Sains Malaysiana 55(1)(2026): 155-168
http://doi.org/10.17576/jsm-2026-5501-12

Osteoinductive Potential of Non-Polar *Piper sarmentosum* Extract in a Stem Cell-Based Bone Regeneration Model

(Potensi Osteoinduktif Ekstrak Tidak-Berkutub *Piper sarmentosum* dalam Model Pembentukan Semula Tulang Berasaskan Sel Stem)

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Received: 17 September 2025/Accepted: 14 January 2026

ABSTRACT

Piper sarmentosum (PS), a medicinal plant with pharmacological properties, has yet to be investigated for its potential in stem cell-based osteogenesis using non-polar extracts. This study investigated the osteoinductive potential of a hexane-based extract of PS on human peripheral blood stem cells (PBSCs). PBSCs were cultured for 14 days and characterized using *SLAMF1* and *CD34* stem cell markers. PBSCs were treated with several concentrations of the PS hexane extract to induce osteoblast differentiation. Osteoblast differentiation was assessed through alkaline phosphatase (ALP) activity, mineralization assays, and osteoblast-specific gene expression profiling (*ALP, RUNX2, OPN, OCN*). Gas chromatography-mass spectrometry (GC-MS) analysis identified 13 non-polar bioactive compounds in the extract, with β-asarone and phytol as predominant constituents. The 1 µg/mL concentration of the extract demonstrated optimal osteogenic potential, as evidenced by ALP activity comparable to positive controls, increased mineral deposition, and upregulation of osteogenic genes. These findings suggest that non-polar compounds in PS extract, especially β-asarone and phytol, possess osteoinductive properties, offering a potential natural approach for bone regeneration applications via stem cell-mediated pathways.

Keywords: Bone regeneration; natural product; osteoblast differentiation; peripheral blood stem cells; *Piper sarmentosum*

ABSTRAK

Piper sarmentosum (PS) merupakan tumbuhan ubatan yang mempunyai ciri farmakologi. Walau bagaimanapun, potensi ekstrak tidak-berkutub PS melibatkan aruhan sel stem kepada osteogenesis masih belum dikaji. Oleh itu, penyelidikan ini adalah untuk menentukan potensi osteoinduktif ekstrak PS berasaskan pelarut heksana terhadap proses osteogenesis melibatkan sel stem darah periferi manusia (PBSC). PBSC telah dikulturkan selama 14 hari serta dicirikan menggunakan penanda sel stem *SLAMF1* dan *CD34*. PBSC kemudiannya dirawat menggunakan beberapa kepekatan ekstrak heksana PS untuk mengaruh pembezaan osteoblas. Pembezaan osteoblas pula ditentukan melalui aktiviti alkali fosfatase (ALP), pemineralan dan profil pengekspresan gen yang khusus kepada osteoblas (*ALP, RUNX2, OPN* dan *OCN*). Analisis kromatografi gas-spektrometri jisim (GC-MS) pula telah mengenal pasti sebanyak 13 sebatian bioaktif tidak-berkutub dalam ekstrak PS dengan β-aseron dan fitol sebagai bahan utama. Kepekatan ekstrak pada 1 µg/mL telah menunjukkan potensi osteogen yang optimum, melalui aktiviti ALP yang setanding dengan kawalan positif, peningkatan pemendapan mineral dan peningkatan pengawalaturan gen osteogen. Penemuan ini mencadangkan bahawa sebatian tidak-berkutub yang terdapat di dalam ekstrak PS, terutamanya β-aseron dan fitol mempunyai ciri osteoinduktif. Ini menunjukkan pendekatan bahan semula jadi berpotensi untuk diaplikasikan dalam penjanaan semula tulang menggunakan sel stem.

Kata kunci: Pembentukan-semula tulang; pembezaan osteoblas; *Piper sarmentosum;* produk semula jadi; sel stem darah periferi

INTRODUCTION

Stem cells are unspecialized cells that can self-renew and differentiate into other types of cells, ultimately leading to the development of tissues and organs (Hagar et al. 2021), offering a potential natural-based approach to tissue engineering and regeneration. Stem cells have been used in tissue engineering, cellular therapy, and regenerative medicine. Stem cells have also been frequently used to repair cells and to test the toxicity of novel medications (Feng et al. 2011; Luz & Tokar 2018). Cell differentiation is important for ensuring a well-balanced supply of healthy cells. The differentiation of stem cells towards bone cells, such as osteoblasts or osteoclasts is important in maintaining bone cell homeostasis. Osteoporosis, a condition characterised by brittle bones and fractures, is a bone disorder caused by an imbalance in this differentiation process (Chapurlat & Genant 2016). Several plant-derived compounds have been shown to stimulate cell differentiation. Thus, replicating bone healing by inducing the differentiation of stem cells into desirable lineage-committed progenitors, triggered by certain plant extracts, can be utilised in regenerative medicine and therapy (Park et al. 2019).

Piper sarmentosum (PS) is a member of the Piperaceae family and a tropical and subtropical herb found in Malaysia, the Philippines, and Indonesia (Hussain et al. 2012). This plant is frequently utilised as a complementary medicine to treat a variety of illnesses. Malay people, for example, ate or decocted the leaves to treat hypertension, diabetes, and joint pain (Subramaniam et al. 2003). On the other hand, the root is thought to be capable of curing coughs, flu, and pleurisy. Additionally, a toothache can be relieved by combining the root and table salt (Hussain et al. 2009). PS extracts have also been demonstrated in earlier studies to possess biological activities, including anti-cancer, antibacterial, antioxidant, tissue regeneration, and fracture healing characteristics (Hussain, Hamdan & Sim 2020; Intan Zarina et al. 2020; Shahrul Hisham et al. 2009).

Osteoporosis is an ailment widely treated with alternative medicine and involves two main cell types, osteoclasts and osteoblasts, which regulate proper bone development. Osteoclasts degrade bone, whereas osteoblasts regenerate bone by secreting extracellular proteins (Kermani et al. 2014; Shahrul Hisham et al. 2022). In osteoporosis, bone mass is lost, and the microarchitecture of bone tissue is compromised due to an imbalance in the bone cell activity (Intan Zarina, Yamuna & Shahrul Hisham 2025). This can lead to fractures and weak bones, which overall lowers the quality of life (Chapurlat & Genant 2016). Currently, osteoporosis is being treated with medications such as estrogen, calcitonin, and bisphosphonates (Intan Zarina, Yamuna & Shahrul Hisham 2025; Rodan & Martin 2000). However, a large number of osteoporosis patients take supplementary products, such as vitamin E, magnesium, glucosamine, plant estrogens, chondroitin, electrotherapy, and primrose oil to reduce their lifelong dependence on these medications (Chong et al. 2007). β-glycerophosphate, frequently used as a positive control in *in vitro* osteogenesis studies, has been shown to reduce cell viability (Coelho & Fernandes 2000).

This study explores the potential of natural resources to enhance bone repair. Non-polar compounds from plant extract have produced significant interest in cellular biology due to their potential to influence various cellular processes, such as cellular differentiation. These extracts were rich in tocopherols, fatty acids, triterpenes, and aroma compounds, all of which have multiple biological roles (Ibrahim et al. 2022). The capability of non-polar compounds to interact with cellular membranes and affect lipid metabolism further enhances their role in inducing biological processes such as cell differentiation, which can act as valuable tools for therapeutic applications in tissue engineering and regenerative medicine. A study by Ezzat et al. (2022) showed that the non-polar compounds from green bean (*Phaseolus vulgaris* L.) combined with injection of mesenchymal stem cells produced an increase of 4.4-fold of serum insulin and 1.75-fold in HDL concentration, in addition to a decrease in blood sugar (73.6%), cholesterol (76.1%) and triglycerides (69.5%) of diabetic mice. We investigated the effect of only the non-polar extracts through hexane from PS on bone regeneration. The ability of the non-polar extract to stimulate bone formation was evaluated using a range of assays, including alkaline phosphatase (ALP) activity as an early osteoblast differentiation marker, osteoblast-specific gene expression, and mineralisation staining to assess calcium deposition in mature osteoblasts. GC-MS has been employed to identify the key bioactive of non-polar components within the extract responsible for its osteogenic activity. Further understanding of these extracts may lead to the development of novel biomaterials that promote stem cell differentiation toward bone regeneration.

