rr retno widyowati <rr-retno-w@ff.unair.ac.id>

JBCPP.2020.0489 - DecisionRevise with Major Modifications

1 message

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Sun, Jan 24, 2021 at 8:24 PM

24-Jan-2021

Dear Dr. Widyowati:

Thank you again for submitting your manuscript ID JBCPP.2020.0489 entitled "The effect of ganitri (Elaeocarpus serratus L.) from Baung Forest on bone formation cell models" to Journal of Basic and Clinical Physiology and Pharmacology (JBCPP). Your manuscript has been reviewed and requires major modifications prior to acceptance. The comments of the reviewer(s) are included at the bottom of this letter.

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Once again, thank you for submitting your manuscript to JBCPP. I look forward to receiving your revision.

Kind regards
Dr. Suciati Suciati
Guest Editor, Journal of Basic and Clinical Physiology and Pharmacology

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author
The authors have found the potential candidate for osteoporosis substance which could improve the bone formation by several mechanism. However there some minor points that need to be added to this papers, such as:
1. the abstract should describe more clearly especially the aim and conclusion
so the correlation among them will be understand easily.
2. In the background section should be explained the relation between the tested-parameter with the aim of study.
3. any necessary revision point in the paper was described in the note in the text.

Reviewer: 2

Comments to the Author
The article describes the effect of E. serratus on bine formation cell models. The results are quite representative for this journal, however, there are many details as well as clarification needed prior to acceptance of this manuscript for publication.

The major point in the article which needs clarification, refinement, reanalysis, rewrites and or additional information and suggestion for what could be done to improve the article:
1. English is one of the major concerns of the article. I suggest the author check the language since there are many grammatical errors as well as ambiguous sentences. Article is in need of proofreading by a native speaker or someone fluent in English
2. Title: The title does not reflect the whole content of the article. The manuscript describe the results for exploration for several plants from the Baung forest. It is better to reflect these results rather than focusing on one plant in the title.
3. Introduction:
- Some references are very dated to be used as a reference for current data/statistical claim
- The relation between antioxidant and ALP inhibition to bone cell formation should be briefly explained in the introduction
4. Material and methods:
- Identification of plant material should be stated as well as the drying method for plant materials
- Statistical analysis: author mention that t-test analysis was used in the research, but I can not find the results for statistical analysis in the manuscript
5. Results: The author stated that there IC50 measurement for the most active extract, however, there is no result for IC50 measurement? Figures 1 and 2 only present the %radical scavenging effect
6. Discussion: Please consider to discuss the results for screening for 36 plants before discussing the results for E. serratus

Minor points
1. Please check the use of symbols, some symbols are missing as well spelling
2. Check the number for concentration used
3. Table 1: include rendement values, and family of the plants.
4. Figure 1. Better to use a table rather than a figure for these results
5. Figures 4 and 5 can be combined, so that easier to compare the data
6. Other comments please see file attached

Editors Comment to author:
- Based on the result of similaity check your article has 44% similarity index (file attached), which is above the requirement of the journal (30%). We suggest you to rewrite/paraphrase some sentences to fulfill this requirement.
- Author should proof read the article prior to submission of the revised manuscript

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3 attachments

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The effect of ganitri (Elaeocarpus serratus L.) from Baung Forest on bone formation cell models

DOI: https://doi.org/xxxxx/xxxxxxxxxx
Received: Month Day, Year; **Accepted**: Month Day, Year

Abstract

Objectives: Osteoporosis is a disease described by a skeletal degradation of bone tissue dominating to increased risk of fracture.In order to find out the bone formation agents from Baung Forest plants, this research analyzed the effects of 96% ethanol extract of several plants from Baung Forest on antioxidant activity and the effect of osteoblast differentiation related to bone formation on the most potent extract.
Methods: The antioxidant effect and osteoblast differentiation of 96% ethanol extracts were evaluated by measuring DPPH scavenging and alkaline phosphatase in *p*-nitrophenyl phosphate effects by Elisa reader method, respectively.
Results: The 96% ethanol extract of *Elaeocarpus serratus*L. from Baung Forest had the strongest DPPH radical scavenging as anti-oxidant (82.17%) and stimulated osteoblast differentiation (116%). Then, this extract had been fractionated based on polarity to become hexane, ethyl acetate, butanol, and aqueous fractions. All the fractions stimulated their ALP activity to 138.11±9.72%, 108±5.05%, 148.56±8.47, and 144.58±+1.04, respectively.
Conclusions: The 96% ethanol extract and hexane, butanol and aqueous fractions of *Elaeocarpus serratus*L can successfully reduce expression of antioxidant markers on osteoblasts and maintain osteoblast functions by stimulated alkaline phosphatase.

Keywords: alkaline phosphatase; bone formation; DPPH scavenging; 96% ethanol extract;*Elaeocarpus serratus*

Introduction

Osteoporosis is a bone disorder described by a skeletal degradation of bone tissue dominating to an increased risk of fracture and being a silent disease in many complicated situations [1]. This disease can occur because an imbalance of bone resorption relative to bone formation results in negative bone balance at the tissue level. During growth, bone formation surpassess bone resorption, resulting in bone expansion [2]. It is an important problem of elderly and expected to rise with increased age and life span. At present, 200 million people worldwide are estimated to suffer from osteoporosis [3]. The latest statistical data from the International Osteoporosis Foundation showed that 1 out of 3 women over the age of 50 and 1 out of 5 men will suffer osteoporosis fractures for the rest of their lives [4]. This problem also occurs in Indonesia, which has reached a level of caution because the number of osteoporosis sufferer is far greater than the latest data (>19.7%). The number of elderly people in Indonesia is expected to rise by 14% in the period of 1990-2025, while menopausal women in 2000 contributed to an increase of 15.5 million to 24 million in 2015 [5].
In this study, we have found out bone formation agents from Baung Forest plants. Baung forest is a nature tourism park with an area of 195.5 ha [6]. This forest has its natural biodiversity, beauty, and geology. In the forest, there is a unique plant community, namely bamboo forest that is commonly used by local residents for health therapy. The 36 plant extracts from this forest were screened for antioxidant activity and the most potent extract was analyzed for its effect on osteoblast proliferation, differentiation, and expression of inflammatory markers by measuring alkaline phosphatase (ALP) and DPPH inhibition values.

Materials and methods

Cell Culture and Reagents. Reagent chemicals, such as Alkaline Phosphatase Colorimetric Assay Kit, Acid Phosphatase Leukocyte Kit, and so on, were acquired from Sigma-Aldrich Co. (St Louis, MO, USA). All cell culture materials and solvents were bought from Thermo Fisher Scientific (Waltham, MA, USA) and analytical grade (J.T. Baker, USA). Mouse osteoblast-like cells (7F2) were obtained from Department of Biochemical Sciences & Technology, National Chiayi University, Taiwan, and refined in Dulbecco's Modified Eagle's Medium (DMEM). They were further strengthened with 10% v/v Fetal Bovine Serum (FBS), 100 µg/mLstreptomycin, and 100 units/mL penicillin. Cells were refined in a dabbled incubator with 5% CO_2 at 37°C.

Materials. The plants were collected in middle July 2018 in Baung Forest Indonesia, and voucher specimens were deposited at Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Indonesia.

Extraction. The Baung Forest plants were powdered (100-200 g) and extracted with 96% ethanol-aqueous (100 mL x 3) by maceration method. Each of 96% ethanol solution was concentrated using a rotary evaporator to get each of 96% ethanol

Comment [S1]: Title does not reflect the whole content of the article. The manuscript describe the results for exploration for several plants from Baung forest. It is better to reflect this results rather than focusing on one plant in the title

Comment [S2]: The year of your reference is 1992. It is very aged reference to consider it as present situation

Comment [S3]: This reference also year 2000. Please find recent reference.

Comment [S4]: This sentence is confusing, consider to rewrite

Comment [S5]: What is the relation of bamboo plant with this research? Is this plant also part of the study? If there is no relation better to rewrite the sentence

Comment [S6]: The relation between antioxidant and ALP inhibition to bone cell formation should be briefly explain in the introduction

Comment [S7]: Better to describe or mention the name of chemicals, or maybe replace with " and all other chemicals"

Comment [S8]: Identification of plants need to be stated

Comment [S9]: How did the plant material dried? This process should be stated

extract (E) (Table 1). The potent extract was sequentially fractioned with hexane, ethyl acetate, butanol, and aqueous to provide hexane-soluble (EH), ethyl acetate-soluble (EE), butanol-soluble (EB), and aqueous-soluble (EA) fractions.

Tabel 1. Baung Forest Plants Collection

No.	Name of plant	Indonesian name	Part of plant	Extract (mg)
1	*Ixora nigricans*	Jejarum	Leaves	752
2	*Brucea javanica*	Buah makasar	Leaves	787
3	*Mitrephora polypyrena*	Janglot, kalak	Leaves	614
4	*Hypoestes phyllostachya*	Polkadot	Leaves	876
5	*Eranthemum nervosum*	-	Aerial part	807
6	*Protium javanicum*	Trenggulum	Aerial part	444
7	*Urena lobata*	Pulutan	Leaves	234
8	*Blumea lacera*	Sembung kuwuk	Leaves	583
9	*Allophylus serratus*	-	Leaves	667
10	*Melicope latifolia*	Parijoto	Leaves	1,146
11	*Plumbago zaelanica*	Daun encok	Leaves	223
12	*Parameria leivigata*	Kayu rapet	Leaves	249
13	*Elaeocarpus serratus*	Genitri	Leaves	1,232
14	*Reulia tuberosa*	Pletekan	Leaves	745
15	*Dracaena elliptica*	Drakaena	Leaves	394
16	*Garuga floribunda*	Kilangit	Leaves	376
17	*Sida acuta*	Sidaguri	Aerial part	263
18	*Plumeria acuatifolia*		Leaves	376
19	*Memecylon myrsinoides*	Baho	Leaves	319
20	*Solanum torvum*	Takokak	Leaves	216
21	*Solanum verbascifolium*	Terong teter	Leaves	327
22	*Lantana camara*	Saliara	Aerial part	682
23	*Polyscias nodosa*	Tirotasi	Leaves	1,070
24	*Harrisonia perforata*	Rui	Aerial part	388
25	*Hibiscus surattensis*	Waru	Leaves	429
26	*Lantana camara*	Saliara	Flos	508
27	*Melanolepis multiglandulosa*	Daun kapur	Leaves	168
28	*Rauvolfia tetraphylla*	Pule pandak	Leaves	558
29	*Gloriosa superba*	Kembang sungsang	Leaves	278
30	*Centrosema pubescens*	Centro	Flos	496
31	*Centrosema pubescens*	Centro	Aerial part	397
32	*Voacanga glandiflora*	Kalantong	Leaves	920
33	*Phaleria octandra*	Mut	Leaves	269
34	*Melia azedarach*	Mindi kecil	Leaves	552
35	*Hypoestes phyllostachya*	Polkadot	Leaves	243
36	*Aglaia lawii*	-	Leaves	1199

DPPH Measurement. The antioxidant activity of 96% ethanol extracts was defined by di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) assay. The 0.25 mM DPPH solution was prepared by dissolving DPPH powder in methanol. The 100 □ g/mL of 96% ethanol extracts was mixed with 0.25 mM DPPH reagent in equal amounts (100 □ L) in 96 well plates. Blank solution was the mixture of sample solvent (ethanol, 100 □ L) and methanol (100 □ L). DPPH reagent (100 □ L) was mixed with methanol (100 □ L) to serve as control. The reaction mixtures were shaken gently in the dark for 15-30 minutes at 25°C. After the incubation, the absorbance was measured at 517 nm by using a microplate reader (Tecan, infinite M200). The measurements were performed in triplicates. The DPPH scavenging effect was calculated by the following equation.

$$\text{DPPH scavenging effect} = \frac{\text{(1-absorbance of sample group – absorbance of blank)}}{\text{absorbance of control group}} \times 100\%$$

Cell Viability Assay. The 7F2 cells were plated for cell growth studies at a density of 10^4 cells/well in 96-well plates. DMEM medium consisting of 100 units/mL penicillin, 10% FBS, and 100 µg/mL streptomycin was used to restore the cell. After 24 hours, the 96% ethanol extract, hexane-soluble (EH), ethyl acetate-soluble (EE), butanol-soluble (EB), and aqueous-soluble (EA) fractions were incubated at various concentrations for another 24 hours at 37°C. The cell supernatants were subsequently extracted, after 200 µL 3-(4,5-dimethylthiazol-2-yl)- and 100 µL of 2,5-Diphenyltetrazolium Bromide (MTT) reagent (100 µg/mL) were incubated for 4 hours. Similarly, to dissolve the formazan crystals, 100 µL of dimethyl sulfoxide (DMSO) was added. The absorbance was ruminated at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader. All experiments were carried out in triplicate, with the relative cell viability (%) declared as a portion relative to the unprocessed control cells [7,8].

Differentiation of Cellular Alkaline Phosphatase Activity (ALP). The 7F2 osteoblast-like cells were cultured in 24-well plates at a density of 10^4 in DMEM containing 5 mM β-glycerol phosphate (β-GP), 10% FBS, and 50 µg/mL of ascorbic acid (2GF medium) with or without 96% ethanol extract, hexane-soluble (EH), ethyl acetate-soluble (EE), butanol-soluble (EB), and aqueous-soluble (EA) fractions for 4 days incubation period at 37°C in a 5% CO_2 atmosphere. Phosphate buffered saline (PBS)

Comment [S10]: I did not find this abbreviation in the table?
If you have put this abbreviation in the the beginning of manucript, it is better to use the abbreviation on the later paragraf, so there is not need to put/write the whole sentence again. Please check in the later paragraf for cell viability assay etc

Comment [S11]: Check spelling

Comment [S12]: It is better to include %rendement rather than the weight of extracts
The Family of the plant should be included. And it is better to catagorize the plants in order of the family

Comment [S13]: Please include reference for this assay

Comment [S14]: Check symbol

Comment [S15]: Check the use of abbreviation, if neede just write the abbreaviation, there is no need to repeat the use of whole sentence/words

Comment [S16]: There is no need to use capital letter

was used to wash the extracted supernatants. After that, a percentage of the v/v triton solution was inserted and incubated for 10 minutes at 37°C. After incubation, the cell lysates were examined for ALP by adding 200 µL of p-nitrophenyl phosphate (PNPP) and di-ethanolamine buffer into each well for a period of 30 minutes and at room temperature. The 50 µL/well stop solution was inserted to end the reaction while ELISA reader at 405 nm was used to evaluate the absorbance [7,8].

Statistical Analysis. The experiments were carried out for three more consecutive times using similar results. It was then presented as means ± standard deviations. The paired t-test was used to illustrate data analysis. The differences proved to be statistically significant at $P<0.05$.

Results

The Effect of 96% Extracts from Baung Forest Plants on DPPH Radical Scavenging. In the course of our project in order to find antiosteoporotic agents from natural resources [8,9,10,11,12], we screened several plants from Baung Forest on antioxidant by measuring DPPH scavenging. Oxidative stress produces a cellular breakage due to structural change of the membranes, lipid oxidation, and oxidation of nucleic acids and proteins. The damage may expand to the organs and become systemic [13]. Many diseases have been related to oxidative stress, including bone diseases (osteoporosis). Antioxidants induce acceleration of bone loss through activation of tumor necrosis factor alpha (TNF) [14]. Based on the screening result,the 96% ethanol extract of *Elaeocarpus serratus* L. (13), *Memecylon myrsinoides* (19), *Hibiscus surattensis* (25), and *Hypoestes phyllostachya* (35) from Baung Forest showed high DPPH radical scavenger (82.17±2.95, 81.02±1.17, 75.38±1.92 and 71.47±3.55%, respectively) (Fig.1). Therefore, the most potent plant as an antioxidant is *Elaeocarpus serratus*L. In Indonesia, the leaves of this plant have been traditionally used to treat arthritis [15] and in India, they are used as Ayurveda of anti-osteoporosis [16] and arthritis [17]. Then, the DPPH radical scavenging toward this plant at different concentration were explored to find the IC_{50} value.

