

THE ROLE OF PLANT EXTRACTS IN THE REPAIR OF *Rattus Norvegicus* MANDIBULAR ALVEOLAR BONE IN A PERIODONTITIS MODEL

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ABSTRACT

The evaluated herbs plants (α -Mangostin, usnic acid, *Piper betle* L, and *Daemonorops draco*) have potency in the alveolar bone repair of mandibular on the periodontitis modeling. Its herbs were evaluated with the flavonoid and phenolic, and ionic values identified by a multi-tester meter. Mol-inspiration software was used to calculate the miLogP value of α -mangostin and usnic acid. The evaluation of alveolar bone repair is based on H and E staining profiles of osteoblasts and osteoclasts. The α -Mangostin and *Piper beetle* L had higher total flavonoid and phenolic concentrations than usnic acid and *Daemonorops draco*. Based on the miLogP value, usnic acid has better absorption than α -mangostin. The ionic amounts of dissolved Oxygen, conductivity, and total dissolved solids have correlated with osteoblast and osteoclast expression in the bone repair process. The herbs extract can increase the stimulation to produce osteoclast and stability in 7 and 14 days. The ionic values, flavonoids, and phenolic totals of herbs affect the mandibular alveolar bone repair, which is marked to increase the new bone formation (osteoblast) and bone resorption (osteoclast).

Keywords: Alveolar Bone, Plant Herbs, Periodontitis, Osteoblast, and Osteoclast.

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INTRODUCTION

Periodontitis is reported as one of the degenerative diseases in adults. This infection can disturb the teeth, soft tissue, and alveolar bone.¹ Subgingival plaque accumulation, dental caries, and gingivitis are the main factor of periodontitis. If it lasts long, was caused losses of the periodontal ligament and alveolar bone.² Immunologically, periodontitis inflammation is caused by pro-inflammatory cytokines. Tumor necrosis factor- α (TNF- α), prostaglandin E2 (PGE2), and interleukin 1 β (IL-1 β), also receptor activator of nuclear factor- κ β ligand (RANKL) and receptor activator of nuclear factor κ β (RANK) were reported as the main factor in periodontitis infect. These proteins reported impairing the balancing between the protective and destructive processes that cause the alveolar bone resorption to suppress osteoblast production and increase the osteoclast.³ Bone proteins such as Osteocalcin, TGF-beta, and BMP (Bone matrix protein) increase bone repair. They have produced osteocytes, osteoblast, and osteoclast through parenchymal cell regeneration. Its method promotes periodontal repair.⁴ TRAP5b was released by osteoclast cells during differentiation and bone resorption. Its protein is a protease enzyme that is abundantly produced by lysosomes. This enzyme is a precursor of osteoblast and osteoclast and is often used as the biomarker of bone resorption.⁵ The handling of bone damage in periodontitis cases requires osteoconductive dan osteoinductive properties. Administration of bone repair drugs will increase osteoinductive bone healing. It still has several problems, like calcifying bone calcium, which is not long-term.⁶ Moreover, curettage,

flap periodontal, and gingivectomy are reported as periodontal therapies in surgery. It has often used by the dentist in periodontitis treatment. Still, its activities have a biological effect on the tissues and health of bone post-surgery and cause the emergence of secondary infection.⁷ The new alternative of material drug bone repair can be osteoinductive. The use of natural material benefits, besides preventing resistance to antibiotics, low prices, quickly obtained, and common side effects. Treatment of Periodontitis for decreasing inflammation removes the gaps between the gums and teeth, reduces the risk of secondary infection, and repairs the periodontal tissue and alveolar bone absorbed during infection.¹ In Indonesia, several herbal plants are widely reported as anti-infection, pyretic, anti-cancer, osteoporosis, and bone healing. Among the many herbs, α -Mangostin, Usnic acid, betel leaf extract (*Piper betle L*), and Jernang extract (*Daemonorops draco*) contain antioxidants. The flavonoids and phenolic totals can help the bone healing process.⁸ As the new medicine concepts, these herbs have a role in bone repair pathogenesis. The guided tissue regeneration stimulates the growth of new bone after bone infection, and the tissue-stimulating proteins encourage the development of around tissue of bone and the formation of new bone. This study's objective was to analyze the biological properties of herbal plants related to the ionic value, flavonoid, and phenolic total in the bone repair of the alveolar bone of rat mandibular on the periodontitis modeling.

