SCREENING FOR VIETNAMESE PLANT EXTRACTS WITH POTENTIAL BENEFIT FOR ANTI-OSTEOCLASTOGENESIS

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Abstract. Bone's homeostasis is only achieved when there is a balance between bone formation and bone resorption. A metabolic disorder of bone-resorbing osteoclasts can lead to osteoporosis. Long-term use of anti-osteoporosis drugs can lead to undesirable side effects so traditional herbs can be a potential source of alternative medicine. In the present study, forty-one Vietnamese plants (seventy methanol extracts) were screened for osteoclastogenesis inhibitory activities on RAW264.7 mouse macrophage cells. For the first time, 29 extracts from 24 species: Thunbergia grandiflora (Roxb. ex Rottl.) Roxb., Saurauia nepaulensis DC, Choerospondias axillaris (Roxb.) Burtt., Xylopia vielana Pierre, Ilex godajam Colebr. ex Hook.f., Ilex kaushue S.Y. Hu, Pothis repens (Lour.) Druce, Carallia brachiate (Lour.) Merr., Oroxyllum indicum (L.) Kurz, Bombax ceiba L., Stixis fasciculata (King) Gagn., Viburnum lutescens Blume, Garcinia multiflora Champ. ex Benth., Garcinia ohiangifolia Champ. ex Benth., Amishclotype mollissima (Blume) Hassk., Trichosanthes rubriflos Thorel ex Cayla, Dichapetalum longepetalum (Turcz.) Engl., Cleistanthus tonkinensis Jabl., Homonoia riparia Lour., Macaranga henryi (Pax & K.Hoffm.) P. Rehder, Croton tiglium L., Chaetocarpus castanocarpus (Roxb.) Thwaites, Phyllanthus emblica L., Hydnocarpus hainanensis (Merr.) Sleum, Hydnocarpus anthelminicus Pierre, Illigera rhodantha Hance, Cratoxylum cochinchenense (Lour.) Blume, Machilus thunbergii Sieb. & Zucc., Saraca dives Pierre, Piper longum L., Nageia fleuryi (Hickel) de Laub., Gouania javanica Miq., Psychotria rubra (Lour.) Poir., Mussaenda pubescens W. T. Aiton, Mycetia balansae Drake, Clausena excavata Burm.f., Zanthoxylum avicennae (Lam.) DC., Turpinia montana (Blume) Kurz, Helicteres hirsuta Lour., Symlocos cambodiana (Pierre) Hallier f., Eurya cerasifolia (D.Don) Kobuski, Pellionia repens (Lour.) Merr. showed potential as effective inhibitors of osteoclastogenesis.

Keywords: Vietnamese plant extracts, osteoclastogenesis, osteoclast, bone resorption, osteoporosis.

Classification numbers: 1.2.1.

1. INTRODUCTION

Bone is a connective tissue that mechanically supports movement and also acts as a metabolic organ with large reserves of calcium and phosphate. Bone remodeling is an important physiological process in which old bones are degraded and replaced, performed by a
combination action of 4 types of bone cells: bone lining cells, osteocytes, osteoclasts, and osteoblasts. The bone surface is made up of bone lining cells and the osteocytes are the main mechanical cells that play a role in controlling the initial stage of bone remodeling. Osteoclasts are large multinucleated cells that dissolve the bone whereas osteoblasts are the bone-forming cells [1]. The balance of bone formation and resorption processes play a key role in bone development and homeostasis. The imbalance between bone forming cells and bone resorbing cells may lead to abnormal bone conditions: too much bone formation (osteopetrosis) or too little bone (osteoporosis) [2]. Osteoporosis is one of the major bone health problems today, and as a result of estrogen deficiency after menopause, it occurs most commonly in women. Approximately 50% of women suffer from osteoporotic fractures throughout their lifetime [3]. Osteoclastogenesis is mainly regulated by two essential cytokines: Macrophage colony-stimulation factor (M-CSF) and Receptor activator of NF-κB (RANK) ligand (RANKL) promotes the proliferation and survival of osteoclast precursors while RANKL participates in the osteoclastogenesis by interacting with RANK on the precursor’s surface and driving the osteoclast precursor into osteoclast [4]. Drugs that target the inhibition of the formation or activity of osteoclasts are valuable for osteoporosis treatment [5]. However, the long-term use of medications such as bisphosphonates and estrogen therapy results in several adverse effects [6,7]. Because of patients’ concerns, it is essential to search for new anti-osteoporotic agents.