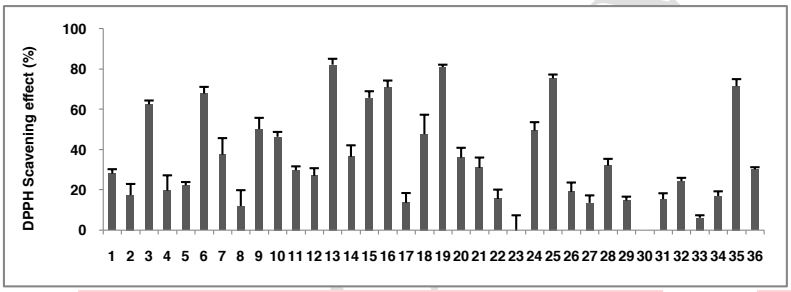

Figure 1: Antioxidant activity using DPPH method of 96% ethanol extracts of Baung Forest plants at 100 mg/mL.

The Effect of 96% Ethanol Extract of *Elaeocarpus serratus* L. Leaves on DPPH Radical Scavenging. In this research, we analyzed the effects of 96% ethanol extract (E), hexane-soluble (EH), ethyl acetate-soluble (EE), butanol-soluble (EB), and aqueous-soluble (EA) fractions of *Elaeocarpus serratus*L. leaves toward antioxidant related to bone turnover. Several researches reported on the pharmacological effects of plant extract (Elaeocarpaceae family) from several countries [15,16,18,19,20,21], but there have been no reports on 96% ethanol extract of *Elaeocarpus serratus*L. from Baung Forest, Indonesia.

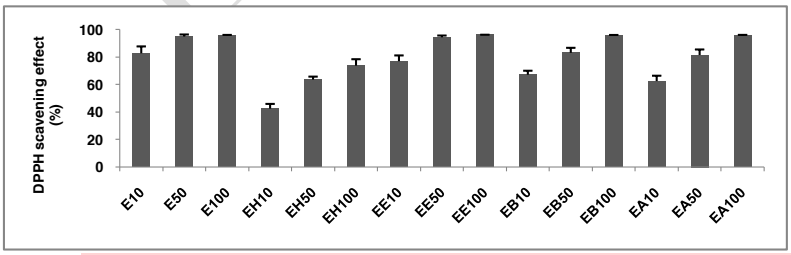

Figure 2: Antioxidant activity using DPPH method of 96% ethanol extract, hexane, ethyl acetate, butanol and aqueous fractions of *Elaeocarpus serratus* leaves.

DPPH is a stable nitrogen that focuses on free radical that can receive an electron or hydrogen radical to finish a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the

Comment [S17]: Is this sentence correct? Using similar results? Not similar method?

Comment [S18]: Which data were analyzed for t-test? I can't find the results for statistical analysis in the manuscript

Comment [S19]: Please check the type of word used in results section. Different from previous section

Comment [S20]: Better to state the number of plants used

Comment [S21]: Is this sentence correct? Please check.
If antioxidant induce acceleration of bone loss than it means better not to use antioxidant

Comment [S22]: It is

Comment [S23]: Where is the results for IC50 measurement? Figures 1 and 2 only present the %radical scavenging effect

Comment [S24]: I think it is better to use table than graph for this results

Comment [S25]: Is this the correct concentration? If yes than this is quite high concentration for the assay

Comment [S26]: Please put explanation for the code E10 etc
What is the axis title?
Example for axis: sample (state the concentration)
This comment also applied for Figures 3, 4, and 5

corresponding hydrazine. Thus, the antioxidant activity of 96% ethanol extract (E), hexane-soluble (EH), ethyl acetate-soluble (EE), butanol-soluble (EB) and aqueous-soluble (EA) fractions with several concentrations (10, 50 and 100 □ g/mL) was detected by DPPH scavenging assay in a range of concentration. Based on the result, the 96% ethanol extract (E), hexane-soluble (EH), ethyl acetate-soluble (EE), butanol-soluble (EB), and aqueous-soluble (EA) fractions had IC_{50} value of 23.27, 42.47, 19.93, 30.12, and 34.90, respectively (Fig.2).

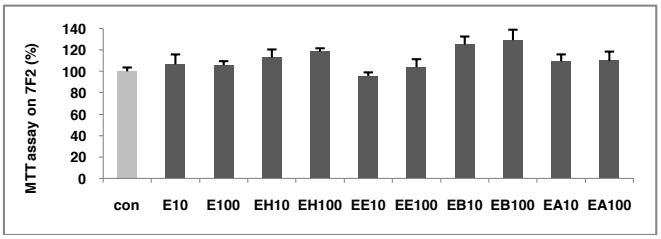

Figure 3: The MTT test of 96% ethanol extract, hexane, ethyl acetate, butanol and aqueous fractions of *Elaeocarpus serratus* leaves on 7F2 osteoblast cells.

The Effect on ALP Stimulation of 7F2 Osteoblasts of 96% Ethanol of *Elaeocarpus serratus* L.The viability effect of 96% ethanol extract (E), hexane-soluble (EH), ethyl acetate-soluble (EE), butanol-soluble (EB), and aqueous-soluble (EA) fractions of *Elaeocarpus serratus*L. from Baung Forest in 7F2 osteoblastic cell lines was carried out using MTT test. The viability cells of their extract and fractions increased in dose-related, in which they showed that high concentration of extract and fractions were not toxic (Fig. 3) and raised cellular uptake. Then, ALPexperiments were continued.

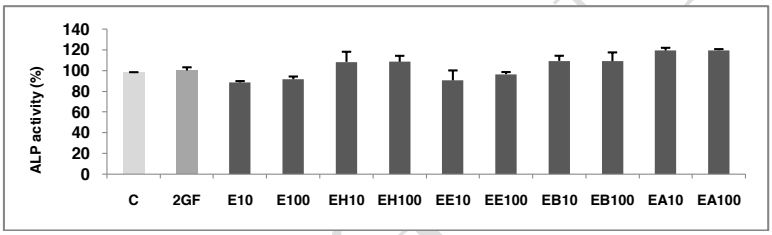

Figure 4: The ALPactivity of 96% ethanol extract, hexane, ethyl acetate, butanol and aqueous fractions of *Elaeocarpus serratus* leaves for 4 days.

The ALP stimulation of 7F2 osteoblast cells using 96% ethanol extract (E), hexane-soluble (EH), ethyl acetate-soluble (EE), butanol-soluble (EB), and aqueous-soluble (EA) fractions of *Elaeocarpus serratus*L. from Baung Forest was incubated for 4 and 7 days. The effects of test samples on the ALP assay increased in the 7F2 osteoblasts opposed to the 2GF group on EH, EB, and EA fractions for 4 days (Fig. 4). After 7 days, the EB, EA and EH fractions stimulated their ALP activity to 148.56±8.47, 144.58±+1.04, and 138.11±9.72%, respectively (Fig. 5).

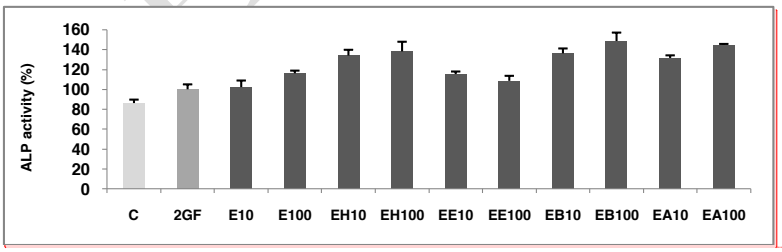

Figure 5: The ALPactivity of 96% ethanol extract, hexane, ethyl acetate, butanol and aqueous fractions of *Elaeocarpus serratus* leaves for 4 days.

Discussion

Comment [S27]: At 4 days incubation

Comment [S28]: Better to combine figures 4 and 5, so it is easier to compare the results

Comment [S29]: At 7 days incubation?

Comment [S30]: For this section please consider to discuss the results for screening for 36 plants before discussing the results foe E. serratus

For thousands of years, natural plants have performed a pivotal part in the development of pharmaceutical drugs and food supplement for the treatment and prevention of diseases [22]. One of such plants with high medicinal value is *Elaeocarpus serratus*L. from Baung Forest which belongs to the Elaeocarpaceae family. Traditionally, it is used to treat migraine, stress, anxiety, depression, lack of concentration, palpitation, nerve pain, epilepsy, asthma, hypertension, liver diseases [15], arthritis [23], Ayurveda of anti-osteoporosis [16], and Ayurveda of osteoarthritis [17]. Several studies have shown that this plant is active and can be functioned as the treatment of arthritis [24], anti-microbial [25], anti-inflammatory, analgesic, pesticide, nematoside, antioxidant [17], antibacterial, diarrhea, and dysentery [26]. The leaves contain flavonoids, carotenoids [23,27], fatty acid [18], myricitrin, and mearnsetin derivatives [28]. Myricitrin has the greatest antioxidant activity in this plant [28]. It was also proved in this study that 96% ethanol extract of *Elaeocarpus serratus*L leaves had a radical scavening DPPH value of 82.17±2.95% (Fig.1). This is the greatest value of its activity compared to other plant extracts from Baung Forest. In several concentration, the 96% ethanol extract (E), hexane-soluble (EH), ethyl acetate-soluble (EE), butanol-soluble (EB) and aqueous-soluble (EA) fractions of this plant had strong DPPH scavenger value (Fig.2). Consequently, we explored this extract for bone formation activity.

Comment [S31]: Is biologically active/ pharmacologically active

Comment [S32]: Is this the correct name?

Comment [S33]: Better to state the concentration

Comment [S34]: How much is strong? Please put reference

Several studies have associated antioxidants with bone metabolism. Lower plasma antioxidants can be found in elderly women or women with osteoporosis. Oxidative stress in estrogen deficiency of postmenopausal osteoporosis has been linked to the activation of NADPH oxidase and/or decreased synthesis of antioxidant enzymes and glutathione (GSH) levels [13,18]. This antioxidant leads the acceleration of bone loss through activation of tumor necrosis factor alpha (TNF) [14]. Converting in the redox state is also linked to the process of bone remodeling that allows continuous bone regeneration through coordinated action of bone cells: osteoclasts, osteoblasts, and osteocytes. Antioxidants directly contribute to activating osteoblast differentiation in bone formation and mineralization processes.

Based on the results, the 96% ethanol extract of *Elaeocarpus serratus*L leaves had a strong antioxidant activity and also played a role in the activation of osteoblast differentiation which is directly related to bone formation. Osteoblast differentiation is characterized by measuring levels of alkaline phosphatase (ALP). ALP is an important enzyme that is a useful biochemical marker of bone formation [29]. The 96% ethanol extract of *Elaeocarpus serratus*L leaves stimulated ALP activity in dose of dependent manner (116% of 100 µg/mL). Among the fractions, butanol-soluble fraction (EB) had the strongest ALP activity (148.56±8.47%). It is a potential fraction for activation of bone formation. Ethanol extract from this plant contains fatty acid ester derivatives such as *n*-dotriacontanol (10.70%), *n*-octadecanol (10.08%), docosanoic acid, 1,2,3-propanetriyl ester (9.07%), *n*-hexadecene (8.52%), bis-(3,5,5-trimethylhexyl) ether (6.30%), ethanone, 1-cyclopentyl- (4.81%), cyclohexane, ethyl- (4.05%), and minor components were hexadecanoic acid methyl ester (0.80%), ricinoleic acid (0.77%), citronellyl isobutyrate (0.69%) and farnesol (0.51%) [18]. Fatty acid has a role in increasing bone formation by stimulated catenin activity in osteoblast and resulting in increased in osteoblastogenesis [30,31]. The mechanisms of fatty acid are complex and involve resolvins and protectins, prostaglandins, growth factors, cytokines, and some other molecular signaling pathways [31]. This plant also contains carotenoids [27], that have a stimulatory effect on osteoblastic bone formation *in vitro*, thereby increasing bone mass. It has an effect on the gene expression of various proteins that is related to osteoblastic bone formation [31]. Thus, the 96% ethanol extract of *Elaeocarpus serratus*L leaves may have a potential effect in the maintaining of bone health and decreasing of bone loss.

Conclusions

The 96% ethanol extract and hexane, butanol and aqueous fractions of *Elaeocarpus serratus*L can successfully reduce expression of antioxidant markers on osteoblasts and maintain osteoblast functions by stimulated alkaline phosphatase.

Comment [S35]: Is this the correct sentence?

References

1. Jennifer JW. Methods in molecular biology: Osteoporosis methods and protocol, Human Press; 2008.
2. Raisz LG. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. J Clin Invest 2005;115:3318–3325.
3. Cooper C, Campion G, Melton LJ. 3rd Hip fracture in the elderly: a world-wide projection. Osteoporos Int 1992;2(6):285–289.
4. Kanis JA, Johnell O, Oden A, Sembo I, Redlund-Johnell I, Dawson A, De Laet C, Jonsson B. Long-term risk of osteoporotic fracture in Malmö. Osteoporos Int 2000;11:669–674.
5. Sudoyo SA, Simadibrata S. Osteoporosis. Buku ajar ilmu penyakit dalam II. 4th ed., Jakarta, FKUI 2006.
6. Balai Besar Konservasi Sumber Daya Alam Jawa Timur: Taman Wisata Alam Gunung Baung (cited 2019 Des 5). Available from: http://bbksdajatim.org/taman-wisata-alam-gunung-baung-1523
7. Yeh CC, Su YH, Lin YJ, Chen PJ, Shi CS, Chen CN, Chang HI. Evaluation of the protective effects of curcuminoid (curcumin and bisdemethoxycurcumin)-loaded liposomes against bone turnover in a cell-based model of osteoarthritis. Drug Des Dev Ther 2015;9:2285–2300.
8. Widyowati R, Suciati, Haryadi DM, Chang H, Suryawan IPGN, Utama AW. The effect of *Rusa unicolor* antler extracts from East Kalimantan in bone turnover cell models. Turk J Pharm Sci2020;17(4):440–445.
9. Widyowati R, Tezuka Y, Miyahara T, Awale S, Kadota S. Alkaline phosphatase (ALP) enhancing iridoid glucosides from the Indonesian medicinal plant *Barleria lupulina*.Nat Prod Comm 2010;5(11):1711–1716.
10. Widyowati R. Alkaline phosphatase activity of *Graptophylum pictum* and *Spilanthes acmella* fractions against MC3T3-E1 cells as marker of osteoblast differentiation cells. Int J Pharm Pharm Sci 2011;3(1):34–37.
11. Laswati H, Subadi I, Widyowati R, Agil M, Pangkahila JA. *Spilanthes acmella* and physical exercise increased testosterone levels and osteoblast cells in glucocortocoid-induced osteoporosis male mice. Bali Medical Journal 2015;4(2):76–81.