EXPERIMENTAL

Material

Medical Faculty, Universitas Andalas, Padang Sumatera Barat, Indonesia, No.412/KEP/FK/2017 approved the research for ethical clearance. Four groups of 24 rat subjects were used, namely α -Mangostin, Usnic acid, *Piper betle L*, and *Daemonorops draco*, with intervals of 7 and 14 days.

Plant Material

The whole plant used in this study was obtained from Sumatera Barat Province, Indonesia. *Gracia mangostana L* was purified α -Mangostin from Batusangkar, Kubang Landau (-0.4555771,100.6409525). *Usnea articulata* was purified with Usnic acid from Gunung Singalang (-0.3804360,100.2852379). *Piper betle L* from Padang, Medicine plant Garden (-0.9044995,100.4620656), and *Daemonorops Draco* from Muaro Bungo (-1.4789265,102.1400109). All of the assay materials were collected by the writer Deddi Prima Putra from the Biota Sumatera Laboratory, Universitas Andalas, Padang, Sumatera Barat, Indonesia.

Extraction and Purification

The α -Mangostin is isolated from mangosteen rind (*Gracia mangostana L*) and Usnic acid purified from the thallus of the wind tree (*Usnea articulata*). The α -Mangostin was extracted and filtered by Andalas Sitawa Fitolab, Andalas University, Padang, Sumatera Barat, Indonesia (GPS Coordinate, -0.9476875,100.4529375), Product codes RC08402. Whereas, Usnic acid was produced by the Biota Sumatera Laboratory, Pharmacy Faculty, Universitas Andalas, Padang, Sumatera Barat, Indonesia, (GPS Coordinates, -0.9094017,100.4607161), CAS Number 7562-61-0. The extracted process of betel leaf (*Piper betle L*) and Jernang peel (*Daemonorops draco*) uses 70% ethanol.⁹ The dry powder of the betel leaf and the Jernang fruit's skin was macerated using 70% ethanol as a solvent. Then, stir 1 kg of each extract material in 10 L of 70% ethanol for the first six h. Then let it stand for 18 h. Furthermore, the filtrate was separated by decantation and filtering. The extraction process was repeated with 5 L of 70% ethanol for 24 h, then decanted and filtered. The extract solution was put into a round bottom flask and attached to a rotary vacuum evaporator. Then add distilled water to the water container to the standard limit. It then turned on the vacuum pump at a temperature of 50 °C, a pressure of 20 Psi, and a rotation of 120 xg. The extract solution was oven to dry at 50 °C to obtain a concentrated extract.

Calculation of Molecular Properties and Bioactivity Score

The efficiency and molecular properties were calculated with the help of the software package Molinspiration property engine. Compound structures were drawn in ChemDraw Professional v.16 by Cambridge Soft and brought into 3D with Chem3D v.16 by Cambridge. In addition, mol-inspiration properties engine v. 2018.10 was used to determine the bioactivity and molecular scores of α -mangostin and usnic acid compounds. First, the molecular structures of the two compounds are imported into the mol-inspiration drawing program. Then, you click the computed properties button to get the atomic count,

molecular weight, particle count, and percentages of the most influential groups, such as N, O, OH, and NH. The second step is determining how well each material tests for host or pathogen response.¹⁰

Total of Flavonoid

The standard catechin solution is made by dissolving 10 mg of catechins in 10 mL of methanol p.a. until a concentration of 1000 ppm is reached. The stock solution was diluted to obtain serial solutions of 10, 20, 30, 40, 50, and 60 (all concentrations expressed in ppm) by adding 1 mL to methanol p.a. to obtain 100 ppm. Then, 3 mL of methanol, 0.2 mL of 10% AlCl₃, 0.2 mL of 1 M potassium acetate, and 10 mL aqua dest are added to each concentration, respectively. The absorbance was then measured using UV-Vis spectrophotometry at a wavelength of 500 nm after being incubated for 30 min at 25 °C. A 0.5 mL ethanol blank containing 1.5 mL 95% ethanol, 0.1 mL 10% AlCl₃, 0.1 mL 1 M potassium acetate, and 2.8 mL Aquadest was used to determine flavonoid concentrations. It was then placed in a 25 °C incubator for 30 minutes. Each absorption reading is matched up against a blank. First, 100 mg α-Mangostin, Usnic acid, *Piper betle* L, and *Daemonorops draco* were dissolved in 10 mL of methanol to determine the total flavonoid content. After adding 0.1 mL of 10% AlCl₃, 0.1 mL of 1 M potassium acetate, and 2.8 mL of aquadest to 10 mL of 95% ethanol in a volumetric flask, the mixture was incubated at 25 degrees Celsius for 30 minutes. Using a UV-Vis spectrophotometer, we determined that the absorbance value was 434.2 nm. Three batches of the sample solution were prepared to ensure the flavonoid concentrations were comparable to catechin.

