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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, *Pothos repens* (Lour.) Druce, *Carallia brachiate* (Lour.) Merr., *Oroxylum indicum* (L.) Kurz, *Bombax ceiba* L., *Stixis fasciculata* (King) Gagn., *Viburnum lutescens* Blume, *Garcinia multiflora* Champ. ex Benth., *Garcinia oblongifolia* Champ. ex Benth., *Amischlotype mollissima* (Blume) Hassk., *Trichosanthes rubriflos* Thorel ex Cayla, *Dichapetalum longepetalum* (Turcz) Engl., *Cleistanthus tonkinensis* Jabl., *Homonoia riparia* Lour., *Macaranga henryi* (Pax & K.Hoffm.) Rehder, *Croton tiglium* L., *Chaetocarpus castanocarpus* (Roxb.) Thwaites, *Phyllanthus emblica* L., *Hydnocarpus hainanensis* (Merr.) Sleum, *Hydnocarpus anthelminhicus* Pierre, *Illigera rhodantha* Hance, *Cratoxylum cochinchinense* (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., *Symplocos 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. Viet Nam 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, Viet Nam 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.

Table 1. List of plants used for screening of anti-osteoclastogenesis effects.

No.	Family	Species	Vietnamese name	Part used*
S1	Acanthaceae	*Thunbergia grandiflora* (Roxb. ex Rottl.) Roxb.	*Dây bông báo*	*L*
S2	Acanthaceae	*Thunbergia grandiflora* (Roxb. ex Rottl.) Roxb.	*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*

S7	Annonaceae	*Xylopia vielana* Pierre	*Giền đỏ*	*L*
S8	Aquifoliaceae	*Ilex godajam* Colebr. ex Hook.f.	*Vỏ rụt*	*S*
S9	Aquifoliaceae	*Ilex kaushue* S.Y. Hu	*Chè đắng*	*S*
S10	Aquifoliaceae	*Ilex kaushue* S.Y. Hu	*Chè đắng*	*Fr*
S11	Araceae	*Pothos repens* (Lour.) Druce	*Ráy le*	*S*
S12	Rhixophoraceae	*Carallia brachiate* (Lour.) Merr.	*Săng mả*	*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.	*Trứng quốc*	*L*
S18	Capparaceae	*Stixis fasciculata* (King) Gagn.	*Trứng quốc*	*S*
S19	Caprifoliaceae	*Viburnum lutescens* Blume	*Vót*	*L*
S20	Caprifoliaceae	*Viburnum lutescens* Blume	*Vót*	*S*
S21	Caprifoliaceae	*Viburnum lutescens* Blume	*Vót*	*F*
S22	Clusiaceae	*Garcinia multiflora* Champ. ex Benth.	*Dọc*	*L*
S23	Clusiaceae	*Garcinia multiflora* Champ. ex Benth.	*Dọc*	*S*
S24	Clusiaceae	*Garcinia oblongifolia* Champ. ex Benth.	*Bứa*	*L*
S25	Clusiaceae	*Garcinia oblongifolia* Champ. ex Benth.	*Bứa*	*S*
S26	Commelinaceae	*Amischlotype mollissima* (Blume) Hassk.	*Thài lài rừng*	*B*
S27	Cucurbitaceae	*Trichosanthes rubriflos* Thorel ex Cayla	*Qua lâu 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.	*Rù rì*	*L*
S32	Euphorbiaceae	*Macaranga henryi* (Pax & K.Hoffm.) Rehder	*Mã rạng*	*S*
S33	Euphorbiaceae	*Macaranga henryi* (Pax & K.Hoffm.) Rehder	*Mã rạng*	*F*
S34	Euphorbiaceae	*Croton tiglium* L.	*Bã đậu*	*S*
S35	Euphorbiaceae	*Croton tiglium* L.	*Bã đậu*	*F*
S36	Euphorbiaceae	*Chaetocarpus castanocarpus* (Roxb.) Thwaites	*Dạ nâu*	*L*
S37	Euphorbiaceae	*Chaetocarpus castanocarpus* (Roxb.) Thwaites	*Dạ nâu*	*S*
S38	Euphorbiaceae	*Chaetocarpus castanocarpus* (Roxb.) Thwaites	*Dạ nâu*	*Fr*
S39	Euphorbiaceae	*Phyllanthus emblica* L.	*Me rừng*	*L*
S40	Euphorbiaceae	*Phyllanthus emblica* L.	*Me rừng*	*S*
S41	Flacourtiaceae	*Hydnocarpus hainanensis* (Merr.) Sleum	*Lọ nồi hải nam*	*B*
S42	Flacourtiaceae	*Hydnocarpus hainanensis* (Merr.) Sleum	*Lọ nồi hải nam*	*Fr*
S43	Flacourtiaceae	*Hydnocarpus anthelminhicus* Pierre	*Chùm bao lớn*	*F*
S44	Hernandiaceae	*Illigera rhodantha* Hance	*Liên đằng hoa nhỏ*	*L*
S45	Hernandiaceae	*Illigera rhodantha* Hance	*Liên đằng hoa nhỏ*	*S*
S46	Hypericaceae	*Cratoxylum cochinchinense* (Lour.)	*Thành ngạch*	*L*