MATERIALS AND METHODS

APPROVAL FROM AN ETHICAL COMMITTEE

This study was conducted in accordance with the 1975 Helsinki Declaration. The Research Ethics Committee of Universiti Kebangsaan Malaysia provided ethical clearance, as referenced by UKM PPI/111/8/JEP-2019-612, before the commencement of the research.

PLANT SUBSTANCES

Fresh *Piper sarmentosum* (PS) leaves obtained at the Forest Research Institute of Malaysia (FRIM), Malaysia (GPS coordinate: 3°14′7.80 N 101°38′9.59 E), were identified by a botanist from the Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA (UiTM). The leaves were washed and dried at 50 °C in the oven before being ground into a fine powder. The extraction processes were completed in the Faculty of Science and Technology Laboratory at Universiti Kebangsaan Malaysia (UKM).

PLANT EXTRACT PREPARATIONS

The non-polar hydrocarbons, i.e., hexane at a ratio of 1:10, were used in the Soxhlet apparatus to extract 20 g of dry, powdered leaves that had been heated in an oven. After that, the extracts were concentrated at lower pressure in a rotary evaporator. For fully evaporated solvent, the extracts were kept in the fume hood for a few days. The dehydrated extracts were stored at -20 °C until needed. For further examination, the concentrations (1-50 µg/mL) were later produced using 1% dimethyl sulfoxide (DMSO).

ISOLATION OF MONONUCLEATED CELLS

With the consent of healthy donors (aged 18-25 years), peripheral blood was collected from 15/10/2019 to 15/10/2021, and mononuclear cells were isolated. Ficoll-Paque™ PLUS was layered over blood samples that had been diluted with Hank's Balanced Salt Solution (HBSS) at a 1:3 ratio. After centrifugation of the samples at 400 × g for 20 min at 27 °C, the density-gradient separation produced four layers, with the mononucleated cells located in the second layer. The mononucleated cell layer was removed and washed 3 times with phosphate buffer saline (PBS) before being cultured at 37 °C in a complete medium comprising alpha medium essential medium (AMEM), 10% (v/v) newborn calf serum (NBCS), and 2% (v/v) penicillin-streptomycin.

PROLIFERATION AND STEMNESS DETERMINATION OF PERIPHERAL BLOOD STEM CELLS (PBSCS)

Peripheral Blood Stem Cells (PBSC) were seeded at a density of 1×10^5 cells/mL in proliferation medium and cultured for 14 days. Cell proliferation was assessed using the trypan blue exclusion assay. Viable and non-viable cells were counted using a hemocytometer under a light microscope, with live cells excluding the dye and dead cells appearing stained.

Stemness properties of isolated PBSCs were evaluated using the reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) technique. Total RNA was extracted on days 0, 7, and 14 utilised TRIzol reagent following the manufacturer's instructions. Reverse transcription was performed using the Sensifast cDNA Synthesis kit. The cDNA product was subsequently amplified using the Thunderbird SYBR QPCR Mix kit under the following thermal cycle conditions: 30 s at 95 °C (initial denaturation), followed by 40 cycles of 5 s at 95 °C and annealing for 10 s at either 60 °C for *GAPDH* and *SLAMF1* or 55 °C (*CD34*).

Primers were designed using the PrimerQuest™ Tool and were gene-specific for the intended target. Table 1 shows the primer sequences that have been employed for the osteoblast markers, i.e., alkaline phosphatase (*ALP*), runt-related transcription factor 2 (*RUNX2*), osteopontin (*OPN*), and osteocalcin (*OCN*), as well as hematopoietic stem cells (signalling lymphocytic activation molecule family member 1 (*SLAMF1*) and *CD34*.

OSTEOBLAST DIFFERENTIATION WITH PS HEXANE EXTRACT

The seeded cells at a density of 1×10^5 cells/mL were treated with the hexane extract of PS at concentrations of 1, 15, 35, and 50 µg/mL. Cells cultured with treated 10 mM β-glycerophosphate and 50 µg/mL ascorbic acid acted as a positive control, whereas cells maintained in complete medium without any supplement served as a negative control. Osteogenic differentiation was performed over 14 days.

TABLE 1. Primer sequences for hematopoietic stem cell characterisation and osteoblast detection

Gene	Sequences (5′–3′)	Extension temperature (°C)
CD34	Forward: TAGCCAAGTCTGCCAACTATTC	55
	Reverse: CCAACATACCACCCTCCATTT	
SLAMF1	Forward: GGAAAGCAGGAAGGAGGA	60
	Reverse: GCAGCCCAGTATCAAGGT	
ALP	Forward: GGAGTATGAGAGTGACGAGAAAG	54
	Reverse: GAAGTGGGAGTGCTTGTATCT	
RUNX2	Forward: CGGAATGCCTCTGCTGTTAT	55
	Reverse: TGTGAAGACGGTTATGGTCAAG	
OPN	Forward: GCTAAACCCTGACCCATCTC	56
	Reverse: ATAACTGTCCTTCCCACGGC	
OCN	Forward: CCTGAAAGCCGATGTGGT	57
	Reverse: GGCAGCGAGGTAGTGAAGA	
GAPDH	Forward: GACCACTTTGTCAAGCTCATTTC	60
	Reverse: CTCTCTTCCTCTTGTGCTCTTG	

ALKALINE PHOSPHATASE (ALP) ENZYME ASSAY

Following the differentiation period, the cells were rinsed with phosphate-buffered saline (PBS) and lysed with 0.1% Triton. Total protein content was quantified using the Bradford assay, with absorbance measured at 595 nm. ALP activity was assessed by incubating the lysates at 37 °C for 30 min in reaction buffer containing 0.1 mol/L sodium bicarbonate-sodium carbonate, 2 mmol/L magnesium sulphate, and 6 mmol/L p-nitrophenyl inorganic phosphate. The reaction was terminated with 1 mol/L sodium hydroxide, and the absorbance was measured at 405 nm. ALP-specific activity was calculated as the amount of p-nitrophenol released per minute per milligram of total protein. Results were expressed as a percentage relative to the negative control group.

VON KOSSA STAINING OF MINERALISED CELLS

To assess mineralisation, the differentiated cells at 1×10^5 cells/mL were fixed on a glass slide with 10% formalin (v/v) in PBS) for 30 min. After rinsing with deionised water, the cells were stained with a 5% silver nitrate (v/v) solution under ultraviolet light for 30 min. Subsequently, the slides were treated with 5% sodium carbonate (v/v) in 25% formalin for 2 min, followed by 5% sodium thiosulfate (v/v) for 2 min. Slides were rinsed thoroughly with deionised water after each step and air-dried before microscopic analysis.

REVERSE TRANSCRIPTASE QUANTITATIVE POLYMERASE CHAIN REACTION (RT-QPCR)

Total RNA was extracted from the treated cells on days 0, 7, and 14 of the differentiation assays using TRIzol reagent, following the manufacturer's protocol. Complementary DNA (cDNA) was synthesised using the SensiFAST cDNA Synthesis Kit under the following thermal conditions: 10 min at 25 °C, 15 min at 42 °C, and 5 min at 85 °C. Quantitative PCR was performed using Thunderbird Next SYBR qPCR Mix. The amplification protocol included an initial denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and gene-specific annealing/extension for 10 s. Gene expression was normalised against the housekeeping gene *GAPDH*. The primer sequences used for osteoblast-related genes, i.e., *ALP, RUNX2, OPN* and *OCN,* are listed in Table 1.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS

The chemical composition of the PS hexane extract was analysed using GC-MS equipped with a 30 m × 0.25 mm ID × 0.25 µm capillary column. The injection port temperature was maintained at 290 °C and operated in splitless mode. Helium (99.999%) was used as the carrier gas at a flow rate of 36.3 cm/s. Samples diluted in DMSO were injected using the AOC-20i + s autosampler. The temperature programme began at 50 °C (held for 5 min), increased at 2 °C/min to 300 °C, and held for 10 min. Mass spectra were recorded over the m/z range of 30-700. Compound identification was based on comparison with the NIST spectral database.