The formula of flavonoid total: $F = \frac{CxVxf \times 10^2}{m} \times 100\%$, Where, F = Amount flavonoid of AlCl₃ method; c = Cathecin equivalence (µm/ml); V = volume total of extract; f = Dilution factor; and m = Sample weight (g).¹¹

Total of Phenolic

10 mg gallic acid was dissolved in methanol p.a to 10 mL (1000 ppm). Furthermore, 2.5 mL aliquot was diluted with methanol p.a to a 25 mL volume resulting in a concentration of 100 ppm. Moreover, the solution is pipette 1, 2, 3, 4, and 5 mL in methanol p.a to 10 mL, so the resulting concentrations (ppm) of 10, 20, 30, 40, 50, and 60. To each gallic acid solution, 0.4 mL of Folin-Ciocalteau reagent was added, shaken, and left for 4-8 min, and added 4 mL of 7% Na₂CO₃ solution was homogeneous. Then 10 mL of aqua dest was added, and the solutions were left to stand for two h at room temperature. The absorbance was measured at a wavelength of 744.8 nm. A calibration curve was generated by plotting the absorbance against concentration. Determination of total phenolic levels of α-Mangostin, Usnic acid, *Piper betle* L, and *Daemonorops draco* was conducted by dissolving 100 mg of each sample in 10 mL methanol. Then, 1 mL aliquot was added to a test tube. Subsequently, 0.4 mL of Folin-Ciocalteau reagent shook and left for five min. 4 mL of 7% Na₂CO₃ solution was added and shaken until homogeneous. 10 mL aqua dest was poured, and the solution was let stand for 2 h at room temperature. The absorption was measured at a wavelength of 744.8 nm. The examination was repeated three times, and the phenolic total was expressed as mg of gallic acid equivalent / g of extract. The total of Phenolic compounds is calculated using a formula: Phenolic total = $\frac{C \times v}{m}$. where, c = gallic acid equivalent (mg/GAE/L); v = volume of extract solution used (ml) and m = used extraction mass (gram).¹¹

Ionic Assay

Ionic tests comprised dissolved Oxygen, conductivity, and total dissolved solids (TDS) against α-Mangostin, Usnic acid, *Piper betle* L, and *Daemonorops Draco*. Conductivity checks were carried out with a conductivity meter, dissolved Oxygen with a DO meter, and total dissolved solids with a TDS meter (CyberScan DO 300, Eutech Instruments Pte Ltd, Singapura).¹² It is evaluated using an electrode base with various probes, voltage sources, and amperage meters. The electrodes are separated by a particular distance (usually 1 cm). During the measurement, these two electrodes are immersed in the sample solution. The test was carried out three times to get the optimal value.

Periodontitis Model

Male Wistar rats weighing 250-300 grams, aged 2-3 months, were acclimatized for a week. The mice were injected with Xylaxine base at a 10 mg/kg body weight dose to cause a sedative effect. Ten minutes later, the mice were anesthetized intramuscularly with ketamine in 10 mg/kg body weight. The periodontitis was

induced by non-resorbable silk ligature threads 3/0 (3 metrics). It is used in the mandibular incisors' subgingival cervical area, forming a Fig.-8 pattern. On the seventh day of ligation removed, the gingiva turned red. Gingival recession, edema, and changes in the gingiva contour indicated periodontitis. One mouse was taken randomly, representing the population, decapitated for its jaws, then fixed in a 10% formalin solution. After that, x-rays were performed using a periapical technique to see any loss of alveolar bone in the interdental rats.¹³ The rat-periodontitis applied the α -Mangostin 2.5%, Usnic acid 2.5%, *Piper betle* L 2.5%, and *Daemonorops Draco* 2.5% on the gingival area experiencing periodontitis. They are used daily, from 1-14 days, morning and evening. After the seventh and fourteenth days, the mice were euthanized with ketamine in 10 mg/kg body weight intramuscularly and then dislocated the neck. Mandibular bone was immersed in 10% formalin for histopathological preparations.