12. Widyowati R, Ekasari W, Purwitasari N. An amine derivative from the aerial part of *Spilanthes acmella* Murr. and their ALP activity. The Nat Prod J 2020;10(5):571–577.
13. Naka K, Muraguchi T, Hoshii T, Hirao A. Regulation of reactive oxygen species and genomic stability in hematopoietic stem cells. Antioxid Redox Signal 2008;10:1883–1894.
14. Domazetovic V,Marcucci G, Lantomasi T, Brandi ML, Vincenzini MT.Oxidative stress in bone remodeling: role of antioxidants. Clin Cases Miner Bone Metab 2017;14(2):209–216.
15. Das P, Kar P, Hasnu S, Nath S, Tanti B. Phytochemical screening and antioxidant activity of *Elaeocarpus serratus* L. of Assam. J Pharmacognosy & Phytochemistry 2017;6(4):866–869.
16. Hardainiyan S, Nandy BC, Kumar K. *Elaeocarpus Ganitrus* (Rudraksha): A Reservoir Plant with their Pharmacological Effects. Int J Pharm Sci Rev Res 2015;34(1):55–64.
17. Sreelekshmi SG, Manoj GS, Lawarence B,Murugan K. Ameliorative potentials of plant-derived phytochemicals against arthritis. Trends in Biosciences 2018;11(7):1714–1720.
18. Geetha DH, Rajeswari M, Indhiramuthu J, Chemical profiling of *Elaeocarpus serratus* L. by GC-MS. Asian Pac J Trop Biomed 2013;3(12):985–987.
19. Kumar TS, Shanmugam S, Palvannan T, Kumar VMB. Evaluation of antioxidant properties of *Elaeocarpus ganitrus* Roxb. Leaves. Iranian J Pharm Res 2008;7(3):211–215.
20. Sarananda KH, Thillakawardane TU, Alexander B. Production of health-friendly, ready-to-serve fruit drinks from under- utilized local fruits from Sri Lanka. Sri Lanka J Food & Agriculture 2017;3(2):37–48.
21. Arivu I, Muthulingam M. Detailed study on *Elaeocarpus ganitrus* (Rudraksha) for its medicinal importance - A review. Int J Curr Sci 2017;20(1): E 16–30.
22. Megraj KVK, Koneri RR, Meenakshisundaram K. Biological activities of some Indian medicinal plants. JAPER2011;1:12–44.
23. Manoj GS, Lawarence B,Sreelekshmi SG,Murugan K.Review of ameliorative potentials of plant-derived phytochemicals against arthritics. Nov Tech Arthritis Bone Res 2017;1(2):1–6.
24. Geetha DH, Jayashree I, Rajeswari M. In vivo anti-arthritic activity of ethanolic extracts of *Elaeocarpus serratus* L. Int J Pharm Sci Rev Res 2018;48(2):92–97.
25. Lima FF, Breda CA, Cardoso CAL, Duarte MCT, Sanjinez-Argandoña EJ.Evaluation of nutritional composition, bioactive compounds and antimicrobial activity of *Elaeocarpus serratus* fruit extract. Afr J Food Sci 2019;13(1):30–37.
26. Sharker SMD, Shahid IJ. Assesment of antibacterial and cytotoxic activity of some locally used medicinal plants in Sunderban mangrove forest region. African J. Pharma. Pharmacol 2010;4(4):66–69.
27. Otero DM, Ferreira-Ribeiro CD. Potential bioactive compounds of unconventional food plants. Agri Res & Tech 2019;23(2):257–259.
28. Jayasinghe L, Amarasinghe NR, Arundathie BG, Rupasinghe GK, Jayatilake NH, Fujimoto Y.Antioxidant flavonol glycosides from *Elaeocarpus serratus* and *Filicium decipiens*. Nat Prod Res 2012;26(8):717–21.
29. Golub EE, Boesze-Battaglia K. The role of alkaline phosphatese in mineralizeation. Current Opinion in Orthopaedics 2007;18:444–448.
30. Ahn SH, Park S, Baek J, Lee SY, Baek WY, Lee SY, Lee YS, Yoo HJ, Kim H, Lee SH, Im DS, Lee SK, Kim BJ, Koh JM. Free fatty acid receptor 4 (GPR120) stimulates bone formation and suppresses bone resorption in the presence of elevated n-3 fatty acid levels. Endocrinology 2016;157(7):2621–2635.
31. Kajarabille N, Díaz-Castro J, Hijano S, López-Frías M, López-Aliaga I, Ochoa JJ. A new insight to bone turnover: Role of □-3 polyunsaturated fatty acids. Hindawi Publishing Corporation The Scientific World Journal 2013;1–16.

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Exploration of several plants from Baung Forest on bone formation cell models

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Abstract

Objectives: Osteoporosis is an ailment described by a skeletal degradation of bone skeletal dominating to increases the chance of fracture. In order to find out the bone formation agents from Baung Forest plants, this research analyzed the effects of 96% ethanol extract of several plants from Baung Forest on antioxidant activity and the effect of osteoblast differentiation-related to the bone formation on the most potent extract.
Methods: The antioxidant effect and osteoblast differentiation of 96% ethanol extracts were evaluated by measuring DPPH scavenging and alkaline phosphatase in *p*-nitrophenyl phosphate effects by the Elisa reader method, respectively.
Results: The 96% ethanol extract of *Elaeocarpus serratus* L. from Baung Forest had the strongest DPPH radical scavenging as anti-oxidant (82.17%) and stimulated osteoblast differentiation (116%). Then, this extract had been fractionated based on polarity to become hexane, ethyl acetate, butanol, and aqueous fractions. All the fractions stimulated their ALP activity to 138.11±9.72%, 108±5.05%, 148.56±8.47, and 144.58±1.04, respectively.
Conclusions: The extract and fractions of *Elaeocarpus serratus* L can successfully inhibit DPPH radical scavenging value and increase ALP activities as markers of osteoblast functions.

Keywords: alkaline phosphatase; bone formation; DPPH scavenging; 96% ethanol extract; *Elaeocarpus serratus*

Introduction

Osteoporosis is an ailment described by a skeletal degradation of bone skeletal dominating to increases the chance of fracture and being a quiet ailment in many complex situations [1]. This ailment can occur because an disproportion of bone resorption relative to bone formation products in effectiess bone equilibrium at the tissue. During growth, bone formation surpassess bone resorption, resulting in bone elaboration [2]. It is a prominent matter of elderly and estimated to increase with rising age and life span. At 1992, the 200 million populace global were expected to endure from osteoporosis [3]. Then in 2000, statistical data from the International Osteoporosis Foundation represented that 1 out of 3 women over 50 years old and 1 out of 5 men will endure osteoporosis fractures for the spend of their lives [4]. This problem too occurs in Indonesia, which has reached a level of caution because the amount of osteoporosis sufferer has increased from the latest data (>19.7%). The amount of elderly in Indonesia is estimated to increase by 14% during of 1990-2025, while in the 2000-2015 period, menopausal women donated to an intensify of osteoporosis sufferers by 8.5 million [5]. WHO estimates that in 2050 the number of fracture sufferers will increase by 2 times in women and 3 times in men [6,7].

In this study, we have found out bone formation agents from Baung Forest plants. Baung forest is a nature tourism park with an area of 195.5 ha [8]. This forest has its natural biodiversity, beauty, and geology. In the forest, there are various types of plants that are commonly used by local residents for health therapy. The 36 plant extracts from this forest were screened for antioxidant activity (DPPH inhibition values) and the most potent extract was analyzed for its effect on osteoblast proliferation and differentiation by evaluating alkaline phosphatase (ALP). Oxidative stress in bone cells results in the production of reactive oxygen species (ROS) from lipoxygenase and oxidase [9]. ROS can affect bone cells through decreased production of bone matrix protein (characterized by decreased ALP value) [10]. ALP is an identified biochemical marker of bone formation on the osteoblast plasma membrane reflecting osteoblastic activity on bone remodeling process [11] and plays an important role in osteoid formation and bone mineralization [12].

Materials and methods

Cell Culture and Reagents. Reagent chemicals, such as Alkaline Phosphatase Colorimetric Assay Kit, Acid Phosphatase

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Leukocyte Kit, and all other chemicals, were acquired by Sigma-Aldrich Co. (St Louis, MO, USA). All cell culture materials and solvents were purchased from Thermo Fisher Scientific (Waltham, MA, USA) and analytical grade (J.T. Baker, USA). Mouse osteoblast-like cells (7F2) were obtained from Department of Biochemical Sciences & Technology, National Chiayi University, Taiwan, and refined in Dulbecco's Modified Eagle's Medium (DMEM). They were farther strengthened by 10% v/v Fetal Bovine Serum (FBS), 100 µg/mL streptomycin, and 100 units/mL penicillin. Cells were incubated in a dabbled incubator with 5% CO_2 at 37°C.

Materials. The plants were collected in middle July 2018 in Baung Forest Indonesia, and voucher samples were stored at Pharmacognosy Laboratory, Faculty of Pharmacy, Universitas Airlangga, Indonesia. The plants were identified by the Plant Conservation Institution, Purwodadi Botanical Garden.

Extraction. Fresh plants obtained from Baung Forest Purwodadi were cleaned and washed with clean running water, then dried under indirect sun to dry. After drying, the particle sizes were reduced by grinding until a powder was obtained. A total of 100-200 g of plant powders were extracted with 96% ethanol-aqueous (100 mL x 3) by maceration method. Each of 96% ethanol solution was evaporated using a rotary evaporator to get each of 96% ethanol extract (E) (Table 1). The potent extract was sequentially fractioned with hexane, ethyl acetate, butanol, and aqueous to provide hexane-soluble (EH), ethyl acetate-soluble (EE), butanol-soluble (EB), and aqueous-soluble (EA) fractions.

Table 1. Baung Forest Plants Collection and their DPPH radical scavenger effect (%)

No.	Name of plant	Indonesian name	Family name	Part of plant	E (% yields)	DPPH at 100 µg/mL (%)
1	*Ixora nigricans*	Jejarum	Rubiaceae	Leaves	7.52	28.06±2.19
2	*Brucea javanica*	Buah makasar	Simaraubaceae	Leaves	7.87	17.34±5.56
3	*Mitrephora polypyrena*	Janglot, kalak	Annonaceae	Leaves	6.14	62.47±1.92
4	*Hypoestes phyllostachya*	Polkadot	Acanthaceae	Leaves	8.76	19.60±7.60
5	*Eranthemum nervosum*	-	Acanthaceae	Aerial part	8.07	22.22±1.65
6	*Protium javanicum*	Trenggulum	Burseraceae	Aerial part	8.88	67.86±3.30
7	*Urena lobata*	Pulutan	Malvaceae	Leaves	4.68	37.46±8.24
8	*Blumea lacera*	Sembung kuwuk	Asteraceae	Leaves	11.66	11.87±7.95
9	*Allophylus serratus*	-	Sapindaceae	Leaves	6.67	50.15±5.61
10	*Melicope latifolia*	Parijoto	Rutaceae	Leaves	14.61	46.35±2.42
11	*Plumbago zaelanica*	Daun encok	Plumbaginaceae	Leaves	4.46	29.73±1.91
12	*Parameria leivigata*	Kayu rapet	Apocynaceae	Leaves	7.49	26.90±3.85
13	*Elaeocarpus serratus*	Genitri	Elaeocarpaceae	Leaves	12.32	82.17±2.95
14	*Reulia tuberosa*	Pletekan	Acanthaceae	Leaves	7.45	36.39±5.72
15	*Dracaena elliptica*	Drakaena	Asparagaceae	Leaves	9.85	65.71±3.30
16	*Garuga floribunda*	Kilangit	Burseraceae	Leaves	8.36	70.95±3.37
17	*Sida acuta*	Sidaguri	Malvaceae	Aerial part	6.58	13.59±4.82
18	*Plumeria acuatifolia*	Kemboja	Apocynaceae	Leaves	7.52	47.66±9.66
19	*Memecylon myrsinoides*	Baho	Melastomataceae	Leaves	7.09	81.02±1.17
20	*Solanum torvum*	Takokak	Solanaceae	Leaves	5.40	36.01±4.88
21	*Solanum verbascifolium*	Terong teter	Solanaceae	Leaves	7.27	30.91±5.14
22	*Lantana camara*	Saliara	Verbenaceae	Aerial part	6.82	15.45±4.65
23	*Polyscias nodosa*	Tirotasi	Araliaceae	Leaves	10.70	-
24	*Harrisonia perforata*	Rui	Rutaceae	Aerial part	8.62	49.45±4.18
25	*Hibiscus surattensis*	Waru	Malvaceae	Leaves	8.58	75.38±1.92
26	*Lantana camara*	Saliara	Verbenaceae	Flos	11.29	18.94±4.69
27	*Melanolepis multiglandulosa*	Daun kapur	Euphorbiaceae	Leaves	6.72	13.52±3.72
28	*Rauvolfia tetraphylla*	Pule pandak	Apocynaceae	Leaves	11.16	32.06±3.33
29	*Gloriosa superba*	Kembang sungsang	Liliaceae	Leaves	9.26	14.68±1.92
30	*Centrosema pubescens*	Centro	Fabaceae	Flos	4.96	-
31	*Centrosema pubescens*	Centro	Fabaceae	Aerial part	7.94	15.35±2.93
32	*Voacanga glandiflora*	Kalantong	Apocynaceae	Leaves	9.20	24.32±1.63
33	*Phaleria octandra*	Mut	Thymelaeaceae	Leaves	8.96	6.09±1.25
34	*Melia azedarach*	Mindi kecil	Meliaceae	Leaves	5.52	16.94±2.32
35	*Hypoestes phyllostachya*	Polkadot	Acanthaceae	Leaves	6.08	71.47±3.55
36	*Aglaia lawii*	-	Meliaceae	Leaves	11.99	30.12±1.11

DPPH Measurement. The antioxidant activity of 96% ethanol extracts was defined by di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) assay. The 0.25 mM DPPH solution was processed using DPPH solution in methanol. The 100 µg/mL of 96% ethanol extracts was mixed with 0.25 mM DPPH reagent in equal amounts (100 µL) in 96 well plates. Blank solution was the mixture of sample solvent (ethanol, 100 µL) and methanol (100 µL). DPPH reagent (100 µL) was mixed with methanol (100 µL) to serve as control. The reaction mixtures were shaken gently in the dark for 15-30 minutes at 25°C. After the incubation, the absorbance was evaluated at 517 nm using a Tecan, infinite M200 microplate reader. The measurements were performed in triplicates. The DPPH radical scavenging was counted by equation [13,14].

DPPH radical scavenging effect = $\frac{\text{(1-sample groups absorbance – blank absorbance)}}{\text{control group absorbance}}$ x 100%

Cell Viability Assay. The 7F2 cells were plated for cell growth studies at a density of 10^4 cells/well in 96-well plates. DMEM medium composing 100 units/mL penicillin, 10% FBS, and 100 µg/mL streptomycin was used to restore the cell. After 24 hours, the E extract, EH, EE, EB, and EA fractions of *Elaeocarpus serratus* L. from Baung Forest were incubated at various concentrations for another 24 hours at 37°C. The cell supernatants were subsequently extracted, after 200 µL 3-(4,5-dimethylthiazol-2-yl)- and 100 µL of 2,5-diphenyltetrazolium bromide (MTT) reagent (100 µg/mL) were incubated during 4 hours. Similarly, to dissolve the formazan crystals, 100 µL of dimethyl sulfoxide (DMSO) was added. The absorbance was ruminated at 570 nm by an enzyme-linked immunosorbent assay (ELISA) reader. All experiments were performed in triplicate, with the relative cell viability (%) declared as a portion relative to the unprocessed control cells [15,16].

Differentiation of Cellular Alkaline Phosphatase Activity (ALP). The 7F2 osteoblast-like cells were plated in 24-well plates at 10^4 in DMEM containing 5 mM β-glycerol phosphate (β-GP), 10% FBS, and 50 µg/mL of ascorbic acid (2GF medium) with or without E extract, EH, EE, EB, and EA fractions of *Elaeocarpus serratus* L. from Baung Forest for 4 and 7 days incubation period at 37°C in a 5% CO_2 atmosphere. Phosphate buffered saline (PBS) was applied to clean the supernatants. After that, a percentage of the v/v triton solution was inserted and incubated for 10 minutes at 37°C. After incubation, the cell lysates were examined for ALP by adding 200 µL of p-nitrophenyl phosphate (PNPP) and di-ethanolamine buffer into each well for a period of 30 minutes and at room temperature. The 50 µL/well stop solution was inserted to stop the reaction while ELISA reader at 405 nm was applied to measure the absorbance [15,16].