STATISTICAL ANALYSIS

Statistical analysis was performed using two-way analysis of variance (ANOVA), followed by Dunnett's post hoc test to compare each treatment group with the positive control. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

CHARACTERISATION OF PERIPHERAL BLOOD STEM CELLS (PBSCs)

To verify the maintenance of PBSCs, a 14-day proliferation assay was conducted. Cell counts showed a consistent increase over the 14-day culture period, indicating sustained cellular proliferation (Figure 1(a)). Concurrently, upregulation of both hematopoietic stem cell markers *CD34* and *SLAMF1* was observed on day 14 (Figure 1(b)), confirming the preservation of stemness. The combination of sustained proliferation and continued expression of stemness markers indicates the successful isolation and maintenance of PBSCs, rendering them suitable for subsequent osteogenic differentiation assay.

VIABILITY AND ALP-SPECIFIC ACTIVITY OF PBSCs TREATMENT USING PS HEXANE EXTRACT

Treatment of PBSCs with various concentrations of PS hexane extract influenced cell viability over a 14-day culture period (Figure 2(a)). The lowest concentration, 1 µg/mL, resulted in the lowest cell viability by day 14, similar to that of the positive control. Higher concentration at 15-50 µg/mL produced a moderate increase in viability (33-87-fold), while the untreated group (negative control) showed the highest proliferation (377-fold), suggesting an absence of differentiation property.

ALP is a well-established marker of osteoblast formation (Hagar et al. 2021; Kermani et al. 2014). ALP-specific activity was markedly elevated at lower extract concentrations, particularly at 1 µg/mL (Figure 2(b)). Normalisation against the negative control showed that reduced proliferation corresponded with enhanced ALP activity, suggesting that lower extract doses promote osteogenic differentiation of PBSCs.

CELL MINERALISATION OF DIFFERENTIATED CELLS

von Kossa staining was used to assess mineral deposition in PBSC-derived osteoblasts following 14 days of treatment with PS hexane extract (Figure 3(a)). No mineralisation

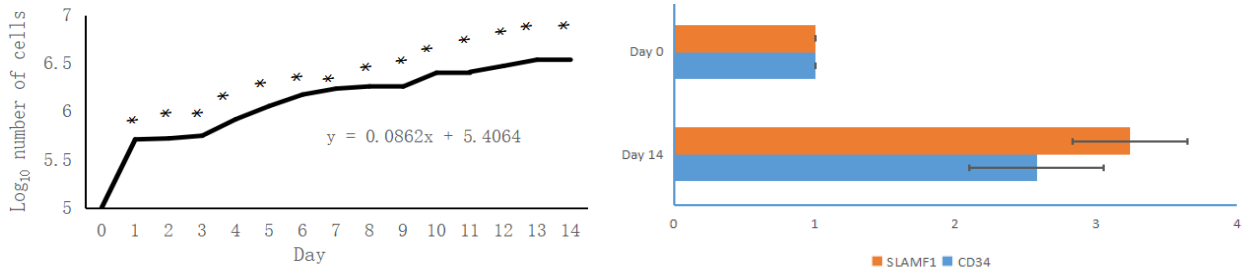
was detected in day 0 samples, which remained unstained (Figure 3(ai)). In contrast, treatment with 1 µg/mL of the extract exhibited prominent dark staining indicative of calcium phosphate deposits (Figure 3(aii)), confirming osteogenic differentiation.

Quantitative analysis demonstrated an inverse relationship between extract concentration and mineralisation (Figure 3(b)). The 1 µg/mL treatment group showed the highest percentage of mineralised cells, surpassing both higher doses and the positive control. Statistically significant differences were observed from day 3, 5, and 7 (day 3: $p < 0.0001$; day 5: $p < 0.001$; day 7: $p < 0.05$), demonstrating the potent osteoinductive effect of the low-dose extract.

EXPRESSION OF OSTEOBLAST GENE MARKERS

RT-qPCR analysis, normalised by *GAPDH* (housekeeping gene), showed dynamic regulation of osteoblast-related genes *ALP, RUNX2, OPN,* and *OCN* during the differentiation period (Figure 4). *ALP* expression increased consistently over time, reaching its peak at 1 µg/mL treatment by day 14, indicating sustained osteoblastic activity (Figure 4(a)). *RUNX2* expression peaked at day 7 and declined thereafter, consistent with its known role as a transcriptional regulator in the early phase of osteogenic differentiation (Figure 4(b)).

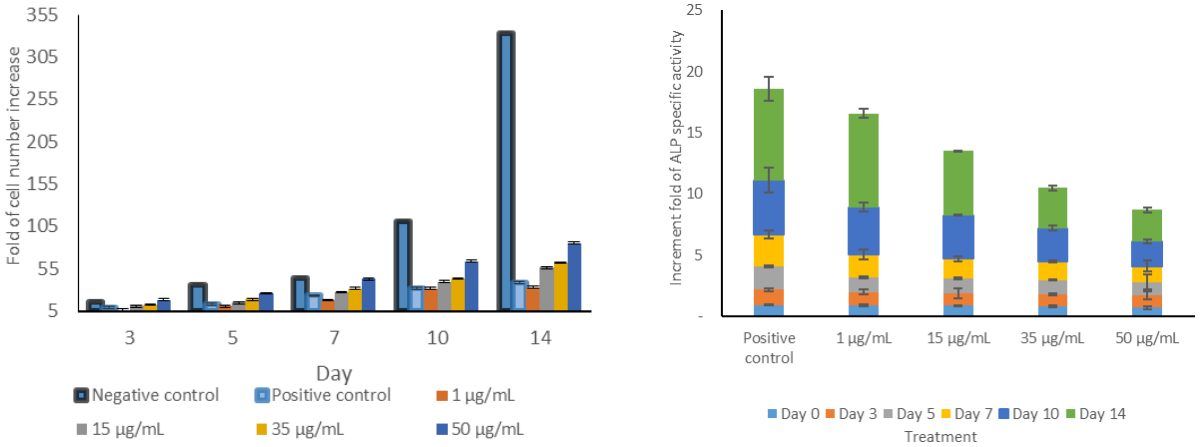
Both *OPN* and *OCN* expression levels followed a similar pattern to *ALP*, with the highest induction at 1 µg/mL on day 14 (34- and 21-fold increases,



(a) (b)

The mean ± standard error of the mean was given for 3 independent experiments (n=3). * Denotes significant differences ($p < 0.05$) as compared to Day 0

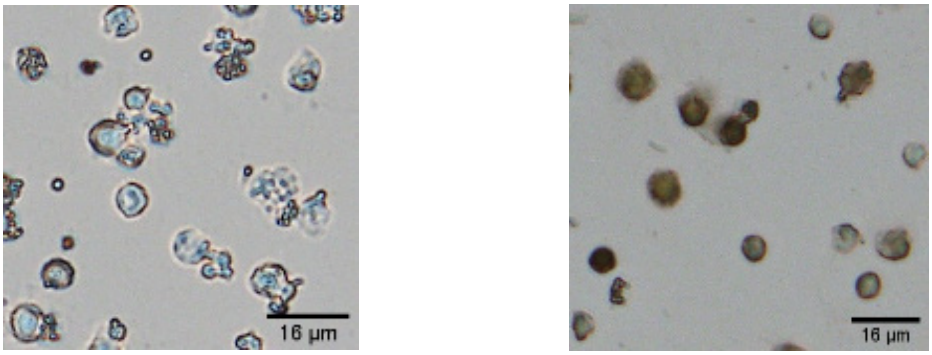
FIGURE 1. Proliferation and marker expression of PBSC in culture. a) The proliferation of PBSC for 14 days and b) The expression profiles of *SLAMF1* and *CD34* on days 0 and 14 of the assays. The expression of both genes was normalised to the housekeeping gene (*GAPDH*). The value of the fold change on day 0 is 1



(a) (b)

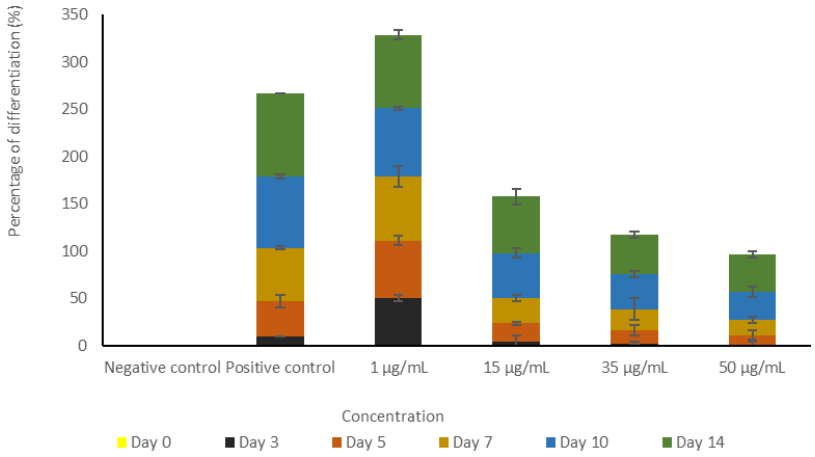
The mean ± standard error of the mean was given from 3 independent experiments (n=3)