In developing countries, medicinal plants may bring important sources of health care. Vietnam has high biodiversity as a source of medicine with more than 12,000 species. In this study, forty-one Vietnamese plants (seventy methanol extracts) were collected from the Me Linh station for Biodiversity and screened for the inhibitory activities of osteoclastogenesis in RAW264.7 murine macrophages.

### 2. MATERIALS AND METHODS

#### 2.1. Materials

**Plant materials**

A total of 41 plants were randomly collected in Me Linh Station for Biodiversity, Vinh Phuc, Vietnam in Spring 2019. The plant samples were identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher of specimens was deposited at the Department of Life Sciences, University of Science and Technology of Hanoi.

| No. | Family       | Species                                  | Vietnamese name | Part used* |
|-----|--------------|------------------------------------------|-----------------|------------|
| S1  | Acanthaceae  | *Thunbergia grandiflora* (Roxb. ex Rottl.) | Dây bông báo    | L          |
| S2  | Acanthaceae  | *Thunbergia grandiflora* (Roxb. ex Rottl.) | Dây bông báo    | S          |
| S3  | Actinidiaceae| *Saurauia nepaulensis* DC.               | Nóng hoa nhọn    | F          |
| S4  | Anacardiaceae| *Choerospondias axillaris* (Roxb.) Burtt. | Xoan nhừ        | L          |
| S5  | Anacardiaceae| *Choerospondias axillaris* (Roxb.) Burtt. | Xoan nhừ        | S          |
| S6  | Anacardiaceae| *Choerospondias axillaris* (Roxb.) Burtt. | Xoan nhừ        | Fr         |
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S7 Annonaceae Xylopia vielana Pierre Giễn dộ L
S8 Aquifoliaceae Illex godajam Colebr. ex Hook.f. Vố rat S
S9 Aquifoliaceae Illex kaushue S.Y. Hu Chè dâng S
S10 Aquifoliaceae Illex kaushue S.Y. Hu Chè dâng Fr
S11 Araceae Pothis repens (Lour.) Druce Rày le S
S12 Rhizophoraceae Carallia brachiate (Lour.) Merr. Sâng mà S
S13 Bignoniaceae Oroxylum indicum (L.) Kurz Núc nác L
S14 Bignoniaceae Oroxylum indicum (L.) Kurz Núc nác Fr
S15 Bignoniaceae Oroxylum indicum (L.) Kurz Núc nác S
S16 Bombacaceae Bombax ceiba L. Gạo S
S17 Capparaceae Stixis fasciculata (King) Gagn. Trùng quốc L
S18 Capparaceae Stixis fasciculata (King) Gagn. Trùng quốc S
S19 Caprifoliaceae Viburnum lutescens Blume Vót S
S20 Caprifoliaceae Viburnum lutescens Blume Vót L
S21 Caprifoliaceae Viburnum lutescens Blume Vót F
S22 Clusiaceae Garcinia multiflora Champ. ex Benth. Độc L
S23 Clusiaceae Garcinia multiflora Champ. ex Benth. Độc S
S24 Clusiaceae Garcinia oblongifolia Champ. ex Benth. Bỉa L
S25 Clusiaceae Garcinia oblongifolia Champ. ex Benth. Bỉa S
S26 Commelinaceae Amischlotype mollissima (Blume) Hassk. Thái lăi rừng B
S27 Cucurbitaceae Trichosanthes rubriflos Thorel ex Cayla Quả láu hoa đỏ B
S28 Dichapetalaceae Dichapetalum longepetalum (Turcz.) Engl. A tràng cánh hoa dài B
S29 Euphorbiaceae Cleistanthus tonkinensis Jabl. Cọc rào L
S30 Euphorbiaceae Cleistanthus tonkinensis Jabl. Cọc rào S
S31 Euphorbiaceae Homonoia riparia Lour. Rỉ rỉ L
S32 Euphorbiaceae Macaranga henryi (Pax & K.Hoffm.) Rehder Mả rằng S
S33 Euphorbiaceae Macaranga henryi (Pax & K.Hoffm.) Rehder Mả rằng F
S34 Euphorbiaceae Croton tiglium L. Bả đau S
S35 Euphorbiaceae Croton tiglium L. Bả đau F
S36 Euphorbiaceae Chaetocarpus castanocarpus (Roxb.) Thwaites Dạ nau L
S37 Euphorbiaceae Chaetocarpus castanocarpus (Roxb.) Thwaites Dạ nau S
S38 Euphorbiaceae Chaetocarpus castanocarpus (Roxb.) Thwaites Dạ nau Fr
S39 Euphorbiaceae Phyllanthus emblica L. Me riêng L
S40 Euphorbiaceae Phyllanthus emblica L. Me riêng S
S41 Flacourtiaceae Hydnocarpus hainanensis (Merr.) Sleum Lô noni hài nam B
S42 Flacourtiaceae Hydnocarpus hainanensis (Merr.) Sleum Lô noni hài nam Fr
S43 Flacourtiaceae Hydnocarpus anthelminthicus Pierre Chùm bao lớn F
S44 Hernandiaceae Illigera rhodantha Hance Liên dân họa nhớ S
S45 Hernandiaceae Illigera rhodantha Hance Liên dân họa nhớ S
S46 Hypericaceae Cratoxylum cochinchenense (Lour.) Thành ngạch L
2.2. Methods