		Blume		
S47	Hypericaceae	*Cratoxylum cochinchinense* (Lour.) Blume	*Thành ngạch*	*S*
S48	Lauraceae	*Machilus thunbergii* Sieb. & Zucc.	*Kháo*	*L*
S49	Lauraceae	*Machilus thunbergii* Sieb. & Zucc.	*Kháo*	*S*
S50	Leguminosae: Caesalpinioideae	*Saraca dives* Pierre	*Vàng anh*	*S*
S51	Piperaceae	*Piper longum* L.	*Tiêu dài*	*L*
S52	Piperaceae	*Piper longum* L.	*Tiêu dài*	*S*
S53	Piperaceae	*Piper longum* L.	*Tiêu dài*	*Fr*
S54	Podocarpaceae	*Nageia fleuryi* (Hickel) de Laub.	*Kim giao*	*L*
S55	Rhamnaceae	*Gouania javanica* Miq.	*Dây đòn gánh*	*L*
S56	Rubiaceae	*Psychotria rubra* (Lour.) Poir.	*Lấu đỏ*	*L*
S57	Rubiaceae	*Psychotria rubra* (Lour.) Poir.	*Lấu đỏ*	*S*
S58	Rubiaceae	*Psychotria rubra* (Lour.) Poir.	*Lấu đỏ*	*F*
S59	Rubiaceae	*Mussaenda pubescens* W. T. Aiton	*Bướm bạc*	*L*
S60	Rubiaceae	*Mussaenda pubescens* W. T. Aiton	*Bướm bạc*	*S*
S61	Rubiaceae	*Mussaenda pubescens* W. T. Aiton	*Bướm bạc*	*Fr*
S62	Rubiaceae	*Mycetia balansae* Drake	*Lấu quả*	*B*
S63	Rutaceae	*Clausena excavata* Burm.f.	*Hồng bì dại*	*L*
S64	Rutaceae	*Zanthoxylum avicennae* (Lam.) DC.	*Muồng truổng*	*L*
S65	Staphyleaceae	*Turpinia montana* (Blume) Kurz	*Côi*	*B*
S66	Staphyleaceae	*Turpinia montana* (Blume) Kurz	*Côi*	*Fr*
S67	Sterculiaceae	*Helicteres hirsuta* Lour.	*Thâu kén lông*	*S*
S68	Symplocaceae	*Symplocos cambodiana* (Pierre) Hallier f.	*Dung*	*L*
S69	Theaceae	*Eurya cerasifolia* (D.Don) Kobuski	*Sum lá sơ ri*	*S*
S70	Urticaceae	*Pellionia repens* (Lour.) Merr.	*Phu lệ bò*	*S*

**Branches (B), leaves (L), roots (R), stems (S), fruits (Fr), and flowers (F).*

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 by15 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 % CO_2.

2.2.3. Assay for in vitro differentiation of osteoclasts

RAW264.7 cells were seeded at a density of 5×10^4 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:

