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Metabolite Profiling of Avocado Seed Extract by LC-QToF-MS/MS: A Natural Approach to Enhancing Bone Healing

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ABSTRACT

Avocado is a plant that easily grows in Southeast Asia, especially in Indonesia. Research on the biological foundations of medicinal plant treatment qualities has become popular in recent years. Avocado seeds usually become organic waste that is not utilized, but they can reduce inflammation and increase wound healing after tooth extraction. More polyphenols are found in avocado seeds than in flesh or skin. There is no study about metabolite profiling on avocado seed that lives in Indonesia, especially in Kediri City East Java. This study aims to prove the potential effect of avocado seeds for wound healing by increasing woven bone formation after tooth extraction. This study is experimental research *in vivo* that analyzes metabolite profiles of avocado seeds using LC-QToF-MS/MS. The research samples were 20 white male Rattus norvegicus and were divided into two groups: control (K) and treated (KE). Then, the lower left incisives of the samples were extracted for H&E staining to analyze the woven bone area at days 7 and 14. The results LC-QToF-MS/MS show the avocado seed extract found many major compounds including catechin, cyanidin, chlorogenic acid, and quinic acid. The results show that the woven bone area was significantly higher in the treatment group. The conclusion is that Avocado seeds have a potential compound that can enhance bone formation.

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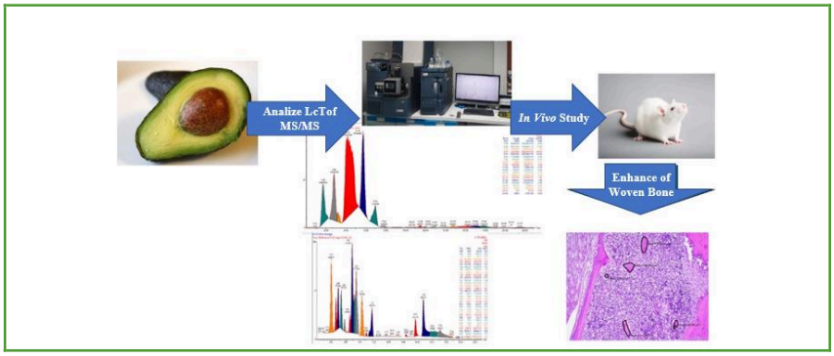
Graphical Abstract



Introduction

The avocado is a plant that comes from Central America and Mexico. Growing avocados in Southeast Asia, especially in Indonesia, is simple because they thrive in tropical and subtropical conditions. In recent years, research on the biological foundations of medicinal plant treatment qualities has grown in popularity. Avocado seed (*Persea americana Mill*) can reduce inflammation and increase wound healing after tooth extraction [1-3]. The total antioxidant content is 1350 µmol Trolox Equivalent (TE) per half fruit, or 600 µmol TE per 30 g. This puts avocados in the middle of the fruit phenolic spectrum, possessing the highest capacity for lipophilic antioxidants. More polyphenols are found in avocado seeds than in flesh or skin [2-4]. Avocado seeds usually become organic waste that is not utilized. Seeds consist of various polyphenols, such as catechin, procyanidin, chlorogenic acid, and quinic acid, but each plant contains different compounds depending on where the plant lives. There is no study about metabolite profiling on avocado seeds in Indonesia, especially in Kediri City, East Java. Some herbal plants have the potential to speed up the healing process, and one of them is avocado seeds. The chemical content of avocado seeds can regulate genes involved in antioxidant activity by inhibition of pro-inflammatory genes expression in addition to bone regeneration signalling pathways [5,6]. Polyphenols also regulate genes involved in antioxidant activity by inhibition of pro-inflammatory genes expression in addition to bone regeneration signaling pathways [5]. The content of these compounds can be used to treat problems faced by diabetes sufferers. Diabetes mellitus (DM) is one of the systemic diseases that can cause a delay and an uncoordinated healing process. DM is one of metabolic diseases which characterized by hyperglycemia that occurs due to abnormalities either in insulin secretion, insulin action, cells insensitivity, or any of them. The International Diabetes Federation (IDF) organization estimated that at least 463 million people aged 20-79 in the world had diabetes in 2019, equivalent to a prevalence rate of 9.3% of the total population of the same age [7,8]. Whereas tooth extraction is the act of removing tooth and tooth root from the socket, either under procedure or spontaneously, which will involve the hard and soft tissues in the oral