Statistical Analysis. The experiments were perfomed for three times using similar methods. It was then expressed as means ± standard deviations. The one-way ANOVA and LSD test were used to illustrate data analysis. The differences proved to be statistically significant at $P<0.05$.

Results

The Effect of 96% Extracts from Baung Forest Plants on DPPH Radical Scavenging. During our project in order to discover antiosteoporotic delegates from natural sources [16,17,18,19,20], we screened 36 plants from Baung Forest on antioxidant by measuring DPPH scavenging. Oxidative stress produces a breakage of cellular owing to membranes structural change, lipid oxidation, and oxidation of nucleic acids and proteins. The breakage may expand to the organs and become systemic [21]. Many ailments have been related to oxidative stress, inserting bone diseases (osteoporosis). Antioxidants reduce acceleration of bone damage thru encoragement of tumor necrosis factor alpha (TNFα) [22]. Based on the screening result, the 96% ethanol extract of *Elaeocarpus serratus* L. (13), *Memecylon myrsinoides* (19), *Hibiscus surattensis* (25), and *Hypoestes phyllostachya* (35) from Baung Forest showed high DPPH radical scavenging (82.17±2.95, 81.02±1.17, 75.38±1.92 and 71.47±3.55%, respectively) (Table.1). Therefore, the most potent plant as an antioxidant is *Elaeocarpus serratus* L. In Indonesia, the leaves of this plant are used traditionally to treat arthritis [23] and in India, it is used as Ayurveda of anti-osteoporosis [24] and arthritis [25]. Then, the % DPPH radical scavenging toward this plant at different concentration were explored.

The Effect of 96% Ethanol Extract of *Elaeocarpus serratus* L. Leaves on DPPH Radical Scavenging. In this research, we analyzed the effects of E extract, EH, EE, EB, and EA fractions of *Elaeocarpus serratus* L. from Baung Forest leaves toward antioxidant related to bone turnover. Several researches reported on the pharmacological effects of plant extract (Elaeocarpaceae family) from several countries [23,24,26,27,28,29], but there have been no reports on 96% ethanol extract of *Elaeocarpus serratus* L. from Baung Forest, Indonesia.

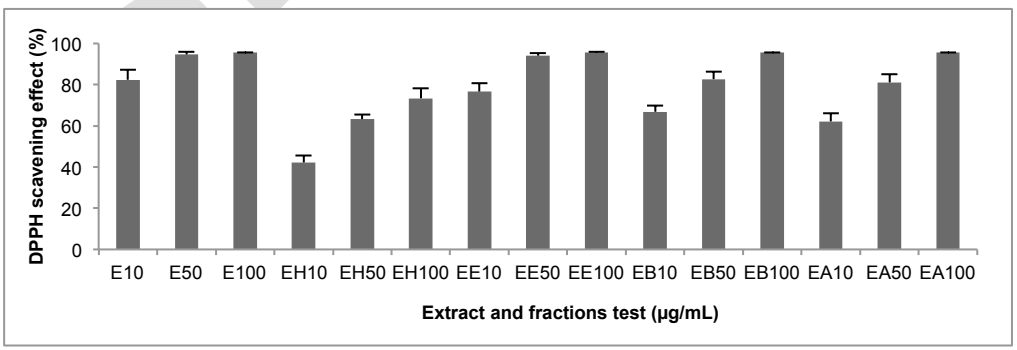

Figure 1: Antioxidant activity using DPPH method of 96% ethanol extract, hexane, ethyl acetate, butanol and aqueous fractions of *Elaeocarpus serratus* leaves. The number of 10, 50 & 100 following extract (E) and fractions (EH, EE, EB, EA) indicate their concentrations at 10, 50 & 100 µg/mL.

DPPH is steady nitrogen that focuses on free radical that can receive hydrogen radical or electron to finish a steady diamagnetic molecule. DPPH radicals respond with appropriate reducing agents as a yield of which the electrons get couple off becoming the corresponding hydrazine. Thus, the antioxidant activity of E extract, EH, EE, EB and EA fractions of *Elaeocarpus serratus* L. from Baung Forest with several concentrations (10, 50 and 100 µg/mL) was detected by DPPH scavenging assay in a range of concentration. Based on the result, the E extract, EH, EE, EB and EA fractions had IC_{50} value of 23.27, 42.47, 19.93, 30.12, and 34.90, respectively (Fig.1).

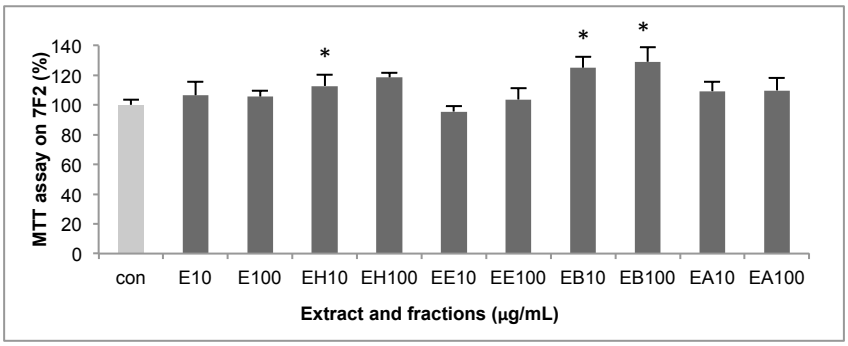

Figure 2: The MTT test of 96% ethanol extract, hexane, ethyl acetate, butanol and aqueous fractions of *Elaeocarpus serratus* leaves on 7F2 osteoblast cells. The number of 10, 50 & 100 following extract (E) and fractions (EH, EE, EB, EA) indicate their concentrations at 10, 50 & 100 µg/mL.

The ALP Stimulation Effect of 7F2 Osteoblasts of 96% Ethanol of *Elaeocarpus serratus* L. The viability results of E extract, EH, EE, EB and EA fractions of *Elaeocarpus serratus* L. from Baung Forest in 7F2 osteoblastic cell lines was carried out using MTT test. The viability cells of their extract and fractions increased in dose-related, in which they showed that high concentration of extract and fractions were not toxic (Fig. 2) and elevated cellular uptake. Then, ALP experiments were proceed.

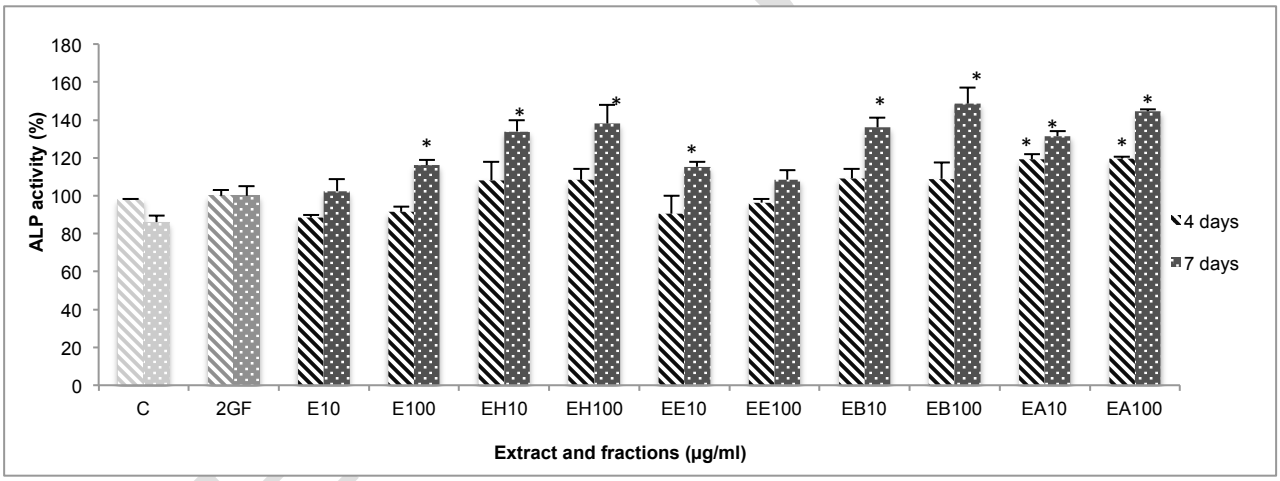

Figure 3: The ALP activity of 96% ethanol extract, hexane, ethyl acetate, butanol and aqueous fractions of *Elaeocarpus serratus* leaves for 4 and 7 days incubation. The number of 10, 50 & 100 following extract (E) and fractions (EH, EE, EB, EA) indicate their concentrations at 10, 50 & 100 µg/mL. The sign * means $p<0.05$ to 2GF.

The ALP stimulation of 7F2 osteoblast cells using E extract, EH, EE, EB and EA fractions of *Elaeocarpus serratus* L. from Baung Forest was incubated for 4 and 7 days. The results of samples on increasing ALP assay in the 7F2 osteoblasts againsted to the 2GF group on EH, EB, and EA fractions for 4 days (Fig. 3). After 7 days, the EB, EA and EH fractions stimulated their ALP activity to 148.56±8.47, 144.58±1.04, and 138.11±9.72%, respectively (Fig. 3).

Discussion

Geographically, the Baung Purwodadi forest area is located between 7°49'9"-7°47'23" South Latitude and 112°16'23"-112°17'17" East Longitude with the topography in general being bumpy to hilly, the altitude of this area ranges from between 200 - 501 masl, red yellow mediterranean soil types and latosols, soil derived from old quarter rock with the main material in the form of metamorphic sediment, climate type D rainfall with a value of Q = 81.82%, the average annual amount of 2.654, 10 mm/year with an average number of rainy days of 141.05 days [30]. In the forest there are plant communities. Potential flora in the TWA Gunung Baung area, including *Brucea javanica*, *Urena lobata*, *Plumbago zaelanica*, *Parameria leivigata*, *Garuga floribunda*, *Plumeria acuatifolia*, *Lantana camara*, *Rauvolfia tetraphylla*, *Gloriosa superba*, *Melia azedarach* and others (Table 1).

These plants are used by the local community for treatment such as lowering sugar levels, fever, inflammation, high blood pressure, treating stomach aches, relieving joint pain, headaches, worming and urination.

The use of these plants as traditional medicine is only based on inheritance from ancestors without knowing the chemical content that plays a role in treatment [31]. Therefore, to determine the exact chemical content for treatment, it is necessary to explore plants, especially forest plants that have a large enough potential. The initial screening was antioxidant potential because the assay is simple and easy for large quantities. Oxidative stress occurs as a result of overproduction of ROS which is not balanced, which can cause bone disruption. The altered redox state is also associated with the bone remodeling process which enables the continuous regeneration of bone through the coordinated action of bone cells. Changes in ROS and/or the antioxidant system involve in the pathogenesis of bone loss. ROS induces apoptosis (death) of osteoblasts and osteocytes, this encourages osteoclastogenesis and inhibits mineralization and osteogenesis [32]. Based on DPPH Radical Scavenging result on several plants in the Baung forest, *Elaeocarpus serratus* L. has the highest potential in trapping DPPH radical scavening (82.17±2.95). Therefore, it continues the exploration of this plant to determine their ability to increase bone density.

Natural plants have performed a pivotal part in pharmaceutical drugs and dietary supplement developments for the therapy and precaution of ailment [33]. One of them is *Elaeocarpus serratus* L. from Baung Forest which belongs to the Elaeocarpaceae family. Traditionally, it is used to treat migraine, stress, anxiety, depression, lack of concentration, palpitation, nerve pain, epilepsy, asthma, hypertension, liver diseases [23], arthritis [34], Ayurveda of anti-osteoporosis [24], and Ayurveda of osteoarthritis [25]. Several studies have shown that this plant is pharmacologically active and can be functioned as the treatment of arthritis [35], anti-microbial [36], anti-inflammatory, analgesic, pesticide, nematoside, antioxidant [25], antibacterial, diarrhea, and dysentery [37]. The leaves contain flavonoids, carotenoids [34,38], fatty acid [26], myricitrin, and mearnsetin derivatives [39]. Myricitrin has the greatest antioxidant activity in this plant [39]. It was also proved in this study that 96% ethanol extract of *Elaeocarpus serratus* L leaves had a radical scavening DPPH value of 82.17±2.95% (Table 1). This is the greatest value of its activity compared to other plant extracts from Baung Forest. Based on Figure 2, almost all fractions has the ability to trap free radical > 50% at concentration of 10-100 µg/mL but the hexane fraction (EH) at 10 µg/ml cannot trapping DPPH radicals by up to 50%. The greater percentage value of trapping, the better antioksidan activity in DPPH radical scavening [40]. Consequently, we explored this extract for bone formation activity.

Several studies have associated antioxidants with bone metabolism. Lower plasma antioxidants can be found in elderly women or women with osteoporosis. Oxidative stress in estrogen deficiency of postmenopausal osteoporosis has been linked to the activation of NADPH oxidase and/or alleviated synthesis of antioxidant enzymes and glutathione (GSH) levels [21,26]. This antioxidant leads the acceleration of bone loss through activation of tumor necrosis factor alpha (TNFα) [22]. Converting in the redox state is also linked to the process of bone remodeling that permits continuous bone regeneration thru coordinated action of bone cells such as osteoblasts, osteocytes and osteoclasts. Antioxidants directly contribute to activating osteoblast differentiation in bone formation and mineralization processes.

Based on the results, the 96% ethanol extract of *Elaeocarpus serratus* L leaves had a strong antioxidant activity and also played a role in the activation of osteoblast differentiation which is directly related to bone formation. Osteoblast differentiation is characterized by measuring levels of alkaline phosphatase (ALP). ALP is an important enzyme that is a useful biochemical marker of bone formation [41]. This enzyme plays a role in osteoid formation and mineralization. So that the ALP enzyme and bone mineralization have a significant correlation and become a biochemical marker [42]. Bone growth and healing during bone fracture cause high ALP enzymes in bones. However, if the ALP enzyme appears in excess, it can be an indicator of osteosarcoma to bone metastases [43]. The 96% ethanol extract of *Elaeocarpus serratus* L leaves stimulated ALP activity in dose of dependent manner (116% of 100 µg/mL). Among the fractions, butanol-soluble fraction (EB) had the strongest ALP activity (148.56±8.47%). It is a potential fraction for activation of bone formation. Ethanol extract from this plant contains fatty acid ester derivatives such as *n*-dotriacontanol (10.70%), *n*-octadecanol (10.08%), docosanoic acid, 1,2,3-propanetriyl ester (9.07%), *n*-hexadecene (8.52%), bis-(3,5,5-trimethylhexyl) ether (6.30%), ethanone, 1-cyclopentyl- (4.81%), cyclohexane, ethyl- (4.05%), and minor components were hexadecanoic acid methyl ester (0.80%), ricinoleic acid (0.77%), citronellyl isobutyrate (0.69%) and farnesol (0.51%) [26]. Fatty acid has a role in increasing bone formation by stimulated β catenin activity in osteoblast and resulting in increased in osteoblastogenesis [44,45]. The mechanisms of fatty acid are complex and involve protectins and resolvins, prostaglandins, growth elements, cytokines, and few other molecular signaling routes [45]. This plant also contains carotenoids [38], that have a encourage effect on osteoblastic bone formation *in vitro*, therefrom escalating bone mass. This effects the gene expression of various proteins associated to bone formation [45]. Thus, the 96% ethanol extract of *Elaeocarpus serratus* L leaves has potential effect to maintain bone health and decrease bone loss.

Conclusions

The extract and fractions of *Elaeocarpus serratus* L can successfully inhibit DPPH radical scavenging value and increase ALP activities as markers of osteoblast functions.