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

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

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.) Burtt.	Toxic	S39	*Phyllanthus emblica* L.	66.7 %
S5	*Choerospondias axillaris* (Roxb.) Burtt.	< 50 %	S40	*Phyllanthus emblica* L.	97.5 %
S6	*Choerospondias axillaris* (Roxb.) Burtt.	Toxic	S41	*Hydnocarpus hainanensis* (Merr.) Sleum	Toxic
S7	*Xylopia vielana* Pierre	91 %	S42	*Hydnocarpus hainanensis* (Merr.) Sleum	<50 %
S8	*Ilex godajam* Colebr. ex Hook.f.	< 50 %	S43	*Hydnocarpus anthelminhicus* Pierre	Toxic
S9	*Ilex kaushue* S.Y. Hu	95 %	S44	*Illigera rhodantha* Hance	<50 %
S10	*Ilex kaushue* S.Y. Hu	< 50 %	S45	*Illigera rhodantha* Hance	<50 %
S11	*Pothos repens* (Lour.) Druce	< 50 %	S46	*Cratoxylum cochinchinense* (Lour.) Blume	<50 %
S12	*Carallia brachiate* (Lour.) Merr.	< 50 %	S47	*Cratoxylum cochinchinense* (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	*Stixis fasciculata* (King) Gagn.	92.5 %	S52	*Piper longum* L.	96.5 %
S18	*Stixis fasciculata* (King) Gagn.	< 50 %	S53	*Piper longum* L.	Toxic
S19	*Viburnum lutescens* Blume	62.2 %	S54	*Nageia fleuryi* (Hickel) de Laub.	98 %
S20	*Viburnum lutescens* Blume	91 %	S55	*Gouania javanica* Miq.	Toxic
S21	*Viburnum lutescens* 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	*Amischlotype mollissima* (Blume) Hassk.	Toxic	S61	*Mussaenda pubescens* W. T. Aiton	< 50 %
S27	*Trichosanthes rubriflos* Thorel ex Cayla	97.5 %	S62	*Mycetia balansae* Drake	Toxic
S28	*Dichapetalum longepetalum* (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	*Helicteres hirsuta* Lour.	< 50 %
S33	*Macaranga henryi* (Pax & K.Hoffm.) Rehder	Toxic	S68	*Symplocos cambodiana* (Pierre) Hallier f.	81.6 %
S34	*Croton tiglium* L.	< 50 %	S69	*Eurya cerasifolia* (D.Don) Kobuski	93.5 %
S35	*Croton tiglium* L.	< 50 %	S70	*Pellionia repens* (Lour.) Merr.	94 %

Turpinia montana (Blume) Kurz

Symplocos cambodiana (Pierre) Hallier f.

Eurya cerasifolia (D.Don) Kobuski

Nageia fleuryi (Hickel) de Laub

Stixis fasciculata (King) Gagn

Viburnum lutescens Blume

Figure 1. Pictures of some plants collected in Me Linh Station for Biodiversity, Vinh Phuc, Viet Nam in Spring 2019.

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% osteoclastogensis 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-osteoclastogenetic 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.

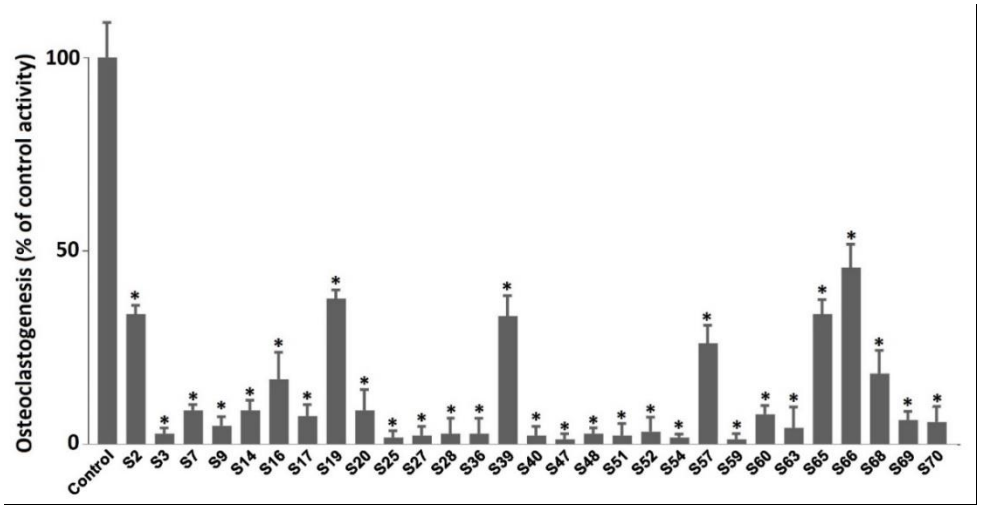

Figure 2. Plant extracts inhibit RANKL-induced osteoclastogenesis in RAW264.7 cells. Only the samples with significant changes in the inhibitory effects are shown. RAW264.7 cells were seeded into 96 well-plates under stimulation of RANKL (100 ng/ml) with or without indicated concentrations of plant extracts 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, ×40). 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-osteoclastogenetic 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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