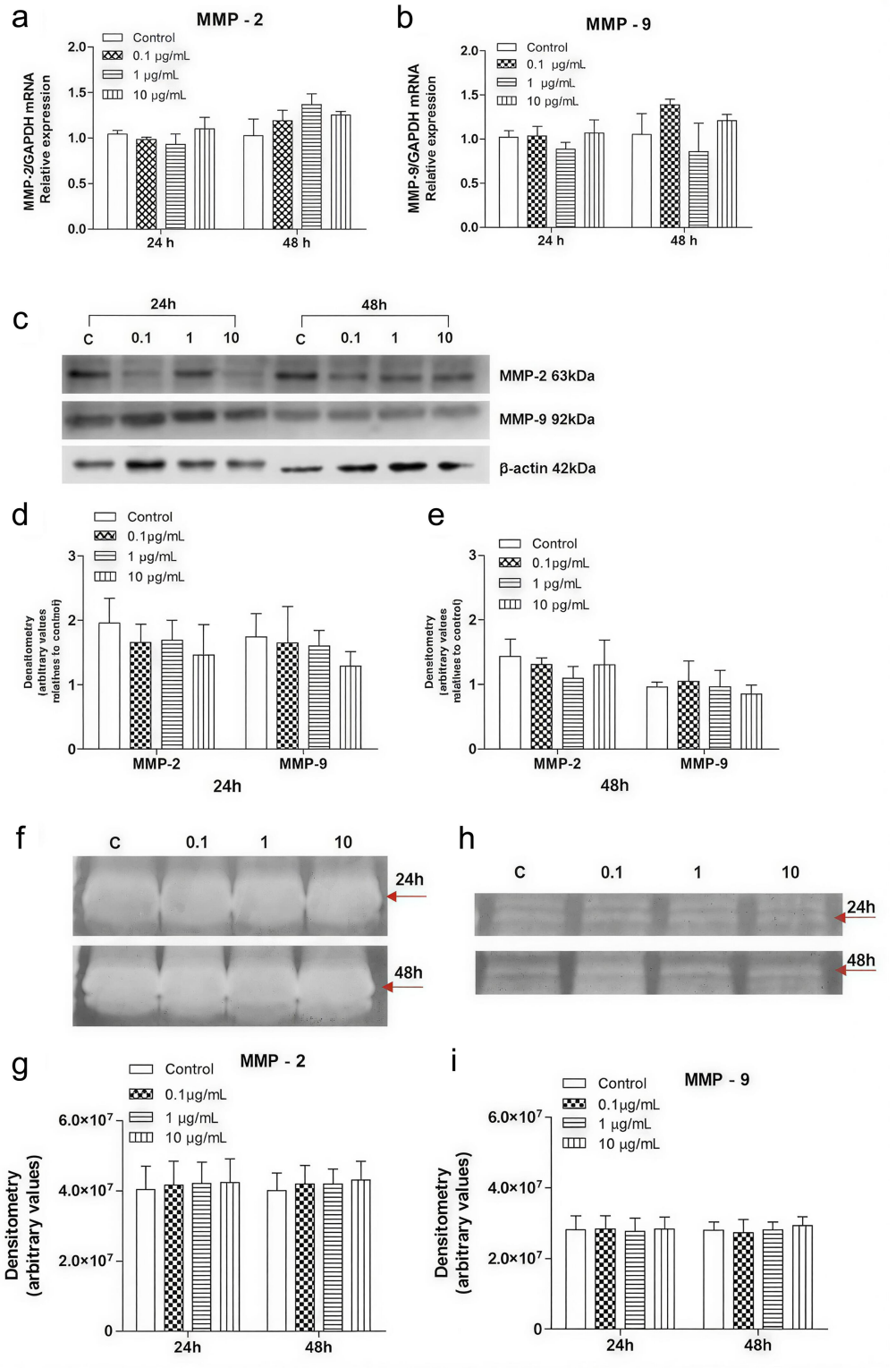


Fig. 2. MMP-2 and MMP-9 expression and activity in MC3T3-E1 cells treated with QG extract. Cells were exposed to QG extract at 0.1, 1.0, or 10.0 µg/mL for 24 or 48 h. *Mmp2* and *Mmp9* gene expression was measured by quantitative polymerase chain reaction, with GAPDH used for normalization (a–b). Protein expression was assessed by western blotting, with β-actin used as a loading control (c–e). MMP-2 and MMP-9 activity was evaluated by

zymography, and gelatin degradation was quantified using ImageJ (f–i). No significant changes were observed in treated groups. Values are represented as mean ± SD, and all experiments were performed in triplicate and independently three times. C, control; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MMP, matrix metalloproteinase; mRNA, messenger RNA; QG, *Qualea grandiflora*; SD, standard deviation.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Study conception and design (CKT, RCO); data acquisition and analysis (CKT, FAO, LLS, GSNO, AAM, PPM, MRS, TJD, MLRS, AGM); data interpretation and manuscript review (MARB, ALD, RCO); funding acquisition (RCO); and manuscript drafting (CKT, RCO). All authors read and approved the final version of the manuscript.