FIGURE 2. Cell viability and ALP-specific activity during differentiation. a) The viability of human PBSC during the differentiation assay and b) The profile of ALP-specific activity has been normalised to the negative control for 14 days



(i) (ii)

(a)



(b)

The mean ± standard error of the mean was given for 3 separate experiments (n=3)

FIGURE 3. Mineralisation of differentiated cells in 14-day culture. a) The representative images of undifferentiated (i) and differentiated (ii) cells (Magnification ×400) and b) The percentage of mineralised cells

respectively) (Figure 4(c) and 4(d)). While other genes continued to increase, *RUNX2* expression significantly decreased on days 10 and 14 ($p < 0.001$). The sequential activation and regulation of these markers support the progression of PBSCs through distinct stages of osteoblastic differentiation.

COMPARATIVE DIFFERENTIATION OF PS EXTRACT AND POSITIVE CONTROL

To compare the osteogenic potential of PS extract with standard osteogenic medium (50 µg/mL ascorbic acid and 10 mM β-glycerophosphate), two-way ANOVA with Dunnett's test was performed across ALP activity, mineralisation, and gene expression outcomes.

ALP-SPECIFIC ACTIVITY

ALP activity at 1 µg/mL was comparable to the positive control throughout the assay ($p > 0.05$), while higher doses (15-50 µg/mL) showed statistically similar activity only during early time points (days 3-7) but declined significantly thereafter (e.g., 50 µg/mL on day 14; $p < 0.0001$) (Figure 5(a)). This suggests that low concentrations maintain osteogenic activity over time, unlike the higher doses.

MINERALISATION

Figure 5(b) demonstrates that during the early phase (days 3-7, all $p < 0.01$), mineralisation levels at 1 µg/mL were significantly higher than those of the positive control. The enhanced differentiation efficacy of the low-dose extract was demonstrated by the consistent underperformance of the 35 and 50 µg/mL treatments compared to the positive control during days 5-14 ($p < 0.0001$).

GENE EXPRESSION

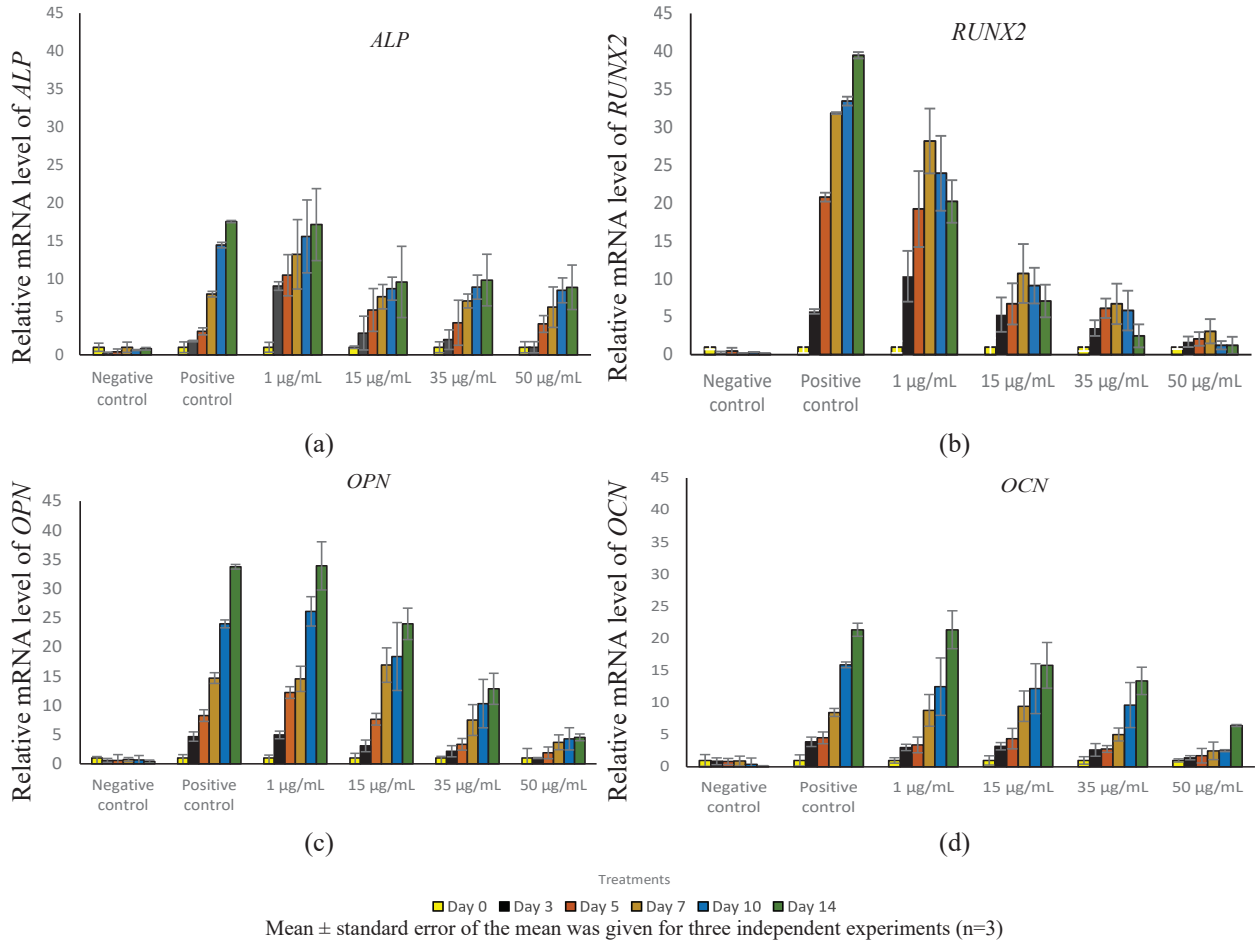
At 1 µg/mL, *ALP*, and *RUNX2* expression levels significantly exceeded the positive control during early

differentiation (*ALP*: days 3-7, $p < 0.05$; *RUNX2*: day 3, $p < 0.05$) (Figure 5(c)). *OPN* and *OCN* expressions remained statistically comparable to the positive control across the entire period. This reinforces the extract's osteoinductive potential, particularly at low concentrations.

COMPOUND AND BIOACTIVITY OF PS HEXANE EXTRACT

GC-MS profiling identified 13 bioactive compounds in the PS hexane extract (Table 2). Neocuproine was the last compound to elute, at 33.735 min, whereas 1-tetradeceneu, which eluted at 13.882 min, was the first compound to be eluted from the hexane extract. The most abundant compounds were 2,4-di-tert-butylphenol (41.86%), followed by γ-asarone and benzene, 1, 2, 3-trimethoxy-5-(2-propenyl (11.01% each).

These compounds exhibit diverse bioactivities, grouped into five categories: antioxidant (9 compounds), antimicrobial (8 compounds), anticancer (5 compounds), antifungal (4 compounds), and cell differentiation (2 compounds). Several compounds demonstrated multifunctionality; for example, β-asarone and phytol were linked to cell differentiation, antioxidant, and antimicrobial effects. Figure 6 illustrates the distribution of these biological functions. The observed differentiation-inducing activity of the extract, especially at low concentration, may be attributed to the presence of compounds with known osteogenic and antioxidant activities, such as β-asarone and phytol.