References

1. Jennifer JW. Methods in molecular biology: Osteoporosis methods and protocol, Human Press; 2008.
2. Raisz LG. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. J Clin Invest 2005;115:3318–3325.
3. Cooper C, Campion G, Melton LJ. 3rd Hip fracture in the elderly: a world-wide projection. Osteoporos Int 1992;2(6):285–289.
4. Kanis JA, Johnell O, Oden A, Sembo I, Redlund-Johnell I, Dawson A, De Laet C, Jonsson B. Long-term risk of osteoporotic fracture in Malmö. Osteoporos Int 2000;11:669–674.
5. Sudoyo SA, Simadibrata S. Osteoporosis. Buku ajar ilmu penyakit dalam II. 4th ed., Jakarta, FKUI 2006.
6. Kementerian Kesehatan RI Pusat Data dan Informasi. Data & Kondisi Penyakit Osteoporosis di Indonesia. Jakarta Selatan: Kementerian Kesehatan RI Pusat Data dan informasi 2015.
7. Kementerian Kesehatan Republik Indonesia. Germas Osteoporosis. s.l.:Kementerian Kesehatan Republik Indonesia 2017.
8. Balai Besar Konservasi Sumber Daya Alam Jawa Timur: Taman Wisata Alam Gunung Baung (cited 2019 Des 5). Available from: http://bbksdajatim.org/taman-wisata-alam-gunung-baung-1523
9. Chen JR, Lazarenko OP, Haley RL, Blackburn ML, badger TM, Ronis MJJ. Ethanil impairs estrogen receptor signaling and activates senescence pathways in osteoblasts protection by estradiol. J Bone Min Res 2009;24:221–230.
10. Gilbert L, He X, Farmer P, Boden S, Kozlowski M, Rubin J, Nanes MS. Inhibition od osteoblast differentiation by tumor necrosis factor-alpha. Endorcinology 2000;141(11):3956–3964.
11. Rekha SP. Comparitive study of biochemical bone turnover markers in pre & post-menopausal women. International Journal of Applied Research 2015;1(5):185.
12. Vaithialingam A, Lakshmi TM, Suryaprakash G, Edukondalu AD, Reddy EP. Alkaline Phosphatase levels in rheumatoid arthritis and osteoporosis in clinical practice. Journal of Current Trends in Clinical Medicine & Laboratory Biochemistry 2013;1(2):20–23.
13. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity LWT. Food Science and Technology 1995;28:25–30.
14. Kim DO, Lee KW, Lee HJ, Lee CY. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. Journal of Agricultural and Food Chemistry 2020;50:3713–3717.
15. Yeh CC, Su YH, Lin YJ, Chen PJ, Shi CS, Chen CN, Chang HI. Evaluation of the protective effects of curcuminoid (curcumin and bisdemethoxycurcumin)-loaded liposomes against bone turnover in a cell-based model of osteoarthritis. Drug Des Dev Ther 2015;9:2285–2300.
16. Widyowati R, Suciati, Haryadi DM, Chang H, Suryawan IPGN, Utama AW. The effect of *Rusa unicolor* antler extracts from East Kalimantan in bone turnover cell models. Turk J Pharm Sci 2020;17(4):440–445.
17. Widyowati R, Tezuka Y, Miyahara T, Awale S, Kadota S. Alkaline phosphatase (ALP) enhancing iridoid glucosides from the Indonesian medicinal plant *Barleria lupulina.* Nat Prod Comm 2010;5(11):1711–1716.
18. Widyowati R. Alkaline phosphatase activity of *Graptophylum pictum* and *Spilanthes acmella* fractions against MC3T3-E1 cells as marker of osteoblast differentiation cells. Int J Pharm Pharm Sci 2011;3(1):34–37.
19. Laswati H, Subadi I, Widyowati R, Agil M, Pangkahila JA. *Spilanthes acmella* and physical exercise increased testosterone levels and osteoblast cells in glucocortocoid-induced osteoporosis male mice. Bali Medical Journal 2015;4(2):76–81.
20. Widyowati R, Ekasari W, Purwitasari N. An amine derivative from the aerial part of *Spilanthes acmella* Murr. and their ALP activity. The Nat Prod J 2020;10(5):571–577.
21. Naka K, Muraguchi T, Hoshii T, Hirao A. Regulation of reactive oxygen species and genomic stability in hematopoietic stem cells. Antioxid Redox Signal 2008;10:1883–1894.
22. Domazetovic V, Marcucci G, Lantomasi T, Brandi ML, Vincenzini MT. Oxidative stress in bone remodeling: role of antioxidants. Clin Cases Miner Bone Metab 2017;14(2):209–216.
23. Das P, Kar P, Hasnu S, Nath S, Tanti B. Phytochemical screening and antioxidant activity of *Elaeocarpus serratus* L. of Assam. J Pharmacognosy & Phytochemistry 2017;6(4):866–869.
24. Hardainiyan S, Nandy BC, Kumar K. *Elaeocarpus Ganitrus* (Rudraksha): A Reservoir Plant with their Pharmacological Effects. Int J Pharm Sci Rev Res 2015;34(1):55–64.
25. Sreelekshmi SG, Manoj GS, Lawarence B, Murugan K. Ameliorative potentials of plant-derived phytochemicals against arthritis. Trends in Biosciences 2018;11(7):1714–1720.
26. Geetha DH, Rajeswari M, Indhiramuthu J, Chemical profiling of *Elaeocarpus serratus* L. by GC-MS. Asian Pac J Trop Biomed 2013;3(12):985–987.
27. Kumar TS, Shanmugam S, Palvannan T, Kumar VMB. Evaluation of antioxidant properties of *Elaeocarpus ganitrus* Roxb. Leaves. Iranian J Pharm Res 2008;7(3):211–215.
28. Sarananda KH, Thillakawardane TU, Alexander B. Production of health-friendly, ready-to-serve fruit drinks from under- utilized local fruits from Sri Lanka. Sri Lanka J Food & Agriculture 2017;3(2):37–48.
29. Arivu I, Muthulingam M. Detailed study on *Elaeocarpus ganitrus* (Rudraksha) for its medicinal importance - A review. Int J Curr Sci 2017;20(1): E 16–30.
30. Sofiah S, Setiadi D, Widyatmoko D. Pola Penyebaran, Kelimpahan dan Asosiasi Bambu pada Komunitas Tumbuhan di Taman Wisata Alam Gunung Baung Jawa Timur. Berita Biologi 2013;12(2).
31. Mannito P. Biosintesis produk alami. Cetakan Pertama. Terjemahan Koensoemardiyah dan Sudarto. New York : Ellis Horwood Limited 1992..
32. Domazetovic V, Gemma M, Teresa I, Maria LB, Maria TV. Oxidative stress in bone remodeling: role of antioxidants. Clinical Cases in Mineral and Bone Metabolism 2017;14(2):209–216.
33. Megraj KVK, Koneri RR, Meenakshisundaram K. Biological activities of some Indian medicinal plants. JAPER 2011;1:12–44.
34. Manoj GS, Lawarence B, Sreelekshmi SG, Murugan K. Review of ameliorative potentials of plant-derived phytochemicals against arthritics. Nov Tech Arthritis Bone Res 2017;1(2):1–6.
35. Geetha DH, Jayashree I, Rajeswari M. In vivo anti-arthritic activity of ethanolic extracts of *Elaeocarpus serratus* L. Int J Pharm Sci Rev Res 2018;48(2):92–97.
36. Lima FF, Breda CA, Cardoso CAL, Duarte MCT, Sanjinez-Argandoña EJ. Evaluation of nutritional composition, bioactive compounds and antimicrobial activity of *Elaeocarpus serratus* fruit extract. Afr J Food Sci 2019;13(1):30–37.
37. Sharker SMD, Shahid IJ. Assesment of antibacterial and cytotoxic activity of some locally used medicinal plants in Sunderban mangrove forest region. African J. Pharma. Pharmacol 2010;4(4):66–69.
38. Otero DM, Ferreira-Ribeiro CD. Potential bioactive compounds of unconventional food plants. Agri Res & Tech 2019;23(2):257–259.
39. Jayasinghe L, Amarasinghe NR, Arundathie BG, Rupasinghe GK, Jayatilake NH, Fujimoto Y. Antioxidant flavonol glycosides from *Elaeocarpus serratus* and *Filicium decipiens*. Nat Prod Res 2012;26(8):717–21.
40. Karadag A, Ozcelik B, Saner S. Review of methods to determine antioxidant capacities. Food Analytical Methods 2009;2:41–60.
41. Golub EE, Boesze-Battaglia K. The role of alkaline phosphatese in mineralizeation. Current Opinion in Orthopaedics 2007;18:444–448.
42. Sharma U, Pal D, Prasad R. Alkaline phosphatase:An overview. Indian J Clin Biochem 2014;29(3):269–278.
43. Sarac F, Saygili FM. Causes of high bone alkaline phosphatase, Biotechnology & Biotechnological Equipment 2007;21(2):194-197.
44. Ahn SH, Park S, Baek J, Lee SY, Baek WY, Lee SY, Lee YS, Yoo HJ, Kim H, Lee SH, Im DS, Lee SK, Kim BJ, Koh JM. Free fatty acid receptor 4 (GPR120) stimulates bone formation and suppresses bone resorption in the presence of elevated n-3 fatty acid levels. Endocrinology 2016;157(7):2621–2635.
45. Kajarabille N, Díaz-Castro J, Hijano S, López-Frías M, López-Aliaga I, Ochoa JJ. A new insight to bone turnover: Role of ω-3 polyunsaturated fatty acids. Hindawi Publishing Corporation The Scientific World Journal 2013;1–16.

DE GRUYTER Journal of Basic and Clinical Physiology and Pharmacology

Exploration of several plants from Baung Forest on bone formation cell models

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Section/Category:	• Phytotherapy
Keywords:	alkaline phosphatase, bone formation, DPPH scavenging, 96% ethanol extract, Elaeocarpus serratus
Abstract:	**Objectives:** Osteoporosis is an ailment described by a skeletal degradation of bone skeletal dominating to increases the chance of fracture. In order to find out the bone formation agents from Baung Forest plants, this research analyzed the effects of 96% ethanol extract of several plants from Baung Forest on antioxidant activity and the effect of osteoblast differentiation-related to the bone formation on the most potent extract. **Methods:** The antioxidant effect and osteoblast differentiation of 96% ethanol extracts were evaluated by measuring DPPH scavenging and alkaline phosphatase in *p*-nitrophenyl phosphate effects by the Elisa reader method, respectively. **Results:** The 96% ethanol extract of *Elaeocarpus serratus* L. from Baung Forest had the strongest DPPH radical scavenging as anti-oxidant (82.17%) and stimulated osteoblast differentiation (116%). Then, this extract had been fractionated based on polarity to become hexane, ethyl acetate, butanol, and aqueous fractions. All the fractions stimulated their ALP activity to 138.11±9.72%, 108±5.05%, 148.56±8.47, and 144.58±1.04, respectively. **Conclusions:** The extract and fractions of *Elaeocarpus serratus* L can successfully inhibit DPPH radical scavenging value and increase ALP activities as markers of osteoblast functions.

AUTHOR FORM

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

INSTRUCTIONS

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. The submitting author is responsible for the accuracy and completeness of the submitted information. The form should be uploaded alongside with the submitted manuscript. The form is in four parts.

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Provide the date of submission and the title of your manuscript and the ID (in case you know it already). Give your full name and if you are NOT the corresponding author please check the box „no". Provide the full name and the initials of all co-authors and indicate, if applicable, the corresponding author.

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This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you and/or any co-authors received, either directly or indirectly (via your institution), to enable the completion of the work. Checking „No" means that you and any co-authors did the work without receiving any financial support from any third party - that is, the work was supported by funds from the same institution that pays the salary and that institution did not receive third-party funds with which to pay you and/or any co-authors. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check „Yes".

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Please add all co-authors of your manuscript. Please ensure that you collected all relevant information of your co-authors correctly, since the submitting author is responsible for the accuracy and completeness of the submitted information.

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Type	No	Recipient Initials	Money paid to you	Money paid to your Institution*	Name of Entity	Comments**
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Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

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Strictlyadhere to the given format.
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Author contributions

We declared that this work was done by Retno Widyowati (RW), Neny Purwitasari (NP), Rice Disi Oktarina (RDO), Wiwied Ekasari (WE), Saarah Khairunnisa (SK) and Hsin-I Chang (HC). NP collected the antioxidant data, WE analyzed the antioxidant data, SK collected the ALP data, RDO analyzed the ALP data, RW designed the study and wrote the manuscript, HC designed the study and analyzed the ALP data. All authors had read and approved the manuscript.

Competing interests

No conflict of interest was associated with this work

Informed consent

All authors have agreed the content of the manuscript.

Ethical approval

Ethical approval was not required.

Reviewer 1

The authors have found the potential candidate for osteoporosis substance which could improve the bone formation by several mechanism. However there some minor points that need to be added to this papers, such as:

1. the abstract should describe more clearly especially the aim and conclusion. so the correlation among them will be understand easily.
   Thank you for the advice and we have revised it.
2. In the background section should be explained the relation between the tested-parameter with the aim of study.
   We have addede their relations as “The 36 plant extracts from this forest were screened for antioxidant activity (DPPH inhibition values) and the most potent extract was analyzed for its effect on osteoblast proliferation and differentiation by measuring alkaline phosphatase (ALP). Oxidative stress in bone cells results in the production of reactive oxygen species (ROS) from lipoxygenase and oxidase [9]. ROS can affect bone cells through decreased production of bone matrix protein (characterized by decreased ALP value) [10]. ALP is an identified biochemical marker of bone formation on the osteoblast plasma membrane reflecting osteoblastic activity on bone remodeling process [11] and plays an important role in osteoid formation and bone mineralization [12].”
3. any necessary revision point in the paper was described in the note in the text.
   Thank you for the correction and we have revised it according to the notes.

Reviewer: 2

The article describes the effect of E. serratus on bine formation cell models. The results are quite representative for this journal, however, there are many details as well as clarification needed prior to acceptance of this manuscript for publication.

The major point in the article which needs clarification, refinement, reanalysis, rewrites and or additional information and suggestion for what could be done to improve the article:

1. English is one of the major concerns of the article. I suggest the author check the language since there are many grammatical errors as well as ambiguous sentences. Article is in need of proofreading by a native speaker or someone fluent in English
   Thank you for your advice.
2. Title: The title does not reflect the whole content of the article. The manuscript describe the results for exploration for several plants from the Baung forest. It is better to reflect these results rather than focusing on one plant in the title.
   Thank you and we have changed it according to reviewer suggestions
3. Introduction:
   - Some references are very dated to be used as a reference for current data/statistical claim. We have added another recent reference.
   - The relation between antioxidant and ALP inhibition to bone cell formation should be briefly explained in the introduction. Thanks for your advice and we've added the antioxidant relate to bone formation.

4. Material and methods:
   - Identification of plant material should be stated as well as the drying method for plant materials. We have added a way of identifying and drying plants
   - Statistical analysis: author mention that t-test analysis was used in the research, but I can't find the results for statistical analysis in the manuscript. We have made a mistake and have corrected it as "It was then presented as means ± standard deviations. The one-way ANOVA and LSD test were used to illustrate data analysis. The differences proved to be statistically significant at $P<0.05$."
5. Results: The author stated that there IC50 measurement for the most active extract, however, there is no result for IC50 measurement? Figures 1 and 2 only present the %radical scavenging effect. We are sorry for mistake and it that calculated in % DPPH radical scavenging.
6. Discussion: Please consider to discuss the results for screening for 36 plants before discussing the results for E. serratus. We have added a discussion on the screening results. Thank you for the advice.