cavity [9]. There is a significant difference in post-extraction complications in DM patients due to the impact of metabolic consequences. The study reported by Power *et al.* showed that 12.5% of patients with DM experienced complication of delayed post-extraction healing [10], because diabetic condition can influence each phase of wound healing such as in coagulation and hemostasis phase, leading to the impairment of healing process [9]. In exemplification, the hyperglycemia state through the AGE-RAGE axis causes excessive bone resorption, thereby disrupting the alveolar bone remodeling process in DM [11,12]. The increase of oxidative stress and inflammation can leads the delayed healing in diabetic and influence the woven bone formation [13-16]. The woven bone is basically mineralized tissue in a connective tissue matrix lined by osteoblasts and containing large numbers of osteocytes [14]. Many research studies have been looking into the effects of avocado seed extract as important bioactive compounds in bone formation by increasing osteoblast proliferation and ALP (Alkaline Phosphatase) activity *in vitro* [1]. Avocado seeds contain polyphenol compounds, including cyanidin, chlorogenic acid, and quinic acid, which are bioactive properties [2]. Polyphenols regulate the expression of transcriptional factors and co-activators, which is important in osteoblast differentiation and bone formation processes, including the expression of RUNX2. Simultaneously, polyphenols downregulated RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand) and TNFα, which are the two major key gene regulators in osteoclast differentiation and inflammatory pathways. In addition, the tissue-damaging effects of hyperglycemia will enhance oxidative products by activation of multiple oxidation pathways, which can influence the inflammatory process and bone formation [3]. In this study, metabolite profiling of avocado seed methanol extract was carried out with LC-QToF-MS/MS. The use of HPLC can produce separations with high sensitivity, selectivity, and resolution, increase compound separation efficiency, accelerate analysis time, separate smaller compounds, and reduce the number of samples required [4,17]. This research aims to analyze the influence of avocado (Persea americana Mill.) seed extract on bone formation post tooth extraction in a diabetes mellitus condition.

Experimental

Materials

The materials for conducting this study included LC QToF-Ms/MS (UPLC: Acquity UPLCH-Class System (Waters), MS: Xevo G2-S QToF (Waters), Methanol (onemed), microscope (Nikon E-100, Tokyo, Japan), Glucometer (OneTouch Select Simple), *Streptozotocin* (Bioworld, USA), buffer citrid acid 0,1 M pH 4,5, Eter (Merck KgaA, Jerman), Ketamine 100 mg (KTM-100, Indonesia), Povidine-Iodine (Onemed), formalin buffer 10% (Makmur Jaya, Indonesia), Akuades steril (Onelab Waterone, Indonesia), Alkohol 70%, 80%, 90%, 96% (Novapharin, Indonesia), Etanol Pro Analisa (Merck KgaA, Jerman), Xylol (Merck KgaA, Jerman), HE (*haematoxiline eosine*), and Staining IHK, RUNX2(F-2, Santacruz Biotechnology Inc).

Methods

This study is experimental research with a posttest-only control group design, which was carried out at Laboratorium Faculty of Dentistry Universitas Hang Tuah Surabaya. The preparation of extracts for the experimental study was performed at Laboratorium Biologi Pharmacy Institut Ilmu Kesehatan Bhakti Wiyata. Metabolit profil analyzed in

Labotarorium Forensik MABES POLRI. The staining and histological observations were performed in Research Center Faculty of Dentistry Universitas Airlangga. The study declared eligible conducted by Universitas Airlangga Faculty of Dental Medicine Health Research Ethical Clearance (No. 0063/HRECC.FODM/II/2024). Twenty white male Rattus Norvegicus, ages two to three months, were used as research samples. By making sure the rats' food and water demands were satisfied, their condition was constantly observed. 10 diabetic rats with no treatment administered were used as the control group (K), whereas Group (KE), the therapy group, received avocado seed extract. Each group's samples were divided equally to be sacrificed on days 7 and 14 post-extraction for further analysis.

Methanol extraction

The material used in this research was avocado seeds (Persea americana Mill.) Avocado seeds were obtained from Avocado Plantations, Wates District, Kediri Regency, East Java. Afterwards, characterization was carried out at the Pharmaceutical Biology Laboratory of the Bhakti Wiyata Kediri Institute of Health Sciences and the LCToF MSMS MS metabolite profile test was carried out at the Forensic Laboratory at POLRI Headquarters. The avocado seeds are washed clean and then dried in the oven for 24 hours. After that, grind it until you get dry simplicia powder. A total of 600 g of dried simplicia powder from avocado seeds was macerated with 6 liters of methanol for 3 x 24 hours and then filtered using a Buchner funnel. Next, the filtrate was evaporated using a rotary vacuum evaporator at 70 °C and continued using a water bath at 60 °C until a thick extract was obtained.