Mean ± standard error of the mean was given for three independent experiments (n=3)

FIGURE 4. Osteoblast gene profiles during treatment. The expressions of a) *ALP,* b) *RUNX2*, c) *OPN* and d) *OCN* after normalisation to the housekeeping gene, *GAPDH*

	Day	Concentrations (µg/mL) 1	15	35	50
	3	0.988	0.9419	0.9186	0.9976
	5	0.3667	0.4193	0.325	0.814
	7	0.2367	0.0747	0.0523	0.4602
	10	0.5242	0.181	0.0013	<0,0001
	14	0.9792	<0,0001	<0,0001	0.0001

(a)

	Day	Concentrations (µg/mL) 1	15	35	50
	3	<0,0001	0.3164	0.0195	0.0194
	5	<0,0001	0.1669	<0,0001	<0,0001
	7	0.0081	0.1669	<0,0001	<0,0001
	10	0.7347	0.0853	<0,0001	<0,0001
	14	0.0915	0.0739	<0,0001	<0,0001

(b)

Gene	Day	Concentrations (µg/mL) 1	15	35	50
ALP	3	0.0011	0.8154	0.9957	0.9906
	5	0.0007	0.2862	0.7956	0.9485
	7	0.0065	0.9998	0.9989	0.9691
	10	0.7844	0.0148	0.0198	0.0111
	14	0.9986	0.0006	0.0005	<0,0001
RUNX2	3	0.032	0.9999	0.617	0.1187
	5	0.9306	<0,0001	<0,0001	<0,0001
	7	0.1948	<0,0001	<0,0001	<0,0001
	10	<0,0001	<0,0001	<0,0001	<0,0001
	14	<0,0001	<0,0001	<0,0001	<0,0001
OPN	3	0.9998	0.8246	0.4944	0.1509
	5	0.0611	>0,9999	0.0332	0.0042
	7	0.9944	0.5161	0.0017	<0,0001
	10	0.5751	0.0289	<0,0001	<0,0001
	14	0.9992	<0,0001	<0,0001	<0,0001
OCN	3	0.9327	0.9707	0.8476	0.2791
	5	0.8967	>0,9999	0.5857	0.2443
	7	0.9883	0.8521	0.0872	0.001
	10	0.1672	0.1003	0.001	<0,0001
	14	0.9999	0.003	<0,0001	<0,0001

(c)

FIGURE 5. Comparison of the various concentrations of extract-treated cells to the positive control. Comparison involved a) ALP-specific activity, b) cell mineralisation percentage and c) gene expression values of the extract-treated cells. Dark blue, significantly higher ($p < 0.05$); light blue, similar to the positive control ($P>0.05$); red, significantly lower ($p < 0.05$) compared to the positive control

TABLE 2. Compound content and bioactivity of PS hexane extract

No	Name of compounds	Chemical formula	Bioactivity	Reference	Retention time (Percentage of peak area)
1	1-tetradecene	C14H28	Antioxidant Antimicrobial	(Attia et al. 2021; Seow et al. 2012)	13.882 (2.53%)
2	2,5-cyclohexadiene-1,4-dione,2,6-bis (1,1-dimethylethyl)	C14H20O2	Antifungal	(Gajera et al. 2020)	15.237 (0.19%)
3	2,4-di-tert-butylphenol	C14H22O	Antioxidant Antifungal Anticancer	(Choi et al. 2013; Hematpoor et al. 2017; Nair et al. 2018; Varsha et al. 2015; Yoon et al. 2006)	15.911 (41.86%)
4	γ -asarone	C12H16O3	Antioxidant Antifungal Anticancer	(Hematpoor et al. 2018, 2017)	16.901 (11.01%)
5	Benzene, 1, 2, 3-trimethoxy-5-(2-propenyl)	C12H16O	Antimicrobial	(Nikhila et al. 2016)	16.901 (11.01%)
6	2-tetradecene, (E)	C14H	Antioxidant Antimicrobial	(Nasir et al. 2020)	17.1 (7.16%)
7	Cetene	C16H	Antioxidant Antimicrobial	(Mou et al. 2013)	17.1 (7.16%)
8	Asarone	C12H16O3	Antioxidant	(Kumar et al. 2012)	18.597 (2.06%)
9	β -asarone	C12H16O3	Antioxidant Antimicrobial Antifungal Anticancer	(Lam et al. 2017; Yang et al. 2013)	18.597 (2.06%)
10	1-octadecene	CH2CH(CH2)15CH3	Cell differentiation Antioxidant Antimicrobial Anticancer	(Lee et al. 2007)	19.93 (3.06%)
11	Phytol	C20H40O	Antioxidant Antimicrobial	(De Menezes et al. 2013; Sanjeev et al. 2020)	26.03 (0.31%)
12	Silicic acid, diethyl bis (trimethylsilyl) ester	C10H28O4Si3	Cell differentiation Antimicrobial	(Sharmila Juliet et al. 2018)	30.988 (0.24%)
13	Neocuproine	C14H12N2	Anticancer	(Rajalakshmi et al. 2018)	33.735 (0.98%)

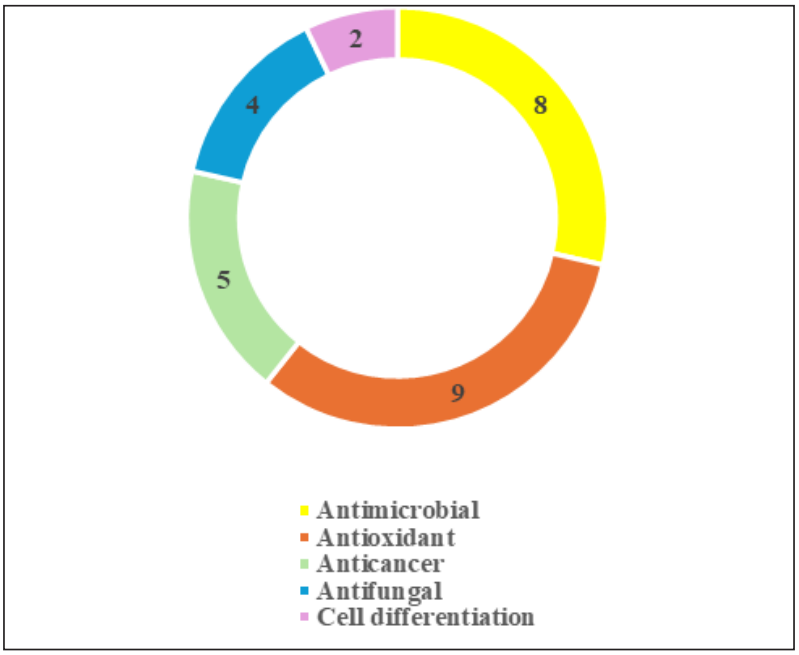


FIGURE 6. The bioactivity frequencies of compounds from *P. sarmentosum* hexane extract

DISCUSSION

OSTEOBLAST DIFFERENTIATION IN PS TREATMENT

Peripheral blood stem cells (PBSCs) were used in this study as a minimally invasive source of stem cells, in accordance with ethical guidelines of the Declaration of Helsinki (1975). Stem cells also express hematopoietic-specific markers, *SLAMF1* and *CD34*, which regulate self-renewal and differentiation (Kent et al. 2009; Shahrul Hisham et al. 2015; Sidney et al. 2014); these markers were upregulated in this study (Figure 1(b)). Previous work has shown that PBSCs respond to conventional osteogenic inducers, such as ascorbic acid and β-glycerophosphate (Muhammad Dain et al. 2011), supporting their potential in bone tissue development studies.

Osteoblasts are a tightly regulated process consisting of four stages: proliferation, extracellular matrix (ECM) production, ECM maturation, and apoptosis (Stein et al. 2004). A decrease in cell proliferation (Figure 2(a)) was observed in our study, especially in cells treated with 1 µg/mL of PS hexane extract, indicating the cells have shifted toward differentiation. This corresponds with established findings that cells cannot simultaneously proliferate and differentiate (Kermani et al. 2014).

Alkaline phosphatase (*ALP*) is the most common marker for osteogenesis, facilitating mineral deposition through hydrolysing phosphate for the formation of hydroxyapatite crystals (Shahrul Hisham et al. 2015). Our results showed a significant increase in ALP-specific activity from day 3 to 14, especially at 1 µg/mL extract (Figure 2(b)), indicating enhanced osteogenic induction. This concentration was more effective than higher doses, suggesting that the low concentration of the PS hexane extract could be the optimal concentration for early differentiation stimulation.