Minor points

1. Please check the use of symbols, some symbols are missing as well spelling. Thank you and we have confirmed the correct symbol
2. Check the number for concentration used. Thank you and we have checked and corrected it.
3. Table 1: include rendement values, and family of the plants. We have added the% yield and family of the plant
4. Figure 1. Better to use a table rather than a figure for these results. We have combined the results in figure 1 into table 1
5. Figures 4 and 5 can be combined, so that easier to compare the data. We have combined figure 4 dan 5
6. Other comments please see file attached. Thank you and we have revised it as attached

Editors Comment to author:

- Based on the result of similaity check your article has 44% similarity index (file attached), which is above the requirement of the journal (30%). We suggest you to rewrite/paraphrase some sentences to fulfill this requirement. Thank you and we have paraphrase and check by turniti (10% similarity index)
- Author should proof read the article prior to submission of the revised manuscript. Thank you and we have done it

Table 1. Baung Forest Plants Collection and their DPPH radical scavenger effect (%)

No.	Name of plant	Indonesian name	Family name	Part of plant	E (% yields)	DPPH at 100 µg/mL (%)
1	*Ixora nigricans*	Jejarum	Rubiaceae	Leaves	7.52	28.06±2.19
2	*Brucea javanica*	Buah makasar	Simaraubaceae	Leaves	7.87	17.34±5.56
3	*Mitrephora polypyrena*	Janglot, kalak	Annonaceae	Leaves	6.14	62.47±1.92
4	*Hypoestes phyllostachya*	Polkadot	Acanthaceae	Leaves	8.76	19.60±7.60
5	*Eranthemum nervosum*	-	Acanthaceae	Aerial part	8.07	22.22±1.65
6	*Protium javanicum*	Trenggulum	Burseraceae	Aerial part	8.88	67.86±3.30
7	*Urena lobata*	Pulutan	Malvaceae	Leaves	4.68	37.46±8.24
8	*Blumea lacera*	Sembung kuwuk	Asteraceae	Leaves	11.66	11.87±7.95
9	*Allophylus serratus*	-	Sapindaceae	Leaves	6.67	50.15±5.61
10	*Melicope latifolia*	Parijoto	Rutaceae	Leaves	14.61	46.35±2.42
11	*Plumbago zaelanica*	Daun encok	Plumbaginaceae	Leaves	4.46	29.73±1.91
12	*Parameria leivigata*	Kayu rapet	Apocynaceae	Leaves	7.49	26.90±3.85
13	*Elaeocarpus serratus*	Genitri	Elaeocarpaceae	Leaves	12.32	82.17±2.95
14	*Reulia tuberosa*	Pletekan	Acanthaceae	Leaves	7.45	36.39±5.72
15	*Dracaena elliptica*	Drakaena	Asparagaceae	Leaves	9.85	65.71±3.30
16	*Garuga floribunda*	Kilangit	Burseraceae	Leaves	8.36	70.95±3.37
17	*Sida acuta*	Sidaguri	Malvaceae	Aerial part	6.58	13.59±4.82
18	*Plumeria acuatifolia*	Kemboja	Apocynaceae	Leaves	7.52	47.66±9.66
19	*Memecylon myrsinoides*	Baho	Melastomataceae	Leaves	7.09	81.02±1.17
20	*Solanum torvum*	Takokak	Solanaceae	Leaves	5.40	36.01±4.88
21	*Solanum verbascifolium*	Terong teter	Solanaceae	Leaves	7.27	30.91±5.14
22	*Lantana camara*	Saliara	Verbenaceae	Aerial part	6.82	15.45±4.65
23	*Polyscias nodosa*	Tirotasi	Araliaceae	Leaves	10.70	-
24	*Harrisonia perforata*	Rui	Rutaceae	Aerial part	8.62	49.45±4.18
25	*Hibiscus surattensis*	Waru	Malvaceae	Leaves	8.58	75.38±1.92
26	*Lantana camara*	Saliara	Verbenaceae	Flos	11.29	18.94±4.69
27	*Melanolepis multiglandulosa*	Daun kapur	Euphorbiaceae	Leaves	6.72	13.52±3.72
28	*Rauvolfia tetraphylla*	Pule pandak	Apocynaceae	Leaves	11.16	32.06±3.33
29	*Gloriosa superba*	Kembang sungsang	Liliaceae	Leaves	9.26	14.68±1.92
30	*Centrosema pubescens*	Centro	Fabaceae	Flos	4.96	-
31	*Centrosema pubescens*	Centro	Fabaceae	Aerial part	7.94	15.35±2.93
32	*Voacanga glandiflora*	Kalantong	Apocynaceae	Leaves	9.20	24.32±1.63
33	*Phaleria octandra*	Mut	Thymelaeaceae	Leaves	8.96	6.09±1.25
34	*Melia azedarach*	Mindi kecil	Meliaceae	Leaves	5.52	16.94±2.32
35	*Hypoestes phyllostachya*	Polkadot	Acanthaceae	Leaves	6.08	71.47±3.55
36	*Aglaia lawii*	-	Meliaceae	Leaves	11.99	30.12±1.11

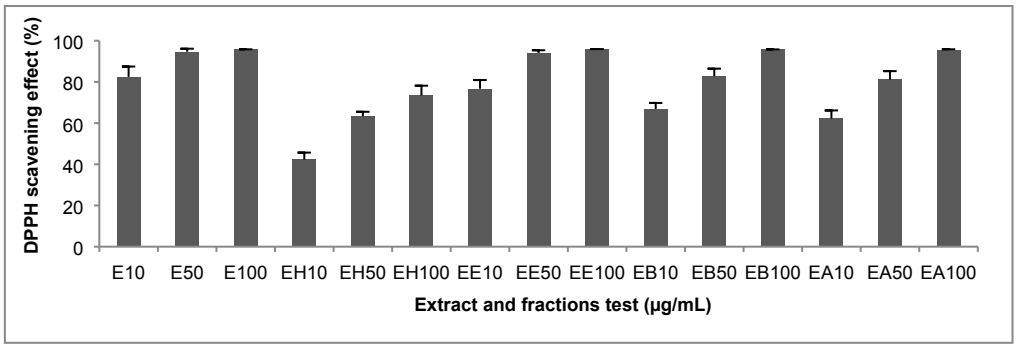

Figure 1: Antioxidant activity using DPPH method of 96% ethanol extract, hexane, ethyl acetate, butanol and aqueous fractions of *Elaeocarpus serratus* L. leaves. The number of 10, 50 & 100 following extract (E) and fractions (EH, EE, EB, EA) indicate their concentrations at 10, 50 & 100 µg/mL.

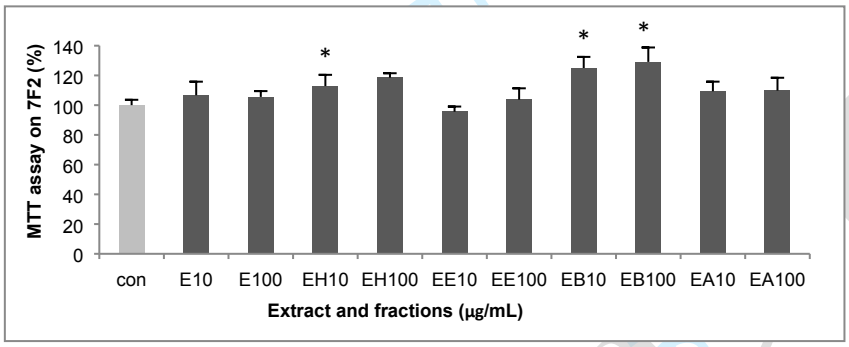

Figure 2: The MTT test of 96% ethanol extract, hexane, ethyl acetate, butanol and aqueous fractions of *Elaeocarpus serratus* L. leaves on 7F2 osteoblast cells. The number of 10, 50 & 100 following extract (E) and fractions (EH, EE, EB, EA) indicate their concentrations at 10, 50 & 100 µg/mL.

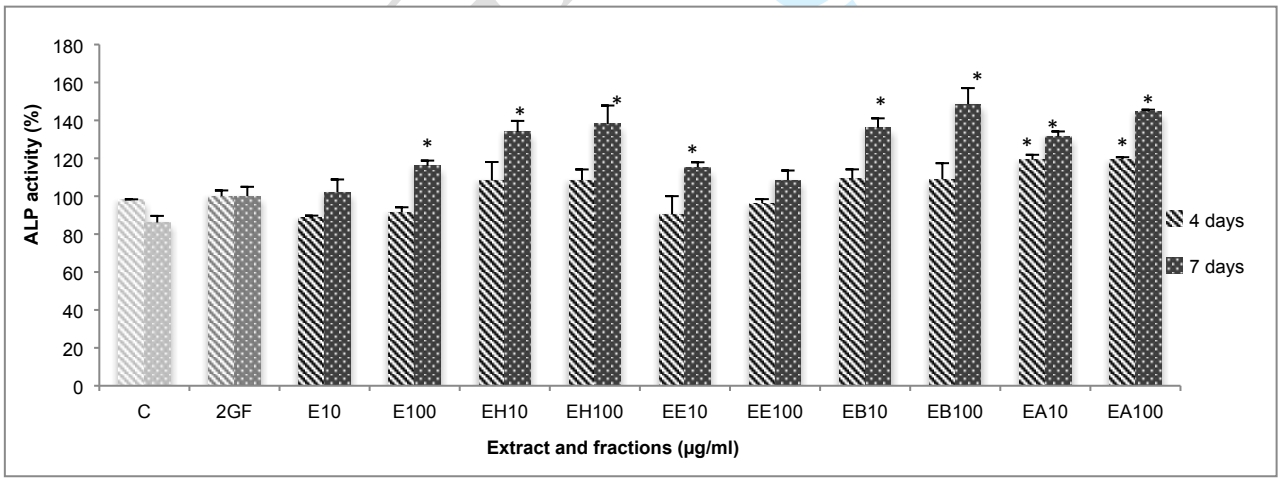

Figure 3: The ALP activity of 96% ethanol extract, hexane, ethyl acetate, butanol and aqueous fractions of *Elaeocarpus serratus* L. leaves for 4 and 7 days incubation. The number of 10, 50 & 100 following extract (E) and fractions (EH, EE, EB, EA) indicate their concentrations at 10, 50 & 100 µg/mL. The sign * means $p<0.05$ to 2GF.

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Exploration of several plants from Baung Forest on bone formation cell models

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Abstract

Objectives: Osteoporosis is an ailment described by a skeletal degradation of bone skeletal dominating to increases the chance of fracture. In order to find out the bone formation agents from Baung Forest plants, this research analyzed the effects of 96% ethanol extract of several plants from Baung Forest on antioxidant activity and the effect of osteoblast differentiation-related to the bone formation on the most potent extract.
Methods: The antioxidant effect and osteoblast differentiation of 96% ethanol extracts were evaluated by measuring DPPH scavenging and alkaline phosphatase in *p*-nitrophenyl phosphate effects by the Elisa reader method, respectively.
Results: The 96% ethanol extract of *Elaeocarpus serratus* L. from Baung Forest had the strongest DPPH radical scavenging as anti-oxidant (82.17%) and stimulated osteoblast differentiation (116%). Then, this extract had been fractionated based on polarity to become hexane, ethyl acetate, butanol, and aqueous fractions. All the fractions stimulated their ALP activity to 138.11±9.72%, 108±5.05%, 148.56±8.47, and 144.58±1.04, respectively.
Conclusions: The extract and fractions of *Elaeocarpus serratus* L can successfully inhibit DPPH radical scavenging value and increase ALP activities as markers of osteoblast functions.

Keywords: alkaline phosphatase; bone formation; DPPH scavenging; 96% ethanol extract; *Elaeocarpus serratus*

Introduction

Osteoporosis is an ailment described by a skeletal degradation of bone skeletal dominating to increases the chance of fracture and being a quiet ailment in many complex situations [1]. This ailment can occur because an disproportion of bone resorption relative to bone formation products in effectiess bone equilibrium at the tissue. During growth, bone formation surpassess bone resorption, resulting in bone elaboration [2]. It is a prominent matter of elderly and estimated to increase with rising age and life span. At 1992, the 200 million populace global were expected to endure from osteoporosis [3]. Then in 2000, statistical data from the International Osteoporosis Foundation represented that 1 out of 3 women over 50 years old and 1 out of 5 men will endure osteoporosis fractures for the spend of their lives [4]. This problem too occurs in Indonesia, which has reached a level of caution because the amount of osteoporosis sufferer has increased from the latest data (>19.7%). The amount of elderly in Indonesia is estimated to increase by 14% during of 1990-2025, while in the 2000-2015 period, menopausal women donated to an intensify of osteoporosis sufferers by 8.5 million [5]. WHO estimates that in 2050 the number of fracture sufferers will increase by 2 times in women and 3 times in men [6,7].

In this study, we have found out bone formation agents from Baung Forest plants. Baung forest is a nature tourism park with an area of 195.5 ha [8]. This forest has its natural biodiversity, beauty, and geology. In the forest, there are various types of plants that are commonly used by local residents for health therapy. The 36 plant extracts from this forest were screened for antioxidant activity (DPPH inhibition values) and the most potent extract was analyzed for its effect on osteoblast proliferation and differentiation by evaluating alkaline phosphatase (ALP). Oxidative stress in bone cells results in the production of reactive oxygen species (ROS) from lipoxygenase and oxidase [9]. ROS can affect bone cells through decreased production of bone matrix protein (characterized by decreased ALP value) [10]. ALP is an identified biochemical marker of bone formation on the osteoblast plasma membrane reflecting osteoblastic activity on bone remodeling process [11] and plays an important role in osteoid formation and bone mineralization [12].

Materials and methods

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Cell Culture and Reagents. Reagent chemicals, such as Alkaline Phosphatase Colorimetric Assay Kit, Acid Phosphatase Leukocyte Kit, and all other chemicals, were acquired by Sigma-Aldrich Co. (St Louis, MO, USA). All cell culture materials and solvents were purchased from Thermo Fisher Scientific (Waltham, MA, USA) and analytical grade (J.T. Baker, USA). Mouse osteoblast-like cells (7F2) were obtained from Department of Biochemical Sciences & Technology, National Chiayi University, Taiwan, and refined in Dulbecco's Modified Eagle's Medium (DMEM). They were farther strengthened by 10% v/v Fetal Bovine Serum (FBS), 100 µg/mL streptomycin, and 100 units/mL penicillin. Cells were incubated in a dabbled incubator with 5% CO_2 at 37°C.

Materials. The plants were collected in middle July 2018 in Baung Forest Indonesia, and voucher samples were stored at Pharmacognosy Laboratory, Faculty of Pharmacy, Universitas Airlangga, Indonesia. The plants were identified by the Plant Conservation Institution, Purwodadi Botanical Garden.

Extraction. Fresh plants obtained from Baung Forest Purwodadi were cleaned and washed with clean running water, then dried under indirect sun to dry. After drying, the particle sizes were reduced by grinding until a powder was obtained. A total of 100-200 g of plant powders were extracted with 96% ethanol-aqueous (100 mL x 3) by maceration method. Each of 96% ethanol solution was evaporated using a rotary evaporator to get each of 96% ethanol extract (E) (Table 1). The potent extract was sequentially fractioned with hexane, ethyl acetate, butanol, and aqueous to provide hexane-soluble (EH), ethyl acetate-soluble (EE), butanol-soluble (EB), and aqueous-soluble (EA) fractions.