Hematoxylin and Eosin Staining

Mandibular bone was decalcified using H₂SO₄ for four weeks in a 37 °C incubator. Furthermore, tissue trimming was done by washing with running water for 120 minutes. The tissue dehydration process uses alcohol with a concentration of 70%, 80%, and 96% for 30 min. Next, Clearing was carried out in toluene alcohol for 20 minutes and pure toluene solution for 60 minutes until the tissue turned transparent. The membrane was implanted in a paraffin block, and the paraffin infiltration process was carried out in an incubator at a temperature of $\pm$ 56 °C. Then the paraffin blocks were cut with a microtome, four μ m thick. Then the pieces are placed on the water's surface in a water bath at 46 °C. Then it is placed on the slide filled with Meyer egg albumin and stored in an incubator at 60 °C until the preparations are ready for staining. The tissue deparaffinization process was done by immersing the slide preparations in xylol for 5 min, then rinsing with running water. The rehydration was done by engaging the slide preparations in 100%, 95%, 90%, 80%, and 70% alcohol for 1 min.

Furthermore, the slides were immersed in hematoxylin for 5 min at room temperature, then washed with running water. The tissue slices were observed under a microscope to ensure the nucleus was well stained. The slide preparations were soaked with eosin for 2 min, then washed with running water. The tissue slices were dehydrated with 50%, 70%, 80%, 90%, and 95% alcohol. The tissue on the slide preparations was dried. The clearing process was by immersing the tissue in xylol for 3 min. Osteoblasts and osteoclasts were viewed and counted using a 400x magnification Olympus BX 51 light microscope. The two bone cells are calculated by looking at five fields of view to see the difference between the two cells.

Statistical Analysis

One-Way ANOVA analyzed the quantitative data of the effect of the test material on osteoblast and osteoclast cells. The independent and paired sample tests examined ionic activity, phenolic total, and flavonoids total. A paired T-test was used to analyze the flavonoids and phenolics totals in the expression of osteoblast and osteoclast. The correlation of their effects was analyzed using paired correlation and Pearson correlation ($r = 1$), a strong relationship, and $p < 0.05$ was the significance limit of the two analysis variables.

RESULTS AND DISCUSSION

The study evaluated several purified Indonesian herbs (α -Mangostin dan Usnic acid) and crude ethanol extract (*Piper betle* L and *Daemonorops Draco*). These plant extracts function as guided tissue regeneration or Tissue-stimulating proteins in the rat mandibular of periodontitis modeling bone repair. The first stage evaluated the molecular properties and bioactivity score calculation of α -mangosteen and usnic acid. The calculated rating can be a reference to accurately measuring the profile of biological properties of both assay materials related to the growth response of bone repair or stimulating the growth of tissues and new bone. In the second step, total flavonoid and phenolic content were measured. This assay evaluates the antioxidant properties of all assay materials utilized in the periodontitis model for alveolar bone repair. The third step measured the ionic composition of the plant extract. It explains the strength of dissolved Oxygen, conductivity, and total dissolved solids of all assay materials that involved bone repair. The last stage is identifying the plant extract effects on the expression of osteoblast and osteoclast cells. Furthermore, it results in a correlation analyzed all of the biological properties of these assay materials. Figure-1 illustrates that the octanol-water partition coefficient or log P used in the QSAR (Quantitative Structure-Activity Relationship) study and drug design are a degree of molecular hydrophobicity.