2.2.1. Preparation of extracts

Different parts of the collected plants including branches (B), leaves (L), roots (R), stems (S), fruits (Fr), and flowers (F) were classified. After drying by oven and cutting into pieces, the samples were extracted with the ratio sample:MeOH 1:1 (w/v) for 20 minutes, followed by 15 minutes in an ultrasonicator. This process was repeated 3 times, the collected solvent was dried by the vacuum evaporator and the corresponding residues were concentrated. The crude extracts were stored at -10°C until assayed.

2.2.2. RAW264.7 cell culture

RAW264.7 cells (murine macrophages) were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were maintained with DMEM supplemented with 10% FBS and penicillin (100 units/ml)-streptomycin (100 μg/ml) at 37°C in a humidified atmosphere of 5% CO₂.
2.2.3. Assay for in vitro differentiation of osteoclasts

RAW264.7 cells were seeded at a density of $5 \times 10^4$ cells/ml onto 96-well plates. For osteoclast differentiation, the cells were stimulated with receptor activator of NF-κB ligand (RANKL, 100 ng/ml; R&D Systems, Minneapolis, MN, USA) in the presence or absence of the extracts at the concentration of 25 µg/ml for 4 days. The extracts and culture media were replaced every two days for four days.

2.2.4. Tartrate-resistant acid phosphatase (TRAP) staining assay

Histochemical staining of the cells was performed with an acid phosphatase leukocyte kit (Sigma-Aldrich; EMD Millipore, Billerica, MA, USA). The assayed cells were fixed with 3.7 % formalin for 15 min, permeabilized with 0.1 % Triton X-100. TRAP staining was performed following the instructions of the manufacturer. TRAP-positive multinucleated cells with more than three nuclei were defined as osteoclasts. The cells were photographed under microscopy model CKX-53 (Olympus, Tokyo, Japan).

2.2.5. Cell viability assay

Cell proliferation and cytotoxic effect of the extracts (25 µg/ml) were assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) - based assay. MTT solution (0.5 mg/mL) was added to each well and incubated for 3 h. Then, the culture supernatants were removed, and formazan crystals were dissolved in DMSO. Absorbance was measured at 540 nm using a microplate reader (Epoch Spectrophotometer, Biotek, VT, USA).