Ethical statement

This study did not involve human participants or live animals. The MC3T3-E1 cell line was obtained from ATCC. Plant collection was approved by SISBIO under approval No. 39825-1.

Data sharing statement

The data are available from the corresponding author upon reasonable request.

References

[1] Zhang L, Guan Q, Wang Z, Feng J, Zou J, Gao B. Consequences of aging on bone. Aging Dis 2024;15(6):2417–2452. DOI: 10.14336/AD.2023.1115, PMID: 38029404.

[2] Zhu S, Chen W, Masson A, Li YP. Cell signaling and transcriptional regulation of osteoblast lineage commitment, differentiation, bone formation, and homeostasis. Cell Discov 2024;10(1):71. DOI: 10.1038/s41421-024-00689-6, PMID: 38956429.

[3] Guntur AR, Rosen CJ. Bone as an endocrine organ. Endocr Pract 2012;18(5):758–762. DOI: 10.4158/EP12141.RA, PMID: 22784851.

[4] Kazemi A, Fathy M, Jahanian A, Khanali J, Ostadi Y, Babajani A, *et al*. The role of MMPs and TIMPs in regenerative medicine: From pathological ECM remodeling to therapeutic applications. Biomed Pharmacother 2025;191:118457. DOI: 10.1016/j.biopha.2025.118457, PMID: 40819542.

[5] Tokuhara CK, Santesso MR, Oliveira GSN, Ventura TMDS, Doyama JT, Zambuzzi WF, *et al*. Updating the role of matrix metalloproteinases in mineralized tissue and related diseases. J Appl Oral Sci 2019;27:e20180596. DOI: 10.1590/1678-7757-2018-0596, PMID: 31508793.

[6] Zambuzzi WF, Yano CL, Cavagis AD, Peppelenbosch MP, Granjeiro JM, Ferreira CV. Ascorbate-induced osteoblast differentiation recruits distinct MMP-inhibitors: RECK and TIMP-2. Mol Cell Biochem 2009;322(1-2):143–150. DOI: 10.1007/s11010-008-9951-x, PMID: 18989628.

[7] Huang B, Cvitkovitch DG, Santerre JP, Finer Y. Biodegradation of resin-dentin interfaces is dependent on the restorative material, mode of adhesion, esterase or MMP inhibition. Dent Mater 2018;34(9):1253–1262. DOI: 10.1016/j.dental.2018.05.008, PMID: 29789163.

[8] Matter AL, Pierce MF, Beal JR, Sahmoun AE. Factors associated with older adult fracture visits to United States emergency departments, 2013-2022. Injury 2026;57(8):113374. DOI: 10.1016/j.injury.2026.113374, PMID: 42214828.

[9] Miranda LL, Guimarães-Lopes VP, Altoé LS, Sarandy MM, Melo FCSA, Novaes RD, *et al*. Plant Extracts in the Bone Repair Process: A Systematic Review. Mediators Inflamm 2019;2019:1296153. DOI: 10.1155/2019/1296153, PMID: 31885494.

[10] Kushioka J, Chow SK, Toya M, Tsubosaka M, Shen H, Gao Q, *et al*. Bone regeneration in inflammation with aging and cell-based immunomodulatory therapy. Inflamm Regen 2023;43(1):29. DOI: 10.1186/s41232-023-00279-1, PMID: 37231450.

[11] Xue W, Yu J, Chen W. Plants and Their Bioactive Constituents in Mesenchymal Stem Cell-Based Periodontal Regeneration: A Novel Prospective. Biomed Res Int 2018;2018:7571363. DOI: 10.1155/2018/7571363, PMID: 30175141.

[12] Chantarawaratit P, Sangvanich P, Banlunara W, Soontornvipart K, Thunyakitpisal P. Acemannan sponges stimulate alveolar bone, cementum and periodontal ligament regeneration in a canine class II furcation defect model. J Periodontal Res 2014;49(2):164–178. DOI: 10.1111/jre.12090, PMID: 23710575.

[13] Corrêa MP. Dicionário das plantas úteis do Brasil e das exóticas cultivadas. Vol. 2. Rio de Janeiro: Ministério da Agricultura, Instituto Brasileiro de Desenvolvimento Florestal; 1978.