Hydrophobicity is correlated with drug absorption, bioavailability, drug receptors, molecular metabolism, and toxicity. The log P coefficient is utilized to assess the lipophilicity of a chemical compound. Additionally, it establishes pharmacokinetic properties. The α -mangostin has a high mi LogP compared to usnic acid. In process drug absorption, usnic acid is better than α -mangostin. It has to describe the calculation of molecular properties and bioactivity scores of usnic acid and α -Mangostin. This value is a reference to predict the involvement of these two chemical compounds in bone repair. The results in Fig.-2 indicate a correlation between the test material properties, osteoblast cell expression increase, and osteoclasts decrease. Based on the miLogP value, Usnic acid responds to bone growth because it has low molecular hydrophobicity. Meanwhile, miLogP α -Mangostin tends to be higher with high molecular hydrophobicity, so this compound stimulates bone tissue growth. Both have different working properties but aim to accelerate the repair of post-infection bones. Savjani (2012)¹⁴ reported that all medicinal substances used for therapeutic purposes must have a lower molecular hydrophobicity. It is related to drugs' solubility when interacting with water or body fluids to be easily absorbed in the pharmacokinetics phase.¹⁵ The line study was made clear by Hermens (2013).¹⁶ The quantitative structure-activity relationship study used the octanol-water partition coefficient or log P to measure molecular hydrophobicity. The R Square (R^2) values of catechin and gallic acid were used as standard curves to determine the levels of flavonoids and phenolics as a reference to determine the effect of the material assay during response and stimulation of bone repair. Aryal (2019)¹⁷ reported that the extract plant's total flavonoid and phenolic levels are associated with their action during interactions with target host cells.

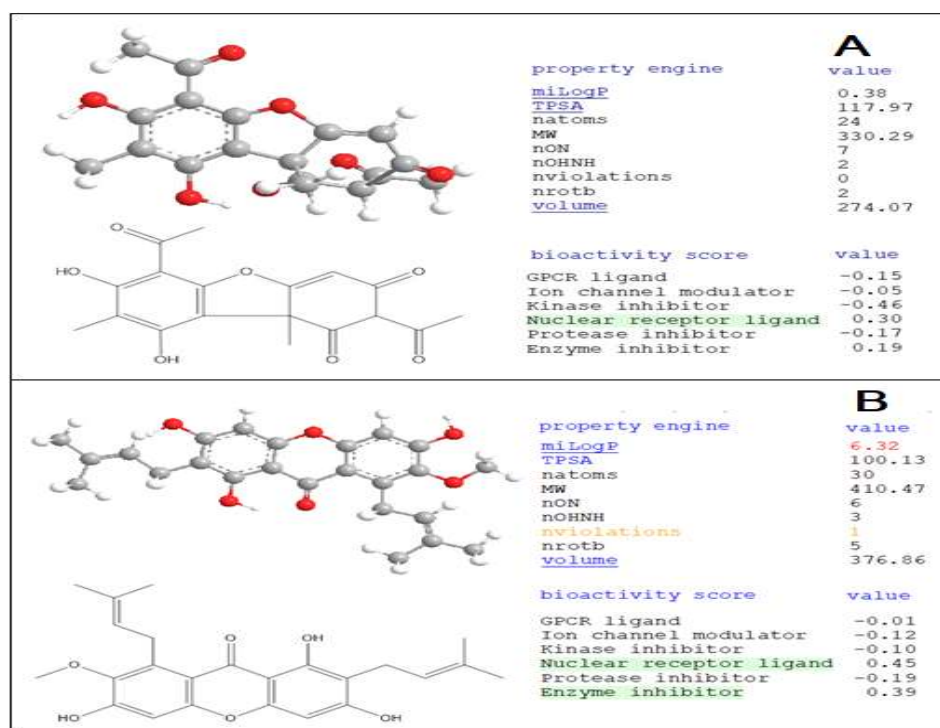


Fig.-1: The Calculation Value of Molecular Properties and Bioactivity. The Usnic Acid Has a Molecular Weight of 330.29 kDa (A), and α -Mangostin Has a Molecular Weight of 410.47 kDa (B). The miLogP Corresponds to the Molecular Hydrophobicity of Drug Absorption.

In Fig.-2, all test materials can stimulate or respond to osteoblasts and osteoclasts in the mandibular alveolar bone repair process. Based on the time interval for the expression of the two bone cells, there was no significant difference ($p > 0.05$), but the bone repair process had a strong relationship with the time interval ($r = 1$). It is assumed that the higher period interval for the bone repair process has correlated with the mandibular alveolar bone's healing after being given the assay material. The test materials have the capacity and quality of involvement in mandibular alveolar bone repair pathogenesis, which is indicated by increasing osteoblasts and maintaining the number of osteoclasts. The study results also reported that the time interval (7 and 14 days) has a significant relationship.