The percentage of cell viability was calculated using the formula:

$$\%\text{Cell viability} = \frac{AS}{AC} \times 100,$$

$$\%\text{Inhibition} = 100\% - \%\text{Cell viability},$$

where: AS is the absorbance of sample, AC is the absorbance of negative control.

2.2.6. Statistical analysis

Data are expressed as mean ± standard error (SE). Statistical significance was assessed by Student’s t test. In all analyses, P values of less than 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

In the present study, 41 plants (Fig. 1) divided into 70 extracts were screened (Table 1) for the inhibition of osteoclastogenesis in RAW264.7 murine macrophages. The cell line has proven to be an important primary osteoclast precursor for in vitro studies of osteoclast formation. By interacting with RANK on the RAW264.7 cell’s surface, RANKL promotes the differentiation of osteoclast precursors into osteoclasts [8]. At the concentration of 25 µg/ml, the negative effects on osteoclastogenesis inhibition were recorded in 23 samples, 17 methanol extracts revealed the cytotoxicity effects (data not shown), and 29 extracts inhibited the osteoclast differentiation of RAW264.7 cells without significant impact on the cell survival (Table 2).
Table 2. The effects of the plant extracts in the inhibition of osteoclastogenesis in RAW264.7 cells.

| No. | Species                          | Effect | No. | Species                             | Effect   |
|-----|----------------------------------|--------|-----|-------------------------------------|----------|
| S1  | Thunbergia grandiflora (Roxb. ex Rottl.) Roxb. | < 50 % | S36 | Chaetocarpus castanocarpus (Roxb.) Thwaites | 97 %     |
| S2  | Thunbergia grandiflora (Roxb. ex Rottl.) Roxb. | 66.2 % | S37 | Chaetocarpus castanocarpus (Roxb.) Thwaites | Toxic    |
| S3  | Saurauia nepaulensis DC.         | 97 %   | S38 | Chaetocarpus castanocarpus (Roxb.) Thwaites | < 50 %   |
| S4  | Choerospondias axillaris (Roxb.) Burt. | Toxic  | S39 | Phyllanthus emblica L.               | 66.7 %   |
| S5  | Choerospondias axillaris (Roxb.) Burt. | < 50 % | S40 | Phyllanthus emblica L.               | 97.5 %   |
| S6  | Choerospondias axillaris (Roxb.) Burt. | Toxic  | S41 | Hydnocarpus hainanensis (Merr.) Sleum Pierre | Toxic    |
| S7  | Xylopia vielana Pierre           | 91 %   | S42 | Hydnocarpus hainanensis (Merr.) Sleum Pierre | < 50 %   |
| S8  | Ilex godajam Celebr. ex Hook.f.  | < 50 % | S43 | Hydnocarpus anthelminticus Pierre     | Toxic    |
| S9  | Ilex kaushae S.Y. Hu             | 95 %   | S44 | Illigera rhodantha Hance             | < 50 %   |
| S10 | Ilex kaushae S.Y. Hu             | < 50 % | S45 | Illigera rhodantha Hance             | < 50 %   |
| S11 | Pothis repens (Lour.) Druce      | < 50 % | S46 | Cratoxylym cochinchnense (Lour.) Blume | < 50 %   |
| S12 | Carallia brachiate (Lour.) Merr. | < 50 % | S47 | Cratoxylym cochinchnense (Lour.) Blume | 98.5 %   |
| S13 | Oroxylum indicum (L.) Kurz       | Toxic  | S48 | Machilus thunbergii Sieb. & Zucc.   | 97 %     |
| S14 | Oroxylum indicum (L.) Kurz       | 91 %   | S49 | Machilus thunbergii Sieb. & Zucc.   | Toxic    |
| S15 | Oroxylum indicum (L.) Kurz       | < 50 % | S50 | Saraca dives Pierre                 | < 50 %   |