[14] Hiruma-Lima CA, Santos LC, Kushima H, Pellizzon CH, Silveira GG, Vasconcelos PC, *et al*. Qualea grandiflora, a Brazilian "Cerrado" medicinal plant presents an important antiulcer activity. J Ethnopharmacol 2006;104(1-2):207–214. DOI: 10.1016/j.jep.2005.09.002, PMID: 16216456.

[15] Strassburg BBN, Brooks T, Feltran-Barbieri R, Iribarrem A, Crouzeilles R, Loyola R, *et al*. Moment of truth for the Cerrado hotspot. Nat Ecol Evol 2017;1(4):99. DOI: 10.1038/s41559-017-0099, PMID: 28812670.

[16] Gaspi FO, Foglio MA, Carvalho JE, Moreno RA. Pharmacological activities investigation of crude extracts and fractions from Qualea grandiflora Mart. J Ethnopharmacol 2006;107(1):19–24. DOI: 10.1016/j.jep.2006.01.023, PMID: 16574359.

[17] Cordeiro TM, Borghetti F, Caldas Oliveira SC, Bastos IMD, de Santana JM, Grellier P, *et al*. Brazilian Cerrado Qualea grandiflora Mart. Leaves Exhibit Antiplasmodial and Trypanocidal Activities In vitro. Pharmacogn Mag 2017;13(52):668–672. DOI: 10.4103/pm.pm_100_17, PMID: 29200731.

[18] Pires JG, Zabini SS, Braga AS, de Cássia Fabris R, de Andrade FB, de Oliveira RC, *et al*. Hydroalcoholic extracts of Myracrodruon urundeuva All. and Qualea grandiflora Mart. leaves on Streptococcus mutans biofilm and tooth demineralization. Arch Oral Biol

2018;91:17–22. DOI: 10.1016/j.archoralbio.2018.04.005, PMID: 29653313.

[19] Alves TM, Silva AF, Brandão M, Grandi TS, Smânia E, Smânia Júnior A, *et al*. Biological screening of Brazilian medicinal plants. Mem Inst Oswaldo Cruz 2000;95(3):367–373. DOI: 10.1590/s0074-02762000000300012, PMID: 10800195.

[20] Rausch RAVQG, Aranha AMF, Damazo AS, Violante IMP, Guimarães CAV, Vieira EMM. Evaluation of Qualea grandiflora Mart in the healing of dry socket in rats (in Portuguese). Rev Odontol Bras Central 2016;25(74):130–134. DOI: 10.36065/robrac.v25i74.1072.

[21] Sasaki Y, Nakatsuka R, Inouchi T, Masutani M, Nozaki T. Inhibition of poly(ADP-ribose) glycohydrolase accelerates osteoblast differentiation in preosteoblastic MC3T3-E1 cells. Int J Mol Sci 2022; 23(9):5041. DOI: 10.3390/ijms23095041, PMID: 35563432.

[22] Feng JN, Zhang CG, Li BH, Zhan SY, Wang SF, Song CL. Global burden of hip fracture: The Global Burden of Disease Study. Osteoporos Int 2024;35(1):41–52. DOI: 10.1007/s00198-023-06907-3, PMID: 37704919.

[23] Bighetti ACC, Cestari TM, Santos PS, Arantes RVN, Paini S, Assis GF, *et al*. In vitro and in vivo assessment of CaP materials for bone regenerative therapy. The role of multinucleated giant cells/ osteoclasts in bone regeneration. J Biomed Mater Res B Appl Biomater 2020;108(1):282–297. DOI: 10.1002/jbm.b.34388, PMID: 31009176.

[24] Oliveira GSN, Tokuhara CK, Oliveira FA, Graeff MSZ, Khan ZN, Saldanha LL, *et al*. Effects of Qualea grandiflora extract on the expression of MMP-14 and HIF-1α in cultured fibroblasts and preosteoblasts. Braz Arch Biol Technol 2021;64:e21200714. DOI: 10.1590/1678-4324-2021200714.

[25] Oliveira FA, Matos AA, Santesso MR, Tokuhara CK, Leite AL, Bagnato VS, *et al*. Low intensity lasers differently induce primary human osteoblast proliferation and differentiation. J Photochem Photobiol B 2016;163:14–21. DOI: 10.1016/j.jphotobiol.2016.08.006, PMID: 27521889.

[26] Matos AA, Oliveira FA, Machado AC, Saldanha LL, Tokuhara CK, Souza LP, *et al*. An extract from Myracrodruon urundeuva inhibits matrix mineralization in human osteoblasts. J Ethnopharmacol 2019;237:192–201. DOI: 10.1016/j.jep.2019.03.052, PMID: 30905790.