Further observation was provided by the von Kossa staining approach, which detects calcium phosphate deposits to indicate matrix mineralisation. Treated cells displayed progressive mineral deposition, distinctly at 1 µg/mL, supporting efficient differentiation (Figure 3(b)). The staining procedure, based on the reaction of silver ions with phosphate and carbonate groups of calcium deposits, produces black precipitates and is widely used for assessing mineralisation during bone development studies (Boyan, Schwartz & Boskey 2000; Intan Zarina et al. 2022, 2021; O'Gorman et al. 2011). Throughout the differentiation induction phase, the increase in black precipitates suggests the presence of a mineralisation process. The highest mineral deposition at 1 µg/mL extract treatment, compared to the higher extract concentrations, indicates that 1 µg/mL is a suitable concentration to be utilised as an inducer for osteoblast differentiation (Figure 3(b)).

Molecular analysis via RT-qPCR confirmed the extract's osteoinductive activity. *ALP* and *RUNX2*, early-stage markers, were upregulated during the initial days of differentiation, with *RUNX2* playing a central role in osteoblast commitment and maturation (Salazar, Gamer & Rosen 2016). Their elevated expression at 1 µg/mL, exceeding that of the positive control during the early stages, emphasises the efficacy of the PS extract in initiating osteogenesis (Figures 4(a)-4(b) & 5).

OCN and *OPN* (Figure 5(c)-5(d)), on the other hand, are associated with the later stages of osteoblast development and matrix maturation (Huang et al. 2007; Lo et al. 2010; Ritter, Farach-Carson & Butler 1992; Shahrul Hisham et al. 2010; Ruzanna et al. 2011). Osteoblast differentiation peaked at day 14 and showed expression at 1 µg/mL, comparable to the positive control (Figures 4(c)-4(d) & 5(c)). *OCN*, as the most abundant osteoblast-secreted non-collagenous protein, further validated the maturation of osteoblast differentiation. These molecular profiles are consistent with prior *in vivo* evidence where aqueous PS extracts enhanced bone healing in fracture rats (Estai et al. 2011).

NON-POLAR COMPOUNDS IN PS EXTRACT CONTRIBUTE TO OSTEOGENIC ACTIVITY

While previous studies have primarily focused on ethanol-based extracts of *Piper sarmentosum* (Intan Zarina et al. 2023), this study is the first to explore the osteogenic potential of its hexane (non-polar) fraction. GC-MS analysis identified 13 non-polar compounds with diverse biological activities. Hexane extracts are known to contain bioactive compounds, including fatty acids, triterpenes, and tocopherols (Ibrahim et al. 2022), which may contribute to cellular differentiation.

The most abundant compound was 2,4-di-tert-butylphenol (41.86%), a known antioxidant and antifungal agent (Hematpoor et al. 2017; Nair et al. 2018; Varsha et al. 2015; Yoon et al. 2006), with potential roles in bone homeostasis. γ-Asarone and benzene, 1,2,3-trimethoxy-5-(2-propenyl), each comprising 11.01%, also exhibited anticancer and antimicrobial activities (Hematpoor et al. 2018, 2017; Nikhila et al. 2016).

Importantly, β-asarone and phytol, both identified in the extract, have been reported to promote cellular differentiation. β-Asarone induces neural cell differentiation (Lam et al. 2017; Yang et al. 2013), whereas phytol has been shown to exert osteoinductive effects in murine mesenchymal stem cells (Sanjeev et al. 2020). Their presence may explain the enhanced osteoblast gene expression and mineralisation observed at 1 µg/mL.

The antioxidant capacity of PS extract likely contributes to its osteogenic efficacy. Oxidative stress inhibits osteoblast differentiation and promotes bone resorption. Antioxidants, including vitamins C and E, that can reduce oxidative damage, can enhance bone formation (Domazetovic et al. 2017; Wong et al. 2019). PS extract contains multiple antioxidant compounds, including β-asarone and phytol, which have the potential to reduce oxidative stress and promote osteogenic development.

The hexane extract of *Piper sarmentosum* is rich in non-polar compounds, which effectively promote PBSC differentiation into functional osteoblasts. The findings highlight the therapeutic potential of the extract in bone regeneration and support further investigation of its active components, particularly β-asarone and phytol, for potential drug development.

CONCLUSION

The hexane extract of *Piper sarmentosum*, comprising non-polar compounds, demonstrated significant osteoinductive properties at an optimal concentration of 1 µg/mL. This extract effectively stimulated early osteoblast differentiation, enhanced extracellular matrix mineralisation, and upregulated key osteogenic genes in peripheral blood stem cells. GC-MS analysis identified β-asarone and phytol as important bioactive compounds, both of which are associated with osteogenic activity. These findings demonstrate that the non-polar compounds of *Piper sarmentosum* may serve as promising natural agents for promoting bone regeneration and have therapeutic potential in the treatment of osteoporosis and related skeletal disorders.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Higher Education, Malaysia (FRGS/1/2018/STG05/CUCMS/02/1), University of Cyberjaya (CRGS/URGS/2023_005) and Universiti Kebangsaan Malaysia (GUP-2024-018).

REFERENCES

Attia, H., Harrathi, J., Alamer, K.H., Alsalmi, F.A., Magné, C. & Khalil, M. 2021. Effects of NACL on antioxidant, antifungal, and antibacterial activities in safflower essential oils. *Plants* 10(12): 2809. https://doi.org/10.3390/plants10122809

Boyan, B.D., Schwartz, Z. & Boskey, A.L. 2000. The importance of mineral in bone and mineral research. *Bone* 27(3): 341-342. doi:10.1016/S8756-3282(00)00347-1

Chapurlat, R.D. & Genant, H.K. 2016. Osteoporosis. In *Endocrinology: Adult and Pediatric* (Seventh ed)., edited by Jameson, J.L., De Groot, L.J., de Kretser, D.M., Giudice, L.C., Grossman, A.B., Melmed, S., & Potts, J.T. & Weir, G.C. Philadelphia: W.B. Saunders. pp. 1184-1213.e6.

Choi, S.J., Kim, J.K., Kim, H.K., Harris, K., Kim, C., Park, G.G., Park, C. & Shin, D. 2013. 2,4-Di-tert-butylphenol from sweet potato protects against oxidative stress in PC12 cells and in mice. *Journal of Medicinal Food* 16(11): 977-983. https://doi.org/10.1089/jmf.2012.2739

Chong, C.A.K.Y., Diaz-Granados, N., Hawker, G.A., Jamal, S., Josse, R.G. & Cheung, A.M. 2007. Complementary and alternative medicine use by osteoporosis clinic patients. *Osteoporosis International* 18(11): 1547-1556. https://doi.org/10.1007/s00198-007-0417-x

Coelho, M.J. & Fernandes, M.H. 2000. Human bone cell cultures in biocompatibility testing. Part II: Effect of ascorbic acid, b-glycerophosphate and dexamethasone on osteoblastic differentiation. *Biomaterials* 21(11): 1095-1102. doi: 10.1016/S0142-9612(99)00192-1

De Menezes Patrício Santos, C.C., Salvadori, M.S., Mota, V.G., Costa, L.M., De Almeida, A.A.C., De Oliveira, G.A.L., Costa, J.P., De Sousa, D.P., De Freitas, R.M. & De Almeida, R.N. 2013. Antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. *Neuroscience Journal* 2013: 949452. https://doi.org/10.1155/2013/949452

Domazetovic, V., Marcucci, G., Iantomasi, T., Brandi, M.L. & Vincenzini, M.T. 2017. Oxidative stress in bone remodeling: Role of antioxidants. *Clinical Cases in Mineral and Bone Metabolism* 14(2): 209-216. https://doi.org/10.11138/ccmbm/2017.14.1.209

Estai, M.A., Suhaimi, F.H., Das, S., Fadzilah, F.M., Alhabshi, S.M.I., Shuid, A.N. & Soelaiman, I. 2011. *Piper sarmentosum* enhances fracture healing in ovariectomized osteoporotic rats: A radiological study. *Clinics* 66(5): 865-872. https://doi.org/10.1590/s1807-59322011000500025

Ezzat, S.M., Rahman, M.F.A., Salama, M.M., Mahrous, E.A. & Bariary, A.E. 2022. Non-polar metabolites of green beans (*Phaseolus vulgaris* L.) potentiate the antidiabetic activity of mesenchymal stem cells in streptozotocin-induced diabetes in rats. *Journal of Food Biochemistry* 46(2): e14083. https://doi.org/10.1111/jfbc.14083