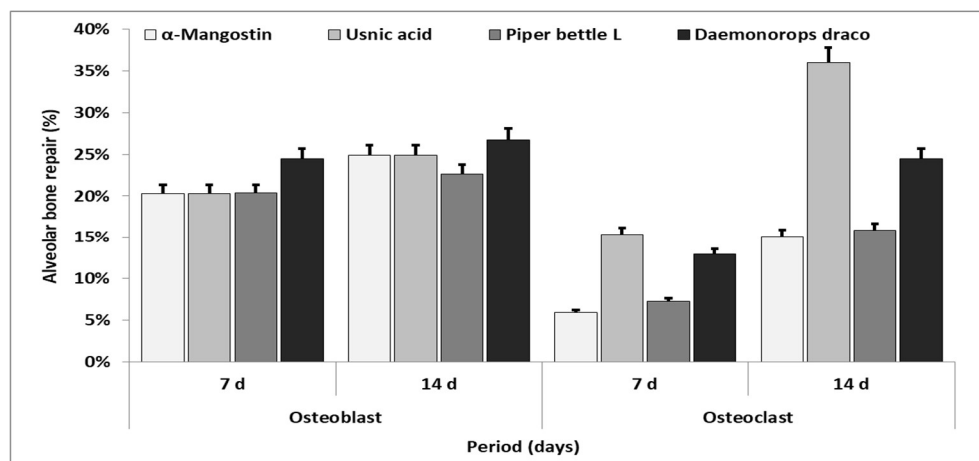


Fig.-2: Histopathological Profile of Osteoblast and Osteoclast Cells of Mandibular Alveolar Bone. The α -Mangostin and Usnic Acid Have a Better Effect on Bone Repair than *Piper betle L* and *Daemonorops draco*. Bar (Alveolar bone repair) and Bar Error (error bar with percentage)

The higher time used for the bone repair process is correlated with bone healing of the mandibular alveolar bone after being given the test material. Therefore it can be assumed that providing the test material does not affect the repair of mandibular alveolar bone. It can determine opacity level based on the bone absorption and desorption processes, as shown in Fig.-3. In Fig.-3, it is shown that the presence of osteoblast cells is more than osteoclast cells as a reflection of bone repair. Chen (2018)¹⁸ reported that bone homeostasis depends on osteoclasts' bone resorption and bone formation by osteoblasts. This process imbalance can cause osteoporosis. Therefore, the communication regulation between osteoclasts and osteoblasts is essential for bone repair. Thus, the involvement of the four herbal plants, besides improving communication between bone cells, also strengthens cooperation during bone resorption. This principle is to create a balance of cytokine and extracellular matrix interactions to increase the role of osteoblasts in bone formation, osteoclast differentiation, or apoptosis.¹⁸ Figures-2 and 3 show that the osteoclast decrease is related to the assay material used to initiate bone formation or repair. Polo-Corrales (2014)¹⁹ reports that communication between bone cells during the construction of new bone (bone repair) is facilitated by ionic activity such as dissolved oxygen volume, conductivity, and total solubility of the test material or drug absorbed during the bone repair response mechanism. This property is related to miLogP , which explains that the better the ionic value, the better the drug's or test material's molecular hydrophobicity. It can help better absorption of the drug.¹⁹ Meanwhile, the total dissolved solid of the test material will determine the pharmacodynamics of the test material and a reference for the inflammatory status during the bone repair process. Less solubility of medicinal substances can cause residual accumulation in the bone repair area. It can affect the quality of healing and cause tissue toxicity due to changes in the medicinal ingredients' molecular properties of chemical compounds.²⁰

In Table-1, it is shown that from the purified test material group, α -Mangostin has a higher total number of flavonoids and phenolics than usnic acid. Meanwhile, *Piper betle L* has a higher total quantity of flavonoids and phenolics from the ethanol extract group than *Daemonorops Draco*. However, *Piper betle L* generally contains flavonoids and total phenolics compared to other test materials. The Paired Sample Test shows that flavonoids and total phenolics of all test materials do not significantly differ in bone repair, with total flavonoids; $p > 0.05$; 0.231 and total phenolic $p > 0.05$; 0.280. both had a negative relationship with bone repair (total flavonoids $r = -0.349$; total phenolics $r = -0.348$).