[27] Pathomwichaiwat T, Ochareon P, Soonthornchareonnon N, Ali Z, Khan IA, Prathanturarug S. Alkaline phosphatase activity-guided isolation of active compounds and new dammarane-type triterpenes from Cissus quadrangularis hexane extract. J Ethnopharmacol 2015;160:52–60. DOI: 10.1016/j.jep.2014.11.026, PMID: 25449449.

[28] Petri AD, Teixeira LN, Crippa GE, Beloti MM, de Oliveira PT, Rosa AL. Effects of low-level laser therapy on human osteoblastic cells grown on titanium. Braz Dent J 2010;21(6):491–498. DOI: 10.1590/s0103-64402010000600003, PMID: 21271038.

[29] da Silva RA, Fuhler GM, Janmaat VT, da C Fernandes CJ, da Silva Feltran G, Oliveira FA, *et al*. HOXA cluster gene expression during osteoblast differentiation involves epigenetic control. Bone 2019; 125:74–86. DOI: 10.1016/j.bone.2019.04.026, PMID: 31054377.

[30] Matsuda SS, Silva TL, Buzalaf MA, Rodrigues AC, de Oliveira RC. Differential effects of fluoride during osteoblasts mineralization in C57BL/6J and C3H/HeJ inbred strains of mice. Biol Trace Elem Res 2014;161(1):123–129. DOI: 10.1007/s12011-014-0086-5, PMID: 25074429.

[31] Islam MT, Jang NH, Lee HJ. Natural Products as Regulators against Matrix Metalloproteinases for the Treatment of Cancer. Biomedicines 2024;12(4):794. DOI: 10.3390/biomedicines12040794, PMID: 38672151.

[32] Mizutani A, Sugiyama I, Kuno E, Matsunaga S, Tsukagoshi N. Expression of matrix metalloproteinases during ascorbate-induced differentiation of osteoblastic MC3T3-E1 cells. J Bone Miner Res 2001;16(11):2043–2049. DOI: 10.1359/jbmr.2001.16.11.2043, PMID: 11697800.

[33] Lieu S, Hansen E, Dedini R, Behonick D, Werb Z, Miclau T, *et al*. Impaired remodeling phase of fracture repair in the absence of matrix metalloproteinase-2. Dis Model Mech 2011;4(2):203–211. DOI: 10.1242/dmm.006304, PMID: 21135056.

[34] BenSaad LA, Kim KH, Quah CC, Kim WR, Shahimi M. Anti-inflammatory potential of ellagic acid, gallic acid and punicalagin A& B isolated from Punica granatum. BMC Complement Altern Med 2017;17(1):47. DOI: 10.1186/s12906-017-1555-0, PMID: 28088220.

[35] Das U, Behera SS, Singh S, Rizvi SI, Singh AK. Progress in the Development and Applicability of Potential Medicinal Plant Extract-Conjugated Polymeric Constructs for Wound Healing and Tissue Regeneration. Phytother Res 2016;30(12):1895–1904. DOI: 10.1002/ptr.5700, PMID: 27530610.

[36] Zhang Y, Ma J, Zhang W. Berberine for bone regeneration: Therapeutic potential and molecular mechanisms. J Ethnopharmacol 2021;277:114249. DOI: 10.1016/j.jep.2021.114249, PMID: 34058315.

[37] Wang X, Tang P, Yang K, Guo S, Tang Y, Zhang H, *et al*. Regulation of bone homeostasis by traditional Chinese medicine active scaffolds and enhancement for the osteoporosis bone regeneration. J Ethnopharmacol 2024;329:118141. DOI: 10.1016/j.jep.2024.118141, PMID: 38570149.

[38] Chen H, Weng Z, Kalinowska M, Xiong L, Wang L, Song H, *et al*. Anti-osteoporosis effect of bioactives in edible medicinal plants: a comprehensive review. Crit Rev Food Sci Nutr 2025;65(22): 4310–4326. DOI: 10.1080/10408398.2024.2386449, PMID: 39093554.

[39] Ouyang X, Ma Q, Zhou C, Tang J, Li M, Qing J, *et al*. Natural bioactive products in the regulation of bone metabolism and regeneration. Front Pharmacol 2025;16:1683279. DOI: 10.3389/fphar.2025.1683279, PMID: 41322298.

[40] Qu Z, Zhao S, Zhang Y, Wang X, Yan L. Natural compounds for bone remodeling: targeting osteoblasts and relevant signaling pathways. Biomed Pharmacother 2024;180:117490. DOI: 10.1016/j.biopha.2024.117490, PMID: 39332184.