Feng, J., Mantesso, A., De Bari, C., Nishiyama, A. & Sharpe, P.T. 2011. Dual origin of mesenchymal stem cells contributing to organ growth and repair. *Proceedings of the National Academy of Sciences* 108(16): 6503-6508. https://doi.org/10.1073/pnas.1015449108

Gajera, H., Hirpara, D.G., Savaliya, D.D. & Golakiya, B. 2020. Extracellular metabolomics of Trichoderma biocontroller for antifungal action to restrain *Rhizoctonia solani* Kuhn in cotton. *Physiological and Molecular Plant Pathology* 112: 101547. https://doi.org/10.1016/j.pmpp.2020.101547

Hagar, M.N., Farinawati, Y., Nur Atmaliya, L., Shahrul Hisham, Z.A. & Rohaya, M.A.W. 2021. Comparative evaluation of osteogenic differentiation potential of stem cells derived from dental pulp and exfoliated deciduous teeth cultured over granular hydroxyapatite based scaffold. *BMC Oral Health* 21(1): 263. https://doi.org/10.1186/s12903-021-01621-0

Hematpoor, A., Paydar, M., Liew, S.Y., Sivasothy, Y., Mohebali, N., Looi, C.Y., Wong, W.F., Azirun, M.S. & Awang, K. 2018. Phenylpropanoids isolated from *Piper sarmentosum* Roxb. induce apoptosis in breast cancer cells through reactive oxygen species and mitochondrial-dependent pathways. *Chemico-Biological Interactions* 279: 210-218. https://doi.org/10.1016/j.cbi.2017.11.014

Hematpoor, A., Liew, S.Y., Azirun, M.S. & Awang, K. 2017. Insecticidal activity and the mechanism of action of three phenylpropanoids isolated from the roots of *Piper sarmentosum* Roxb. *Scientific Reports* 7(1). https://doi.org/10.1038/s41598-017-12898-z

Huang, W., Yang, S., Shao, J. & Li, Y.P. 2007. Signaling and transcriptional regulation in osteoblast commitment and differentiation. *Frontiers in Bioscience: A Journal and Virtual Library* 12: 3068-3092. https://doi.org/10.2741/2296

Hussain, H., Hamdan, N. & Sim, E.U. 2020. Anticancer and antimicrobial peptides from medicinal plants of Borneo Island in Sarawak. *Advances in Traditional Medicine* 21(2): 189-197. https://doi.org/10.1007/s13596-020-00504-z

Hussain, K., Hashmi, F.K., Latif, A., Ismail, Z. & Sadikun, A. 2012. A review of the literature and latest advances in research of *Piper sarmentosum*. *Pharmaceutical Biology* 50(8): 1045-1052. https://doi.org/10.3109/13880209.2011.654229

Hussain, K., Ismail, Z., Sadikun, A. & Ibrahim, P. 2009. Antioxidant, anti-TB activities, phenolic and amide contents of standardised extracts of *Piper sarmentosum* Roxb. *Natural Product Research* 23(3): 238-249. https://doi.org/10.1080/14786410801987597

Ibrahim, N., Abbas, H., El-Sayed, N.S. & Gad, H.A. 2022. *Rosmarinus officinalis* L. hexane extract: Phytochemical analysis, nanoencapsulation, and *in silico, in vitro*, and *in vivo* anti-photoaging potential evaluation. *Scientific Reports* 12(1): 13102. https://doi.org/10.1038/s41598-022-16592-7

Intan Zarina, Z.A., Yamuna, S. & Shahrul Hisham, Z.A. 2025. Southeast Asian medicinal plants in osteoporosis: A systematic review of anti-osteoclastogenesis in preclinical models. *Sains Malaysiana* 54(12): 2925-2936. http://doi.org/10.17576/jsm-2025-5412-11

Intan Zarina, Z.A., Anis Nabilah, J., Muhammad Dain, Y., Zaidah, Z.A., Herryawan Ryadi, E.D. & Shahrul Hisham, Z.A. 2023. Osteogenic potential and bioactive profiles of *Piper sarmentosum* ethanolic extract-treated stem cells. *Pharmaceuticals* 16(5): 708. https://doi.org/10.3390/ph16050708

Intan Zarina, Z.A., Thanaletchumi, M., Rohaya, M.A.W., Farinawati, Y. & Shahrul Hisham, Z.A. 2022. A comparative analysis of ascorbic acid-induced cytotoxicity and differentiation between SHED and DPSC. *Current Stem Cell Research & Therapy* 17(6): 576-588. https://doi.org/10.2174/1574888X17666220124141310

Intan Zarina, Z.A., Shazwana, S.I., Ling, J.P., Zaidah, Z.A. & Shahrul Hisham, Z.A. 2021. The effects of *Piper sarmentosum* aqueous extract on MC3T3-E1 differentiation. *Natural Volatiles & Essential Oils* 8(4): 4063-4079.

Intan Zarina, Z.A., Shazrul, F., Nur Hidayah, J., Herryawan Ryadi, E.D., Zaidah, Z.A., Anis Nabilah, J., Nur Suhanawati, A., Nor Azfa, J., Rohaya, M.A.W. & Shahrul Hisham, Z.A. 2020. The effects of *Piper sarmentosum* aqueous extracts on zebrafish (*Danio rerio*) embryos and caudal fin tissue regeneration. *Scientific Reports* 10: 14165. https://doi.org/10.1038/s41598-020-70962-7

Kent, D.G., Copley, M.R., Benz, C., Wöhrer, S., Dykstra, B.J., Ma, E., Cheyne, J., Zhao, Y., Bowie, M.B., Zhao, Y., Gasparetto, M., Delaney, A., Smith, C., Marra, M. & Eaves, C.J. 2009. Prospective isolation and molecular characterization of hematopoietic stem cells with durable self-renewal potential. *Blood* 113(25): 6342-6350. https://doi.org/10.1182/blood-2008-12-192054

Kermani, S., Rohaya, M.A.W., Intan Zarina, Z.A., Zaidah, Z.A., Sahidan, S. & Shahrul Hisham, Z.A. 2014. Differentiation capacity of mouse dental pulp stem cells into osteoblasts and osteoclasts. *Cell Journal* 16: 31-42.

Kumar, H., Kim, B., Song, S., Kim, J., Kim, I., Kwon, Y., Koppula, S. & Choi, D. 2012. Cognitive enhancing effects of alpha asarone in amnesic mice by influencing cholinergic and antioxidant defense mechanisms. *Bioscience Biotechnology and Biochemistry* 76(8): 1518-1522. https://doi.org/10.1271/bbb.120247

Lam, K.Y.C., Yao, P., Wang, H., Duan, R., Dong, T.T.X. & Tsim, K.W.K. 2017. Asarone from *Acori Tatarinowii* Rhizome prevents oxidative stress-induced cell injury in cultured astrocytes: A signaling triggered by Akt activation. *PLoS ONE* 12(6): e0179077. https://doi.org/10.1371/journal.pone.0179077

Lee, Y., Kang, M., Cho, S. & Jeong, C. 2007. Effects of constituents of *Amomum xanthioides* on gastritis in rats and on growth of gastric cancer cells. *Archives of Pharmacal Research* 30: 436-443. doi:10.1007/BF02980217

Lo, Y., Chang, Y., Wei, B., Huang, Y. & Chiou, W. 2010. Betulinic acid stimulates the differentiation and mineralization of osteoblastic MC3T3-E1 cells: Involvement of BMP/RUNX2 and B-Catenin signals. *Journal of Agricultural and Food Chemistry* 58(11): 6643-6649. https://doi.org/10.1021/jf904158k

Luz, A.L. & Tokar, E.J. 2018. Pluripotent stem cells in developmental toxicity testing: A review of methodological advances. *Toxicological Sciences* 165(1): 31-39. https://doi.org/10.1093/toxsci/kfy174

Mou, Y., Meng, J., Fu, X., Wang, X., Tian, J., Wang, M., Peng, Y. & Zhou, L. 2013. Antimicrobial and antioxidant activities and effect of 1-hexadecene addition on palmarumycin C2 and C3 yields in liquid culture of endophytic fungus *Berkleasmium* sp. Dzf12. *Molecules* 18(12): 15587-15599. https://doi.org/10.3390/molecules181215587

Muhammad Dain, Y., Shahrul Hisham, Z.A., Sahidan, S., Zaidah, Z.A. & Rohaya, M.A.W. 2011. Stem cell heterogeneity of mononucleated cells from murine peripheral blood: Molecular analysis. *The Scientific World Journal* 11: 2150-2159. https://doi.org/10.1100/2011/340278

Nair, R.V.R., Jayasree, D.V., Biju, P.G. & Baby, S. 2018. Anti-inflammatory and anticancer activities of erythrodiol-3-acetate and 2,4-di-tert-butylphenol isolated from *Humboldtia unijuga. Natural Product Research* 34(16): 2319-2322. https://doi.org/10.1080/14786419.2018.1531406

Nasir, N.A.H.A., Roslly, N.A.L., Rosli, N.M. & Razali, Z. 2020. Phytochemical screening and potential DPPH radical scavenging activity of banana peel extract. *Charting the Sustainable Future of ASEAN in Science and Technology 2.* Singapore: Springer. pp. 249-258.