| S16 | Bombax ceiba L.                  | 83.1 % | S51 | Piper longum L.                     | 97.5 %   |
| S17 | Sitis fasciculata (King) Gagn.   | 92.5 % | S52 | Piper longum L.                     | 96.5 %   |
| S18 | Sitis fasciculata (King) Gagn.   | < 50 % | S53 | Piper longum L.                     | Toxic    |
| S19 | Viburnum utesces Blume           | 62.2 % | S54 | Nageia fleuri (Hickel) de Laub.     | 98 %     |
| S20 | Viburnum utesces Blume           | 91 %   | S55 | Gouania javanica Miq.               | Toxic    |
| S21 | Viburnum utesces Blume           | Toxic  | S56 | Psychotria rubra (Lour.) Poir.      | < 50 %   |
| S22 | Garcinia multiflora Champ. ex Benth. | Toxic | S57 | Psychotria rubra (Lour.) Poir.      | 73.6 %   |
| S23 | Garcinia multiflora Champ. ex Benth. | Toxic | S58 | Psychotria rubra (Lour.) Poir.      | < 50 %   |
| S24 | Garcinia oblongifolia Champ. ex Benth. | < 50 % | S59 | Mussaenda pubescens W. T. Aiton     | 95.8 %   |
| S25 | Garcinia oblongifolia Champ. ex Benth. | 98 %  | S60 | Mussaenda pubescens W. T. Aiton     | 92 %     |
| S26 | Antschlotype mollissima (Blume)  | Toxic  | S61 | Mussaenda pubescens W. T. Aiton     | < 50 %   |
| S27 | Trichosanthes rubriflos Thorel ex Cayla | 97.5 % | S62 | Mycetia balansa Drake.              | Toxic    |
| S28 | Dichapetalum longpetalum (Turcz) Engl. | 97 %  | S63 | Clausena excavata Burm.f.           | 95.5 %   |
| S29 | Cleistanthus tonkinensis Jabl.    | < 50 % | S64 | Zanthoxylum avicennae (Lam.) DC.     | Toxic    |
| S30 | Cleistanthus tonkinensis Jabl.    | < 50 % | S65 | Turpinia montana (Blume) Kurz       | 66.2 %   |
| S31 | Homonoia riparia Lour.           | Toxic  | S66 | Turpinia montana (Blume) Kurz       | 54.2 %   |
| S32 | Macaranga henryi (Pax & K.Hoffm.) Rehder | < 50 % | S67 | Heliceres hirsuta Lour.             | < 50 %   |
| S33 | Macaranga henryi (Pax & K.Hoffm.) Rehder | Toxic | S68 | Symplcos cambodianna (Pierre) Hallier f. | 81.6 % |
| S34 | Croton tigilum L.                | < 50 % | S69 | Eurya cerasifolia (D.Don) Kobuski    | 93.5 %   |
| S35 | Croton tigilum L.                | < 50 % | S70 | Pellionia repens (Lour.) Merr.       | 94 %     |
Among 29 positive samples, 23 samples exhibited very strong inhibitory effects with more than 75% osteoclastogenesis inhibition, and 6 samples displayed the moderate effect with the suppression of osteoclastogenesis by 50 - 75% compared to the control (Fig. 2).

According to our results, both leaf and stem extracts of *Phyllanthus emblica* L. (S39 and S40) have osteoclastogenesis inhibitory effects in RAW264.7 cells (66.5% and 97.5% at 25
µg/ml, respectively). The previous study showed geraniin and quercetin were two of the chief components of the fruits of this plant [9]. Geraniin has been shown effective in preventing osteoporosis whereas quercetin is a potent inhibitor of osteoclastogenesis. The ability of quercetin in increasing the new bone formation was also recorded when this compound was presented in a collagen matrix [10 - 12]. Belonging to the Euphorbiaceae family with high inhibitory effects like P. emblica L., but based on our knowledge, there is no information about the chemical components or the effect on the bone disease on C. castanocarpus (Roxb.) Thwaites (S38).