Table 1. Baung Forest Plants Collection and their DPPH radical scavenger effect (%)

No.	Name of plant	Indonesian name	Family name	Part of plant	E (% yields)	DPPH at 100 µg/mL (%)
1	*Ixora nigricans*	Jejarum	Rubiaceae	Leaves	7.52	28.06±2.19
2	*Brucea javanica*	Buah makasar	Simaraubaceae	Leaves	7.87	17.34±5.56
3	*Mitrephora polypyrena*	Janglot, kalak	Annonaceae	Leaves	6.14	62.47±1.92
4	*Hypoestes phyllostachya*	Polkadot	Acanthaceae	Leaves	8.76	19.60±7.60
5	*Eranthemum nervosum*	-	Acanthaceae	Aerial part	8.07	22.22±1.65
6	*Protium javanicum*	Trenggulum	Burseraceae	Aerial part	8.88	67.86±3.30
7	*Urena lobata*	Pulutan	Malvaceae	Leaves	4.68	37.46±8.24
8	*Blumea lacera*	Sembung kuwuk	Asteraceae	Leaves	11.66	11.87±7.95
9	*Allophylus serratus*	-	Sapindaceae	Leaves	6.67	50.15±5.61
10	*Melicope latifolia*	Parijoto	Rutaceae	Leaves	14.61	46.35±2.42
11	*Plumbago zaelanica*	Daun encok	Plumbaginaceae	Leaves	4.46	29.73±1.91
12	*Parameria leivigata*	Kayu rapet	Apocynaceae	Leaves	7.49	26.90±3.85
13	*Elaeocarpus serratus*	Genitri	Elaeocarpaceae	Leaves	12.32	82.17±2.95
14	*Reulia tuberosa*	Pletekan	Acanthaceae	Leaves	7.45	36.39±5.72
15	*Dracaena elliptica*	Drakaena	Asparagaceae	Leaves	9.85	65.71±3.30
16	*Garuga floribunda*	Kilangit	Burseraceae	Leaves	8.36	70.95±3.37
17	*Sida acuta*	Sidaguri	Malvaceae	Aerial part	6.58	13.59±4.82
18	*Plumeria acuatifolia*	Kemboja	Apocynaceae	Leaves	7.52	47.66±9.66
19	*Memecylon myrsinoides*	Baho	Melastomataceae	Leaves	7.09	81.02±1.17
20	*Solanum torvum*	Takokak	Solanaceae	Leaves	5.40	36.01±4.88
21	*Solanum verbascifolium*	Terong teter	Solanaceae	Leaves	7.27	30.91±5.14
22	*Lantana camara*	Saliara	Verbenaceae	Aerial part	6.82	15.45±4.65
23	*Polyscias nodosa*	Tirotasi	Araliaceae	Leaves	10.70	-
24	*Harrisonia perforata*	Rui	Rutaceae	Aerial part	8.62	49.45±4.18
25	*Hibiscus surattensis*	Waru	Malvaceae	Leaves	8.58	75.38±1.92
26	*Lantana camara*	Saliara	Verbenaceae	Flos	11.29	18.94±4.69
27	*Melanolepis multiglandulosa*	Daun kapur	Euphorbiaceae	Leaves	6.72	13.52±3.72
28	*Rauvolfia tetraphylla*	Pule pandak	Apocynaceae	Leaves	11.16	32.06±3.33
29	*Gloriosa superba*	Kembang sungsang	Liliaceae	Leaves	9.26	14.68±1.92
30	*Centrosema pubescens*	Centro	Fabaceae	Flos	4.96	-
31	*Centrosema pubescens*	Centro	Fabaceae	Aerial part	7.94	15.35±2.93
32	*Voacanga glandiflora*	Kalantong	Apocynaceae	Leaves	9.20	24.32±1.63
33	*Phaleria octandra*	Mut	Thymelaeaceae	Leaves	8.96	6.09±1.25
34	*Melia azedarach*	Mindi kecil	Meliaceae	Leaves	5.52	16.94±2.32
35	*Hypoestes phyllostachya*	Polkadot	Acanthaceae	Leaves	6.08	71.47±3.55
36	*Aglaia lawii*	-	Meliaceae	Leaves	11.99	30.12±1.11

DPPH Measurement. The antioxidant activity of 96% ethanol extracts was defined by di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) assay. The 0.25 mM DPPH solution was processed using DPPH solution in methanol. The 100 µg/mL of 96% ethanol extracts was mixed with 0.25 mM DPPH reagent in equal amounts (100 µL) in 96 well plates. Blank solution was the mixture of sample solvent (ethanol, 100 µL) and methanol (100 µL). DPPH reagent (100 µL) was mixed with methanol (100 µL) to serve as control. The reaction mixtures were shaken gently in the dark for 15-30 minutes at 25°C. After the incubation, the absorbance was evaluated at 517 nm using a Tecan, infinite M200 microplate reader. The measurements were performed in triplicates. The DPPH radical scavenging was counted by equation [13,14].

DPPH radical scavenging effect = (1-sample groups absorbance – blank absorbance) x 100%
control group absorbance

Cell Viability Assay. The 7F2 cells were plated for cell growth studies at a density of 10^4 cells/well in 96-well plates. DMEM medium composing 100 units/mL penicillin, 10% FBS, and 100 µg/mL streptomycin was used to restore the cell. After 24 hours, the E extract, EH, EE, EB, and EA fractions of *Elaeocarpus serratus* L. from Baung Forest were incubated at various concentrations for another 24 hours at 37°C. The cell supernatants were subsequently extracted, after 200 µL 3-(4,5-dimethylthiazol-2-yl)- and 100 µL of 2,5-diphenyltetrazolium bromide (MTT) reagent (100 µg/mL) were incubated during 4 hours. Similarly, to dissolve the formazan crystals, 100 µL of dimethyl sulfoxide (DMSO) was added. The absorbance was ruminated at 570 nm by an enzyme-linked immunosorbent assay (ELISA) reader. All experiments were performed in triplicate, with the relative cell viability (%) declared as a portion relative to the unprocessed control cells [15,16].

Differentiation of Cellular Alkaline Phosphatase Activity (ALP). The 7F2 osteoblast-like cells were plated in 24-well plates at 10^4 in DMEM containing 5 mM β-glycerol phosphate (β-GP), 10% FBS, and 50 µg/mL of ascorbic acid (2GF medium) with or without E extract, EH, EE, EB, and EA fractions of *Elaeocarpus serratus* L. from Baung Forest for 4 and 7 days incubation period at 37°C in a 5% CO_2 atmosphere. Phosphate buffered saline (PBS) was applied to clean the supernatants. After that, a percentage of the v/v triton solution was inserted and incubated for 10 minutes at 37°C. After incubation, the cell lysates were examined for ALP by adding 200 µL of p-nitrophenyl phosphate (PNPP) and di-ethanolamine buffer into each well for a period of 30 minutes and at room temperature. The 50 µL/well stop solution was inserted to stop the reaction while ELISA reader at 405 nm was applied to measure the absorbance [15,16].

Statistical Analysis. The experiments were perfomed for three times using similar methods. It was then expressed as means ± standard deviations. The one-way ANOVA and LSD test were used to illustrate data analysis. The differences proved to be statistically significant at $P<0.05$.

Results

The Effect of 96% Extracts from Baung Forest Plants on DPPH Radical Scavenging. During our project in order to discover antiosteoporotic delegates from natural sources [16,17,18,19,20], we screened 36 plants from Baung Forest on antioxidant by measuring DPPH scavenging. Oxidative stress produces a breakage of cellular owing to membranes structural change, lipid oxidation, and oxidation of nucleic acids and proteins. The breakage may expand to the organs and become systemic [21]. Many ailments have been related to oxidative stress, inserting bone diseases (osteoporosis). Antioxidants reduce acceleration of bone damage thru encoragement of tumor necrosis factor alpha (TNFα) [22]. Based on the screening result, the 96% ethanol extract of *Elaeocarpus serratus* L. (13), *Memecylon myrsinoides* (19), *Hibiscus surattensis* (25), and *Hypoestes phyllostachya* (35) from Baung Forest showed high DPPH radical scavenging (82.17±2.95, 81.02±1.17, 75.38±1.92 and 71.47±3.55%, respectively) (Table.1). Therefore, the most potent plant as an antioxidant is *Elaeocarpus serratus* L. In Indonesia, the leaves of this plant are used traditionally to treat arthritis [23] and in India, it is used as Ayurveda of anti-osteoporosis [24] and arthritis [25]. Then, the % DPPH radical scavenging toward this plant at different concentration were explored.

The Effect of 96% Ethanol Extract of *Elaeocarpus serratus* L. Leaves on DPPH Radical Scavenging. In this research, we analyzed the effects of E extract, EH, EE, EB, and EA fractions of *Elaeocarpus serratus* L. from Baung Forest leaves toward antioxidant related to bone turnover. Several researches reported on the pharmacological effects of plant extract (Elaeocarpaceae family) from several countries [23,24,26,27,28,29], but there have been no reports on 96% ethanol extract of *Elaeocarpus serratus* L. from Baung Forest, Indonesia.

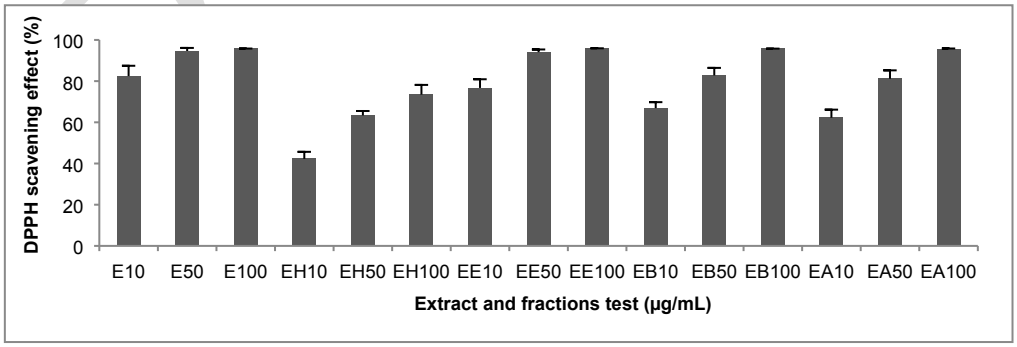

Figure 1: Antioxidant activity using DPPH method of 96% ethanol extract, hexane, ethyl acetate, butanol and aqueous fractions of *Elaeocarpus serratus* leaves. The number of 10, 50 & 100 following extract (E) and fractions (EH, EE, EB, EA) indicate their concentrations at 10, 50 & 100 µg/mL.

DPPH is steady nitrogen that focuses on free radical that can receive hydrogen radical or electron to finish a steady diamagnetic molecule. DPPH radicals respond with appropriate reducing agents as a yield of which the electrons get couple off becoming the corresponding hydrazine. Thus, the antioxidant activity of E extract, EH, EE, EB and EA fractions of *Elaeocarpus serratus* L. from Baung Forest with several concentrations (10, 50 and 100 µg/mL) was detected by DPPH scavenging assay in a range of concentration. Based on the result, the E extract, EH, EE, EB and EA fractions had IC_{50} value of 23.27, 42.47, 19.93, 30.12, and 34.90, respectively (Fig.1).

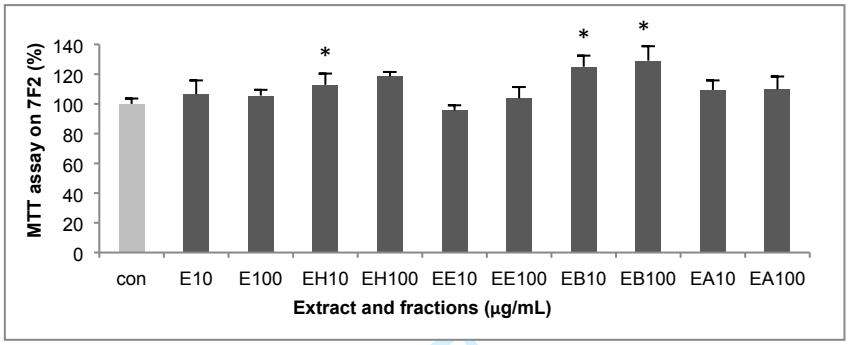

Figure 2: The MTT test of 96% ethanol extract, hexane, ethyl acetate, butanol and aqueous fractions of *Elaeocarpus serratus* leaves on 7F2 osteoblast cells. The number of 10, 50 & 100 following extract (E) and fractions (EH, EE, EB, EA) indicate their concentrations at 10, 50 & 100 µg/mL.

The ALP Stimulation Effect of 7F2 Osteoblasts of 96% Ethanol of *Elaeocarpus serratus* L. The viability results of E extract, EH, EE, EB and EA fractions of *Elaeocarpus serratus* L. from Baung Forest in 7F2 osteoblastic cell lines was carried out using MTT test. The viability cells of their extract and fractions increased in dose-related, in which they showed that high concentration of extract and fractions were not toxic (Fig. 2) and elevated cellular uptake. Then, ALP experiments were proceed.

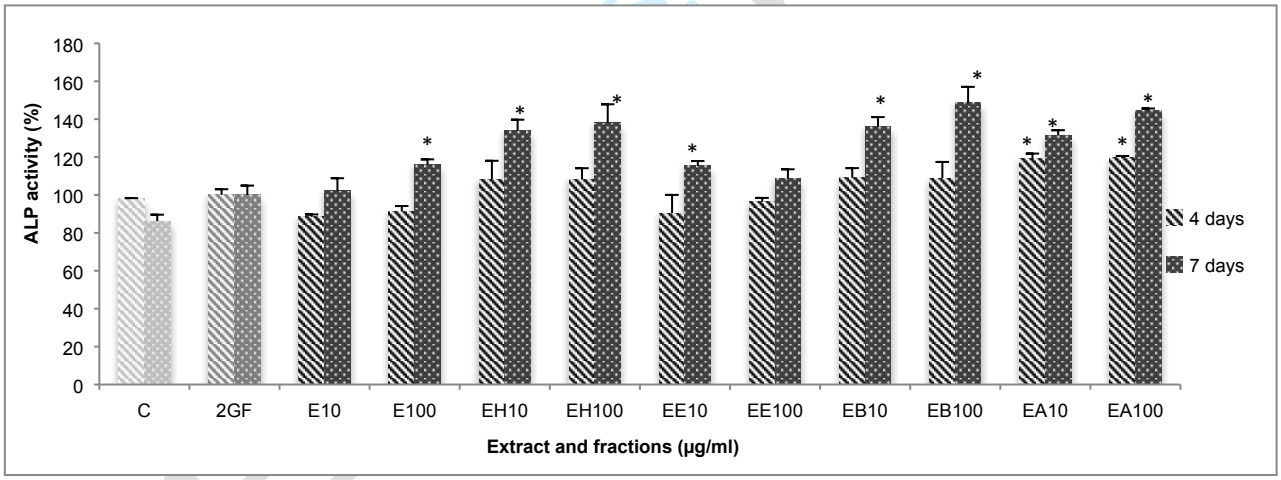

Figure 3: The ALP activity of 96% ethanol extract, hexane, ethyl acetate, butanol and aqueous fractions of *Elaeocarpus serratus* leaves for 4 and 7 days incubation. The number of 10, 50 & 100 following extract (E) and fractions (EH, EE, EB, EA) indicate their concentrations at 10, 50 & 100 µg/mL. The sign * means $p<0.05$ to 2GF.

The ALP stimulation of 7F2 osteoblast cells using E extract, EH, EE, EB and EA fractions of *Elaeocarpus serratus* L. from Baung Forest was incubated for 4 and 7 days. The results of samples on increasing ALP assay in the 7F2 osteoblasts againsted to the 2GF group on EH, EB, and EA fractions for 4 days (Fig. 3). After 7 days, the EB, EA and EH fractions stimulated their ALP activity to 148.56±8.47, 144.58±1.04, and 138.11±9.72%, respectively (Fig. 3).