Nikhila, G.S., Sangeetha, G., Preetha, T.S. & Swapna, T.S. 2016. GC-MS analysis of phytochemical compounds present in the rhizome of *Gloriosa superba* L. *Journal of Pharmacognosy and Phytochemistry* 5(5): 17-20.

O'Gorman, D.M., Tierney, C.M., Brennan, O. & O'Brien, F.J. 2011. The marine-derived, multi-mineral formula, aquamin, enhances mineralisation of osteoblast cells *in vitro. Phytotherapy Research* 26(3): 375-380. https://doi.org/10.1002/ptr.3561

Park, E., Kim, J., Yeo, S., Lim, E., Choi, C.W., Choi, S., Li, W.Y., Lee, J., Park, J., Huh, D. & Jeong, S.Y. 2019. Anti-osteoporotic effects of combined extract of *Lycii Radicis Cortex* and *Achyranthes japonica* in osteoblast and osteoclast cells and ovariectomized mice. *Nutrients* 11(11): 2716. https://doi.org/10.3390/nu11112716

Rajalakshmi, S., Vimalraj, S., Saravanan, S., Preeth, D.R., Shairam, M. & Anuradha, D. 2018. Synthesis and characterization of silibinin/phenanthroline/neocuproine copper(II) complexes for augmenting bone tissue regeneration: An *in vitro* analysis. *JBIC Journal of Biological Inorganic Chemistry* 23(5): 753-762. https://doi.org/10.1007/s00775-018-1566-4

Ritter, N.M., Farach-Carson, M.C. & Butler, W.T. 1992. Evidence for the formation of a complex between osteopontin and osteocalcin. *Journal of Bone and Mineral Research* 7(8): 877-885. https://doi.org/10.1002/jbmr.5650070804

Rodan, G.A. & Martin, T.J. 2000. Therapeutic approaches to bone diseases. *Science (New York, N.Y.)* 289: 1508-1514. doi:10.1126/science.289.5484.1508

Ruzanna, A.K., Shahrul Hisham, Z.A., Rohaya, M.A.W., Sahidan, S. & Fahrul, Z.H. 2011. Differentiation potential of human suspension mononucleated peripheral blood cells. *African Journal of Biotechnology* 10(52): 10756-10764. https://doi.org/10.5897/ajb11.436

Salazar, V.S., Gamer, L.W. & Rosen, V. 2016. BMP signalling in skeletal development, disease and repair. *Nature Reviews Endocrinology* 12(4): 203-221. https://doi.org/10.1038/nrendo.2016.12

Sanjeev, G., Sidharthan, D.S., Pranavkrishna, S., Pranavadithya, S., Abhinandan, R., Akshaya, R., Balagangadharan, K., Siddabathuni, N., Srinivasan, S. & Selvamurugan, N. 2020. An osteoinductive effect of phytol on mouse mesenchymal stem cells (C3H10T1/2) towards osteoblasts. *Bioorganic & Medicinal Chemistry Letters* 30(11): 127137. https://doi.org/10.1016/j.bmcl.2020.127137

Seow, L.J., Beh, H.K., Ibrahim, P., Sadikun, A. & Asmawi, M.Z. 2012. Antimicrobial activity of *Gynura segetum*'s leaf extracts and its active fractions. *CellMed* 2(2): 20.1-20.5.

Shahrul Hisham, Z.A., Lim, K.W., Rohaya, M.A.W., Zaidah, Z.A., Rus Dina, R.D., Muhammad Ashraf, S., Anis Nabilah, J. & Intan Zarina, Z.A. 2022. Gene expression profiles for *in vitro* human stem cell differentiation into osteoblasts and osteoclasts: A systematic review. *PeerJ* 10: e14174. https://doi.org/10.7717/peerj.14174

Shahrul Hisham, Z.A., Nur Akmal, M.R., Rohaya, M.A.W., Sahidan, S., Intan Zarina, Z.A. & Zaidah, Z.A. 2015. Analyses of basal media and serum for in vitro expansion of suspension peripheral blood mononucleated stem cell. *Cytotechnology* 68(4): 675-686. https://doi.org/10.1007/s10616-014-9819-8

Shahrul Hisham, Z.A., Intan Zarina, Z.A., Muhammad Dain, Y. & Rohaya, M.A.W. 2010. Differentiation analyses of adult suspension mononucleated peripheral blood cells of *Mus musculus. Cell Communication and Signaling* 8: 29. https://doi.org/10.1186/1478-811X-8-29

Shahrul Hisham, Z.A., Wan Haifa Haryani, W.O., Zaidah, A.A., Muhd Fauzi, S., Sahidan, S. & Rohaya, M.A.W. 2009. Intrinsic anticarcinogenic effects of *Piper sarmentosum* ethanolic extract on a human hepatoma cell line. *Cancer Cell International* 9: 6. https://doi.org/10.1186/1475-2867-9-6

Sharmila Juliet, Y., Kalimuthu, K., Vajjiram, C. & Ranjitha, V. 2018. Evaluation and comparison of phytochemical, GCMS and FTIR analysis of wild and micropropagated *Cadaba fruticosa* (L.). *World Journal of Pharmaceutical Research* 7(14): 746-760. doi: 10.20959/wjpr201814-12863

Sidney, L.E., Branch, M.J., Dunphy, S.E., Dua, H.S. & Hopkinson, A. 2014. Concise review: Evidence for CD34 as a common marker for diverse progenitors. *Stem Cells* 32(6): 1380-1389. doi:10.1002/stem.1661

Stein, G.S., Lian, J.B., Van Wijnen, A.J., Stein, J.L., Montecino, M., Javed, A., Zaidi, S.K., Young, D.W., Choi, J. & Pockwinse, S.M. 2004. Runx2 control of organization, assembly and activity of the regulatory machinery for skeletal gene expression. *Oncogene* 23(24): 4315-4329. https://doi.org/10.1038/sj.onc.1207676

Subramaniam, V., Adenan, M., Ahmad, A.R. & Sahdan, R. 2003. Natural antioxidants: *Piper sarmentosum* (Kadok) and *Morinda elliptica* (Mengkudu). *Malaysian Journal of Nutrition* 9(1): 41-51.

Varsha, K.K., Devendra, L., Shilpa, G., Priya, S., Pandey, A. & Nampoothiri, K.M. 2015. 2,4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated *Lactococcus* sp. *International Journal of Food Microbiology* 211: 44-50. https://doi.org/10.1016/j.ijfoodmicro.2015.06.025

Wong, S.K., Mohamad, N., Ibrahim, N.'I., Chin, K., Shuid, A.N. & Ima-Nirwana, S. 2019. The molecular mechanism of vitamin E as a bone-protecting agent: A review on current evidence. *International Journal of Molecular Sciences* 20(6): 1453. https://doi.org/10.3390/ijms20061453

Yang, Y., Chen, Y., Zhou, X., Hong, C., Li, C. & Guo, J. 2013. Beta-asarone, a major component of *Acorus tatarinowii* Schott, attenuates focal cerebral ischemia induced by middle cerebral artery occlusion in rats. *BMC Complementary and Alternative Medicine* 13: 236. https://doi.org/10.1186/1472-6882-13-236

Yoon, M., Jeong, T., Park, D., Xu, M., Oh, H., Song, K., Lee, W.S. & Park, H. 2006. Antioxidant effects of quinoline alkaloids and 2,4-Di-tert-butylphenol isolated from *Scolopendra subspinipes. Biological and Pharmaceutical Bulletin* 29(4): 735-739. https://doi.org/10.1248/bpb.29.735

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