*Piper longum* L., known as long pepper, has not only been used as an important spice but also as an important ingredient in traditional medicine. In this study, the methanol extracts from *Piper longum* L. leaves and stems (S51 and S52) showed anti-osteoclastogenesis potential with a very strong effect of more than 95% osteoclastogenesis inhibition (Fig. 1). Piperine – an alkaloid presented in this plant was shown to inhibit the osteoclast formation in RAW264.7 macrophages and human CD14+ monocytes by suppressing the p38/NFATc1/c-Fos signaling axis [13].

*Psychotria* (Rubiaceae) is a genus potentially used in pharmacology due to the discovery of many active compounds. The phytochemical components of *Psychotria rubra* were well investigated but no study about anti-osteoclastogenic activity of these metabolites was recorded [14]. Our results indicated that *P. rubra* stems and leaves (S57 and S59) may be a potential source for the discovery of anti-osteoclastogenic agents. Belong to the same family Rubiaceae as *P. rubra*, however, the information about the chemical contents *Mussaenda pubescens* W. T. Aiton (S59 and S60) still has been undiscovered.

*Figure 2.* Plant extracts inhibit RANKL-induced osteoclastogenesis in RAW264.7 cells. Only the samples with significant changes in the inhibitory effects are shown. RAW264.7 cells were seeded into 96 well-plates under stimulation of RANKL (100 ng/ml) with or without indicated concentrations of plant extracts for 4 days, then the cells were stained for TRAP. The quantities of TRAP-positive multinucleated (> 3 nuclei) osteoclasts were determined following image capture (magnification, x40). Data are presented as the mean ± SE (*P < 0.05, versus vehicle-treated control; n = 3).

Previous study showed that lupeol - one of the chief components of *Pellionia repens* (Lour.) Merr (S70) [15], *Cratoxylum cochinchinense* (Lour.) Blume (S47) [16], and *Bombax ceiba* L. (S16) [17] exhibits strong inhibitory effects on osteoclastogenesis [18]. *Machilus thunbergii* Sieb. & Zucc. (S48) has been used in folk medicine for a long period. Machilin A,
one of the bioactive components of this plant, has been proven to be capable of stimulating osteoblast differentiation in MC3T3-E1 cells [19, 20]. In our study, the extracts of these species significantly inhibited the differentiation to osteoclasts in RAW264.7 cells with the inhibition percentage ranging from 98.5 % and 83.1 % at the tested concentration (Fig. 2).

Oroxylum indicum with the main components from seeds including chrysin, baicalein, and quercetin [21] could be the best explanation of its anti osteoclastogenesis properties. The three compounds have been shown as having promising preventive effects for bone diseases related to excessive bone resorption and bone formation [12, 22, 23] Our experimental data showed that O. indicum fruit extract (S14) has a strong osteoclastogenesis inhibitory effect with 91 % inhibition. Thunbergia grandiflora (S2) is also a promising candidate for anti-osteoporosis drug development. Major constituents of this plant are caffeic acid which showed powerful inhibitory effects on osteoclastogenesis on the rat [24], and other prominent elements are quercetin which enhances bone cell proliferation [25].

Clausena excavata Burm.f. (S63) is widely used in traditional medicine for the cure of many diseases. The morphological, phytochemical, and pharmacological aspects of this plant were profoundly studied. However, the information about the anti-osteoporosis properties of this plant is still limited [26]. Several compounds were isolated from Garcinia oblongifolia Champ. ex Benth. (S25), Ilex kaushue S.Y. Hu (S9), and Xylopia vielana Pierre (S7), however no evidence on bone effects of these chemicals were recorded [27 - 29]. Our data about the positive effects of these samples may be a benefit for further studies on the discovery of the anti-osteoclastogenic components from the plants.

The bioactivities of medicinal plants are mostly contributed by the properties of their constituents. Based on our knowledge, there has been no information about the chemicals or the evaluation of effects on bone diseases related to Turpinia montana (Blume) Kurz (S65 and S66), Symplocos cambodiana (Pierre) Hallier f. (S68), Eurya cerasifolia (D.Don) Kobuski (S69), Nageia fleuryi (Hickel) de Laub (S54), Dichapetalum longepetalum (Turcz) Engl (S28), Saurauia nepaulensis DC. (S3), Viburnum lutescens Blume (S19 and S20), and Stixis fasciculate (King) Gagn. (S17). This is both an opportunity and a challenge for us in further research on these plants.