It means that the faster the bone repair occurs, the fewer total flavonoids and phenolics are needed to achieve a balance in bone repair. The α -Mangostane and *Piper betle L* have more flavonoids and phenolics than usnic acid and *Daemonorops Draco*. So it can be explained that the higher the osteoblast and osteoclast cell expression, the lower the total flavonoid and phenolic values needed for bone repair. Flavonoids control the assay material's role, and phenolics total are managed to respond and stimulate bone repair for 7 and 14 days. Setiawatie (2019)²¹ reported that flavonoids strongly correlate with bone growth. The antioxidants and anti-inflammatory properties are associated with increased bone formation and inhibit bone resorption through the osteoblast and osteoclast differentiation signaling pathways.

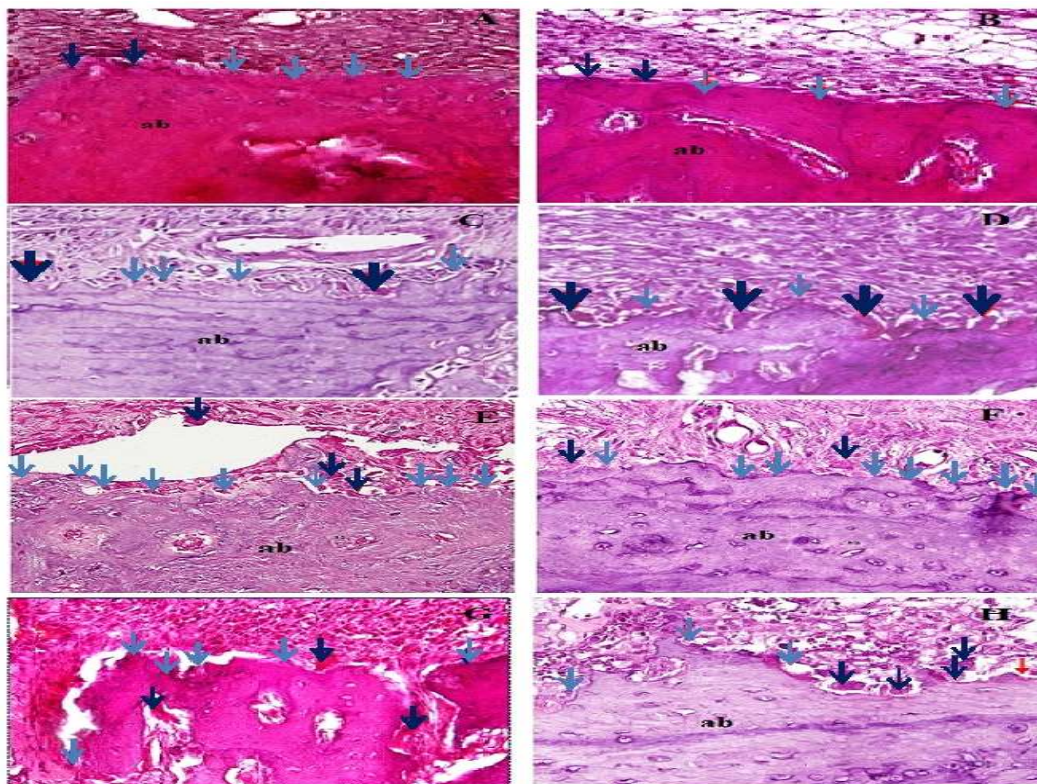


Fig.-3: Histopathological Depiction of Osteoclasts (black arrow) and Osteoblasts (blue arrow) from the Alveolar Bone (ab) in 7 and 14 days. α -Mangostin (A: 7 d and B: 14 d), Usnic Acid (C: 7 d and D: 14 d), Piper betle L Acid (E: 7 d and F: 14 d), and *Daemonorops Draco* (G: 7 d and H: 14 d). Magnification of x400

Table-1: The Flavonoid and Phenolic Totals of Herbs Extract

Herbs	Flavonoid Total					Phenolic Total				
	N	Value (mg CE/g extract)	SDV	Freq (%)	Scale	N	Value (mg GAE/g extract)	SDV	Freq (%)	Scale
α -Mangostin	3	64,376	0,086	26%	Moderate	3	37,26	0,06	26%	Moderate
Usnic acid	3	33,709	0,148	13%	Low	3	16,34	0,06	11%	Low
<i>Piper betle L</i>	3	102,133	0,086	41%	Hight	3	63,30	0,10	44%	Hight
<i>Daemonorops Draco</i>	3	50,194	0,086	20%	Moderate	3	27,47	0,16	19%	Low