Discussion

Geographically, the Baung Purwodadi forest area is located between 7°49'9"-7°47'23" South Latitude and 112°16'23"-112°17'17" East Longitude with the topography in general being bumpy to hilly, the altitude of this area ranges from between 200 - 501 masl, red yellow mediterranean soil types and latosols, soil derived from old quarter rock with the main material in the form of metamorphic sediment, climate type D rainfall with a value of Q = 81.82%, the average annual amount of 2.654, 10 mm/year with an average number of rainy days of 141.05 days [30]. In the forest there are plant communities. Potential flora in the TWA Gunung Baung area, including *Brucea javanica*, *Urena lobata*, *Plumbago zaelanica*, *Parameria leivigata*, *Garuga floribunda*, *Plumeria acuatifolia*, *Lantana camara*, *Rauvolfia tetraphylla*, *Gloriosa superba*, *Melia azedarach* and others (Table 1).

These plants are used by the local community for treatment such as lowering sugar levels, fever, inflammation, high blood pressure, treating stomach aches, relieving joint pain, headaches, worming and urination.

The use of these plants as traditional medicine is only based on inheritance from ancestors without knowing the chemical content that plays a role in treatment [31]. Therefore, to determine the exact chemical content for treatment, it is necessary to explore plants, especially forest plants that have a large enough potential. The initial screening was antioxidant potential because the assay is simple and easy for large quantities. Oxidative stress occurs as a result of overproduction of ROS which is not balanced, which can cause bone disruption. The altered redox state is also associated with the bone remodeling process which enables the continuous regeneration of bone through the coordinated action of bone cells. Changes in ROS and/or the antioxidant system involve in the pathogenesis of bone loss. ROS induces apoptosis (death) of osteoblasts and osteocytes, this encourages osteoclastogenesis and inhibits mineralization and osteogenesis [32]. Based on DPPH Radical Scavenging result on several plants in the Baung forest, *Elaeocarpus serratus* L. has the highest potential in trapping DPPH radical scavening (82.17±2.95). Therefore, it continues the exploration of this plant to determine their ability to increase bone density.

Natural plants have performed a pivotal part in pharmaceutical drugs and dietary supplement developments for the therapy and precaution of ailment [33]. One of them is *Elaeocarpus serratus* L. from Baung Forest which belongs to the Elaeocarpaceae family. Traditionally, it is used to treat migraine, stress, anxiety, depression, lack of concentration, palpitation, nerve pain, epilepsy, asthma, hypertension, liver diseases [23], arthritis [34], Ayurveda of anti-osteoporosis [24], and Ayurveda of osteoarthritis [25]. Several studies have shown that this plant is pharmacologically active and can be functioned as the treatment of arthritis [35], anti-microbial [36], anti-inflammatory, analgesic, pesticide, nematoside, antioxidant [25], antibacterial, diarrhea, and dysentery [37]. The leaves contain flavonoids, carotenoids [34,38], fatty acid [26], myricitrin, and mearnsetin derivatives [39]. Myricitrin has the greatest antioxidant activity in this plant [39]. It was also proved in this study that 96% ethanol extract of *Elaeocarpus serratus* L leaves had a radical scavening DPPH value of 82.17±2.95% (Table 1). This is the greatest value of its activity compared to other plant extracts from Baung Forest. Based on Figure 2, almost all fractions has the ability to trap free radical > 50% at concentration of 10-100 µg/mL but the hexane fraction (EH) at 10 µg/ml cannot trapping DPPH radicals by up to 50%. The greater percentage value of trapping, the better antioksidan activity in DPPH radical scavening [40]. Consequently, we explored this extract for bone formation activity.

Several studies have associated antioxidants with bone metabolism. Lower plasma antioxidants can be found in elderly women or women with osteoporosis. Oxidative stress in estrogen deficiency of postmenopausal osteoporosis has been linked to the activation of NADPH oxidase and/or alleviated synthesis of antioxidant enzymes and glutathione (GSH) levels [21,26]. This antioxidant leads the acceleration of bone loss through activation of tumor necrosis factor alpha (TNFα) [22]. Converting in the redox state is also linked to the process of bone remodeling that permits continuous bone regeneration thru coordinated action of bone cells such as osteoblasts, osteocytes and osteoclasts. Antioxidants directly contribute to activating osteoblast differentiation in bone formation and mineralization processes.

Based on the results, the 96% ethanol extract of *Elaeocarpus serratus* L leaves had a strong antioxidant activity and also played a role in the activation of osteoblast differentiation which is directly related to bone formation. Osteoblast differentiation is characterized by measuring levels of alkaline phosphatase (ALP). ALP is an important enzyme that is a useful biochemical marker of bone formation [41]. This enzyme plays a role in osteoid formation and mineralization. So that the ALP enzyme and bone mineralization have a significant correlation and become a biochemical marker [42]. Bone growth and healing during bone fracture cause high ALP enzymes in bones. However, if the ALP enzyme appears in excess, it can be an indicator of osteosarcoma to bone metastases [43]. The 96% ethanol extract of *Elaeocarpus serratus* L leaves stimulated ALP activity in dose of dependent manner (116% of 100 µg/mL). Among the fractions, butanol-soluble fraction (EB) had the strongest ALP activity (148.56±8.47%). It is a potential fraction for activation of bone formation. Ethanol extract from this plant contains fatty acid ester derivatives such as *n*-dotriacontanol (10.70%), *n*-octadecanol (10.08%), docosanoic acid, 1,2,3-propanetriyl ester (9.07%), *n*-hexadecene (8.52%), bis-(3,5,5-trimethylhexyl) ether (6.30%), ethanone, 1-cyclopentyl- (4.81%), cyclohexane, ethyl- (4.05%), and minor components were hexadecanoic acid methyl ester (0.80%), ricinoleic acid (0.77%), citronellyl isobutyrate (0.69%) and farnesol (0.51%) [26]. Fatty acid has a role in increasing bone formation by stimulated β catenin activity in osteoblast and resulting in increased in osteoblastogenesis [44,45]. The mechanisms of fatty acid are complex and involve protectins and resolvins, prostaglandins, growth elements, cytokines, and few other molecular signaling routes [45]. This plant also contains carotenoids [38], that have a encourage effect on osteoblastic bone formation *in vitro*, therefrom escalating bone mass. This effects the gene expression of various proteins associated to bone formation [45]. Thus, the 96% ethanol extract of *Elaeocarpus serratus* L leaves has potential effect to maintain bone health and decrease bone loss.

Conclusions

The extract and fractions of *Elaeocarpus serratus* L can successfully inhibit DPPH radical scavenging value and increase ALP activities as markers of osteoblast functions.

References

1. Jennifer JW. Methods in molecular biology: Osteoporosis methods and protocol, Human Press; 2008.
2. Raisz LG. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. J Clin Invest 2005;115:3318–3325.
3. Cooper C, Campion G, Melton LJ. 3rd Hip fracture in the elderly: a world-wide projection. Osteoporos Int 1992;2(6):285–289.
4. Kanis JA, Johnell O, Oden A, Sembo I, Redlund-Johnell I, Dawson A, De Laet C, Jonsson B. Long-term risk of osteoporotic fracture in Malmö. Osteoporos Int 2000;11:669–674.
5. Sudoyo SA, Simadibrata S. Osteoporosis. Buku ajar ilmu penyakit dalam II. 4th ed., Jakarta, FKUI 2006.
6. Kementerian Kesehatan RI Pusat Data dan Informasi. Data & Kondisi Penyakit Osteoporosis di Indonesia. Jakarta Selatan: Kementerian Kesehatan RI Pusat Data dan informasi 2015.
7. Kementerian Kesehatan Republik Indonesia. Germas Osteoporosis. s.l.:Kementerian Kesehatan Republik Indonesia 2017.
8. Balai Besar Konservasi Sumber Daya Alam Jawa Timur: Taman Wisata Alam Gunung Baung (cited 2019 Des 5). Available from: http://bbksdajatim.org/taman-wisata-alam-gunung-baung-1523
9. Chen JR, Lazarenko OP, Haley RL, Blackburn ML, badger TM, Ronis MJJ. Ethanil impairs estrogen receptor signaling and activates senescence pathways in osteoblasts protection by estradiol. J Bone Min Res 2009;24:221–230.
10. Gilbert L, He X, Farmer P, Boden S, Kozlowski M, Rubin J, Nanes MS. Inhibition od osteoblast differentiation by tumor necrosis factor-alpha. Endorcinology 2000;141(11):3956–3964.
11. Rekha SP. Comparitive study of biochemical bone turnover markers in pre & post-menopausal women. International Journal of Applied Research 2015;1(5):185.
12. Vaithialingam A, Lakshmi TM, Suryaprakash G, Edukondalu AD, Reddy EP. Alkaline Phosphatase levels in rheumatoid arthritis and osteoporosis in clinical practice. Journal of Current Trends in Clinical Medicine & Laboratory Biochemistry 2013;1(2):20–23.
13. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity LWT. Food Science and Technology 1995;28:25–30.
14. Kim DO, Lee KW, Lee HJ, Lee CY. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. Journal of Agricultural and Food Chemistry 2020;50:3713–3717.
15. Yeh CC, Su YH, Lin YJ, Chen PJ, Shi CS, Chen CN, Chang HI. Evaluation of the protective effects of curcuminoid (curcumin and bisdemethoxycurcumin)-loaded liposomes against bone turnover in a cell-based model of osteoarthritis. Drug Des Dev Ther 2015;9:2285–2300.
16. Widyowati R, Suciati, Haryadi DM, Chang H, Suryawan IPGN, Utama AW. The effect of *Rusa unicolor* antler extracts from East Kalimantan in bone turnover cell models. Turk J Pharm Sci 2020;17(4):440–445.
17. Widyowati R, Tezuka Y, Miyahara T, Awale S, Kadota S. Alkaline phosphatase (ALP) enhancing iridoid glucosides from the Indonesian medicinal plant *Barleria lupulina*. Nat Prod Comm 2010;5(11):1711–1716.
18. Widyowati R. Alkaline phosphatase activity of *Graptophylum pictum* and *Spilanthes acmella* fractions against MC3T3-E1 cells as marker of osteoblast differentiation cells. Int J Pharm Pharm Sci 2011;3(1):34–37.
19. Laswati H, Subadi I, Widyowati R, Agil M, Pangkahila JA. *Spilanthes acmella* and physical exercise increased testosterone levels and osteoblast cells in glucocortocoid-induced osteoporosis male mice. Bali Medical Journal 2015;4(2):76–81.
20. Widyowati R, Ekasari W, Purwitasari N. An amine derivative from the aerial part of *Spilanthes acmella* Murr. and their ALP activity. The Nat Prod J 2020;10(5):571–577.
21. Naka K, Muraguchi T, Hoshii T, Hirao A. Regulation of reactive oxygen species and genomic stability in hematopoietic stem cells. Antioxid Redox Signal 2008;10:1883–1894.
22. Domazetovic V, Marcucci G, Lantomasi T, Brandi ML, Vincenzini MT. Oxidative stress in bone remodeling: role of antioxidants. Clin Cases Miner Bone Metab 2017;14(2):209–216.
23. Das P, Kar P, Hasnu S, Nath S, Tanti B. Phytochemical screening and antioxidant activity of *Elaeocarpus serratus* L. of Assam. J Pharmacognosy & Phytochemistry 2017;6(4):866–869.
24. Hardainiyan S, Nandy BC, Kumar K. *Elaeocarpus Ganitrus* (Rudraksha): A Reservoir Plant with their Pharmacological Effects. Int J Pharm Sci Rev Res 2015;34(1):55–64.
25. Sreelekshmi SG, Manoj GS, Lawarence B, Murugan K. Ameliorative potentials of plant-derived phytochemicals against arthritis. Trends in Biosciences 2018;11(7):1714–1720.
26. Geetha DH, Rajeswari M, Indhiramuthu J, Chemical profiling of *Elaeocarpus serratus* L. by GC-MS. Asian Pac J Trop Biomed 2013;3(12):985–987.
27. Kumar TS, Shanmugam S, Palvannan T, Kumar VMB. Evaluation of antioxidant properties of *Elaeocarpus ganitrus* Roxb. Leaves. Iranian J Pharm Res 2008;7(3):211–215.
28. Sarananda KH, Thillakawardane TU, Alexander B. Production of health-friendly, ready-to-serve fruit drinks from under- utilized local fruits from Sri Lanka. Sri Lanka J Food & Agriculture 2017;3(2):37–48.
29. Arivu I, Muthulingam M. Detailed study on *Elaeocarpus ganitrus* (Rudraksha) for its medicinal importance - A review. Int J Curr Sci 2017;20(1): E 16–30.
30. Sofiah S, Setiadi D, Widyatmoko D. Pola Penyebaran, Kelimpahan dan Asosiasi Bambu pada Komunitas Tumbuhan di Taman Wisata Alam Gunung Baung Jawa Timur. Berita Biologi 2013;12(2).
31. Mannito P. Biosintesis produk alami. Cetakan Pertama. Terjemahan Koensoemardiyah dan Sudarto. New York : Ellis Horwood Limited 1992..
32. Domazetovic V, Gemma M, Teresa I, Maria LB, Maria TV. Oxidative stress in bone remodeling: role of antioxidants. Clinical Cases in Mineral and Bone Metabolism 2017;14(2):209–216.
33. Megraj KVK, Koneri RR, Meenakshisundaram K. Biological activities of some Indian medicinal plants. JAPER 2011;1:12–44.
34. Manoj GS, Lawarence B, Sreelekshmi SG, Murugan K. Review of ameliorative potentials of plant-derived phytochemicals against arthritics. Nov Tech Arthritis Bone Res 2017;1(2):1–6.
35. Geetha DH, Jayashree I, Rajeswari M. In vivo anti-arthritic activity of ethanolic extracts of *Elaeocarpus serratus* L. Int J Pharm Sci Rev Res 2018;48(2):92–97.
36. Lima FF, Breda CA, Cardoso CAL, Duarte MCT, Sanjinez-Argandoña EJ. Evaluation of nutritional composition, bioactive compounds and antimicrobial activity of *Elaeocarpus serratus* fruit extract. Afr J Food Sci 2019;13(1):30–37.
37. Sharker SMD, Shahid IJ. Assesment of antibacterial and cytotoxic activity of some locally used medicinal plants in Sunderban mangrove forest region. African J. Pharma. Pharmacol 2010;4(4):66–69.
38. Otero DM, Ferreira-Ribeiro CD. Potential bioactive compounds of unconventional food plants. Agri Res & Tech 2019;23(2):257–259.
39. Jayasinghe L, Amarasinghe NR, Arundathie BG, Rupasinghe GK, Jayatilake NH, Fujimoto Y. Antioxidant flavonol glycosides from *Elaeocarpus serratus* and *Filicium decipiens*. Nat Prod Res 2012;26(8):717–21.
40. Karadag A, Ozcelik B, Saner S. Review of methods to determine antioxidant capacities. Food Analytical Methods 2009;2:41–60.
41. Golub EE, Boesze-Battaglia K. The role of alkaline phosphatese in mineralizeation. Current Opinion in Orthopaedics 2007;18:444–448.
42. Sharma U, Pal D, Prasad R. Alkaline phosphatase:An overview. Indian J Clin Biochem 2014;29(3):269–278.
43. Sarac F, Saygili FM. Causes of high bone alkaline phosphatase, Biotechnology & Biotechnological Equipment 2007;21(2):194-197.
44. Ahn SH, Park S, Baek J, Lee SY, Baek WY, Lee SY, Lee YS, Yoo HJ, Kim H, Lee SH, Im DS, Lee SK, Kim BJ, Koh JM. Free fatty acid receptor 4 (GPR120) stimulates bone formation and suppresses bone resorption in the presence of elevated n-3 fatty acid levels. Endocrinology 2016;157(7):2621–2635.
45. Kajarabille N, Díaz-Castro J, Hijano S, López-Frías M, López-Aliaga I, Ochoa JJ. A new insight to bone turnover: Role of □-3 polyunsaturated fatty acids. Hindawi Publishing Corporation The Scientific World Journal 2013;1–16.

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