4. CONCLUSIONS

In our present study, 70 plant extracts were screened for the osteoclastogenesis inhibitory effects in RAW264.7 murine macrophages. Twenty-nine extracts from 24 plants have potential as effective agents against osteoclastogenesis. This is the first but important assessment step, providing a preliminary overview of the anti-osteoclastogenesis ability of these plants. In a future study, potential plant species will be studied further, especially species whose chemical constituents have not yet been studied. The phytochemical components of these plants should be analyzed and the active components of these plants should be evaluated.

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Declaration of competing interest. All authors declared that they have no conflicts of interest.
REFERENCES

1. Xu F., Teitelbaum S. L. - Osteoclasts: New insights, Bone Res. 1 (2013) 11-26.
2. Lazner F., Gowen M., Pavasovic D., Kola I. - Osteopetrosis and osteoporosis: two sides of the same coin, Hum Mol Genet. 8 (10) (1999) 1839-1846.
3. Lems W. F., Ratman H. G. - Critical issues and current challenges in osteoporosis and fracture prevention. An overview of unmet needs, Ther. Adv. Musculoskelet Dis. 9 (12) (2017) 299-316.
4. Steven L. T. - Bone resorption by osteoclasts, Science 289 (5484) (2000) 1504-1507.
5. Gideon A. R., Martin T. J. - Therapeutic approaches to bone diseases, Science 289 (5484) (2000) 1508-1514.
6. Judd H. L., Cleary R. E., Creasman W. T., Figge D. C., Kase N., Rosenwaks Z., Tagatz G. E. - Estrogen replacement therapy, Obstet Gynecol 58 (3) (1981) 267-275.
7. Kennel K. A., Drake M. T. - Adverse effects of bisphosphonates: Implications for osteoporosis management, Mayo Clin Proc. 84 (7) (2009) 632-638.
8. Vincent C., Kogawa M., Findlay D. M., Atkins G. J. - The generation of osteoclasts from RAW 264.7 precursors in defined, serum-free conditions, J. Bone Miner. Metab. 27 (1) (2009) 114-119.
9. Liu X., Cui C., Zhao M., Wang J., Luo W. - Identification of phenolics in the fruit of emblica (Phyllanthus emblica L.) and their antioxidant activities, Food Chem. 109 (2008) 909-915.
10. Forte L., Torricelli P., Boanini E., Gazzano M., Rubini K., Fini M., Bigi A. - Antioxidant and bone repair properties of quercetin-functionalized hydroxyapatite: An in vitro osteoblast–osteoclast–endothelial cell co-culture study, Acta Biomater 32 (2016) 298-308.
11. Mo J., Yang R., Li F., He B., Zhang X., Zhao Y., Shen Z., Chen P. - Geraniin promotes osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) via activating β - catenin: a comparative study between BMSCs from normal and osteoporotic rats, J. Nat. Med. 73 (1) (2019) 262-272.
12. Wong R. W. K., Rabie A. B. M. - Effect of quercetin on bone formation, J. Orthop Res. 26 (8) (2008) 1061-1066.
13. Deepak V., Kruger M. C., Joubert A., Coetzee M. - Piperine alleviates osteoclast formation through the p38/c-Fos/NFATc1 signaling axis, BioFactors 41 (6) (2015) 403-413.
14. Calixto N. O., Pinto M. E. F., Ramalho S. D., Burger M. C. M., Bobey A. F., Young M. C. M., Bolzani V. S., Pinto A. C. - The genus Psychotria: phytochemistry, chemotaxonomy, ethnopharmacology and biological properties, J. Braz. Chem. Soc. 27 (8) (2016) 1355-1378.