Torre (2018)²² reported that the process is related to flavonoids' role in activating osteoblast differentiation by activating the mitogen MAP kinase p38. MAP kinases are complex enzymes that regulate protein phosphorylation via redox and phosphorylation reactions. Phosphorylation of p38 explicitly activates the Wnt signaling pathway. This pathway involves a series of dissolved growth factors (Wnts) binding to cell surface receptors (LRP5/6-Frizzled protein) that signal the cytosol kinase GSK3 β becomes phosphorylated. Flavonoids and phenolics from fruit sources have been reported to prevent osteoporosis in ovariectomy. In Table-2, it is explained that the values of ionic dissolved Oxygen, conductivity and total dissolved solid of all assay materials show different percentages. All test materials were calibrated with pH (6.00) and temperature (27 °C). The Usnic acid has a very high ionic dissolved oxygen value compared to other test materials. Meanwhile, *Piper betle L* has a higher conductivity and TDS value than other test materials. Statistical analysis showed that the ionic dissolved oxygen value positively correlated with osteoblast and osteoclast cell expression ($r = 0.33$).

Table-2: The Ionic Value of Herbs Extract

Herbs	Dissolved Oxygen (ppm)				Conductivity (Siemens/m)				Total Dissolved Solids (mg/L)			
	N	Value	SDV	Scale	N	Value	SDV	Scale	N	Value	SDV	Scale
α -Mangostin	3	0,21	0,01	5,3%	3	0,42	0,00	9,8%	3	0,21	0,00	10,4%

Usnic acid	3	2,48	0,13	63,3%	3	0,25	0,00	5,8%	3	0,14	0,00	6,9%
<i>Piper betle L</i>	3	0,69	0,03	17,6%	3	2,44	0,04	57,1%	3	1,11	0,08	55,0%
<i>Daemonorops draco</i>	3	0,54	0,02	13,8%	3	1,16	0,00	27,2%	3	0,56	0,00	27,7%

Meanwhile, ionic conductivity ($r = -0.193$) and total dissolved solids ($r = -0.182$) have a negative relationship. When bone repair occurs, there is a balance between dissolved Oxygen and conductivity of total dissolved solid. It is related to the bone repair process's pharmacokinetics and pharmacodynamic properties. The results showed that the value of ionic dissolved Oxygen had a positive relationship with osteoblast and osteoclast cell expression. Meanwhile, ionic conductivity negatively correlates, meaning a balance between dissolved Oxygen, conductivity, and TDS occurs during bone repair. So it can be explained that the higher the dissolved Oxygen, the lower the conductivity and TDS in the mandibular bone repair process in cases of periodontitis. This study's results align with the principle of ionic activity, namely that the solubility value of Oxygen from the mass of the solution will affect the TDS value and conductivity.²³ The assay materials used in this study have ionic properties that affect bone resorption and absorption during the bone repair, primarily ionic dissolved Oxygen. Oxygen is needed by cells and bone tissue during material adaptation to local tissue in the pathogenesis of bone repair. All these materials' ability to dissolve more Oxygen indicates that the material's oxidation process during bone repair is correlated with oxidation activity and inflammatory response. Oxygen is an essential element in the wound-healing process. In addition to increasing response and communication between cells and tissues, Oxygen helps increase protein synthesis during bone repair or another healing process.²⁴ Based on the result of the study, it could be explained that the process of alveolar bone repair of the rat mandibular in the modeling of periodontitis is influenced by assay materials. Also, every assay material has a different ability on the effect of bone resorption and the periods' intervals of 7 and 14 days. The total flavonoids and phenolics in each test material did not show a significant difference in the development of osteoblasts and osteoclasts, meaning that phenolic and flavonoid levels did not affect the repair of mandibular alveolar bone.²⁵ Still, the presence of these elements was a determinant of the healing rate of post-mandibular alveolar bone. Periodontitis was characterized by a difference in the osteoblast and osteoclast cell expression percentage between 7 and 14 days. The miLogP value of α -mangosteen and usnic acid of these two purified compounds is the basis for assessing the absorption rate involved in bone repair.

CONCLUSION

The α -mangosteen and usnic acid compounds and *Piper betle L* and *Daemonorops Draco* extracts can repair mandibular alveolar bone (osteoblast) and bone resorption (osteoclast) in the periodontitis model. The four herbal have high flavonoid and phenolic totals and stable ionic values.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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