15. Luo Y., Liu Y., Qi H., Zhang G. - A new glucoceramide from the watermelon begonia, Pellionia repens, Lipids 39 (10) (2004) 1037-1042.
16. Nguyen L. H. D., Harrison L. J. - Triterpenoid and xanthone constituents of Cratoxylum cochinchinense, Phytochemistry 50 (3) (1999) 471-476.
17. You Y. J., Nam N. H., Kim Y., Bae K. H., Ahn B. Z. - Antiangiogenic activity of lupeol from Bombax ceiba, Phyther Res. 17 (4) (2003) 341-344.
18. Im N. K., Lee D. S., Lee S. R., Jeong G. S. - Lupeol isolated from *Sorbus commixta* suppresses 1α,25-(OH)2D3-mediated osteoclast differentiation and bone loss in vitro and in vivo, J. Nat. Prod. 79 (2) (2016) 412-420.
19. Lee S. U., Shim K. S., Ryu S. Y., Min Y. K., Kim S. H. - Machilin A isolated from *Myristica fragrans* stimulates osteoblast differentiation, Planta Med. 75 (2) (2009) 152-157.
20. Ma C. J., Sung S. H., Kim Y. C. - Neuroprotective lignans from the bark of *Machilus thunbergii*, Planta Med. 70 (1) (2004) 79-80.
21. Tran T. V. A., Malainer C., Schwaiger S., Hung T., Atanasov A. G., Heiss E. H., Dirsch V. M. - Stupner H. Screening of Vietnamese medicinal plants for NF-κB signaling inhibitors: Assessing the activity of flavonoids from the stem bark of *Oroxylum indicum*, J. Ethnopharmacol 159 (2015) 36-42.
22. Kim M. H., Ryu S. Y., Bae M. A., Choi J. S., Min Y. K., Kim S. H. - Baicalein inhibits osteoclast differentiation and induces mature osteoclast apoptosis, Food Chem. Toxicol 46 (11) (2008) 3375-3382.
23. Xianghe L., Wei L., Junxian H., Jing Y., Xinyun H., Shiwu D., Xianteng Y., Senlei L., Zhihui Y., Yingjie N., Tian X., Sun L. - Chrysin inhibits mouse osteoclastogenesis induced by receptor activator of nuclear factor kappa B ligand, Chinese J. Tissue Engin Res. 23 (25) (2019) 3978-3986.
24. Quan Y. T., Kukita T., Ushijima Y., Kukita A., Nagata K., Sandra F., Watanabe S., Toh K., Okuma Y., Kawasaki S., Okumura Y., Teramachi J., Miyamoto I., Wu Z., Iijima T. - Regulation of osteoclastogenesis by Simon extracts composed of caffeic acid and related compounds: Successful suppression of bone destruction accompanied with adjuvant-induced arthritis in rats, Histochem Cell. Biol. 125 (3) (2006) 215-225.
25. Ibrahim M., Wafa S. A. A. El, Sleem A. - Phytochemical and biological investigation of *Thunbergia grandiflora*, J. Pharmacogn Phytochem 6 (2017) 43-51.
26. Arbab I. A., Abdul A. B., Aspollah M., Abdullah R., Abdelwahab S.I., Mohan S., Abdelmageed A. H. A. - *Clausena excavata* Burm. f. (Rutaceae): A review of its traditional uses, pharmacological and phytochemical properties, J. Med. Plant Res. 5 (33) (2011) 7177-7184.
27. Shan W. G., Lin T. S., Yu H. N., Chen Y., Zhan Z. J. - Polyprenylated xanthones and benzophenones from the bark of *Garcinia oblongifolia*, Helv Chem. Acta. 95 (8) (2012) 1442-1448.
28. Thi H. T., Hoan D., Thi H. Cam H., Pham C., Erik P. - Isolation and biological testing of constituents from *Ilex kaushue* S.Y.Hu (Aquifoliaceae) Viet Nam, Nat. Prod. Chem. Res. 7 (2) (2019) 366.
29. Zhang Y. L., Xu Q. Q., Zhou X. W., Wu L., Wang X. B., Yang M. H., Luo J., Luo J. G., Kong L. Y. - Rare dimeric guaianes from *Xylopia vielana* and their multidrug resistance reversal activity, Phytochemistry 158 (2019) 26-34.