Metabolite profiling

Metabolites identification contained in avoca seeds was done using LC QToF-Ms/MS (UPLC: Acquity UPLCH-Class System (Waters), MS: Xevo G2-S QToF (Waters)). Methanol extract of avocado seeds was injected as much as 0.01 mL into the eluent towards the column under pressure. Afterwards, the compounds in the sample were identified using MS by connecting the UPLC system with summer ion ESI and interpreted using Masslynx 4.1 and web chemspider. The Masslynx 4.1 software was opned, and then File was clicked and the saved folder was selected. Thereafter, the sample spectrum will appear so that the separation is visible, the sample chromatogram display is changed from TIC to BPI. Once the chromatogram is visible, the peak at RT (real time) was clicked on until the m/z chemical composition value appears. The tool was clicked, elemental composition was selected, and spectrum was clicked twice to get the mass elemental composition. Elemental composition is the recommended formula composition of a chemical compound according to the mass of the compound. The target element that may appear was clicked because it uses natural materials; only C, H, N, and O atoms were selected (because these are elements that are commonly found in natural material samples). Next, to find out the name of the compound, we looked through Chemspider by subtracting or adding 1 H atom. Next, the Chemspider website (www.chemspider.com) was opened, and then the column filled with the compound composition obtained from elemental composition. The publication was looked for in the highest order, and then the ID was clicked until the name of the compound appeared. The name of the compound that appeared in Chemspider was a prediction of the compound

found according to the elemental composition of the spectrum.

Diabetic induction and tooth extraction

Diabetes induction was done by intravenous injection of 50 mg/kg rat body weight streptozotocin (STZ) at the rat's tail. Rats were categorized as positive for DM if the blood glucose levels showed ≥ 250 mg/dL on day 1 post-induction by glucometer. We used ketamine 50-80 mg/kg and xylazine 20mg/kg for anaesthesia. The samples' lower left incicives were removed using forceps, and a plastic tool was used to apply the Persea americana Mill. seed extract topically was for one minute in the socket. There was no application to the control group, whilst group E was applied with the extracted gel every day until either 7- or 14-days post extraction.

Tissue preparation

To prepare the tissue, samples from all groups were decapitated on days seven and fourteen while under anaesthesia in general and ketamine-xylazine infusion. The left mandibles were sliced to the side of a tooth socket on each specified day, fixed with 10% formalin, and then decalcified as tissue samples for haematoxylin and eosin (H&E) staining to assess the quality of the woven bone. A light microscope (Nikon E-100, Tokyo, Japan) with 400x magnification was used to view and evaluate the sample slides in five-micrometer fields of view on a binocular light.

Avocado seeds gel preparation

For the preparation of the avocado seed gel, the gel formulation of avocado seed was used. The formulation of the avocado seed gel formulation is extract 10%, CMC Na (Natrium Carboxymethylcellulose) (Sigma Aldrich), Gliserin (Onemed), Propilen Glikol (DOW USP USP GRADEPG), and metil paraben (Nipagin/Ueno). Using the SPSS software package version 17.0, the Mann Whitney. A difference of $p<0.05$ was considered to be statistically significant.

Results and Discussion

The spectrometry result is the interpretation of these metabolite compounds, which shows that there are several dominant compounds or major compounds, the compounds that have higher levels (indicated by percent area) when compared to the concentrations of other substances in the extract. The used method is LC-TOF MS/MS (Liquid chromatography tandem-mass spectrometry). It is an analytical technique that involves the physical separation of a target compound (or analyte) followed by detection based on its mass. In the MS system, the liquid sample turns into droplets through the needle and will be given a positive charge if using positive ESI and a negative charge if using negative ESI. Electrospray Ionisation Source (ESI) is an ionization technique that uses electrospray to produce doubly charged ions. Thus, the resulting ions will be separated by a Q-ToF type analyzer. The ion separation results are detected and will be displayed in the form of a chromatogram, which can be analyzed using the Masslynx 4.1 application so that the m/z spectrum value of each chromatogram peak appears [18]. Figures 1 and 2 depict the chromator image of positive and negative ESI from analysis of avocado seed extract. Each chromatogram peak indicates the presence of a compound and appears according to RT (real time) where the compound is detected, with a certain area percentage. When we click on the peak of the chromatogram in the Masslynx application, the m/z spectrum will appear so that the compound formula can be predicted.

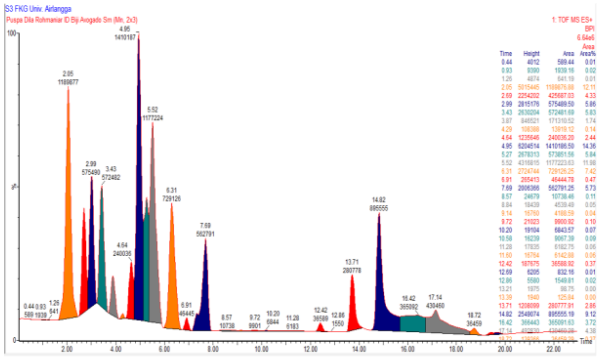


Figure 1. The spectrometry result avocado seeds by ESI positive

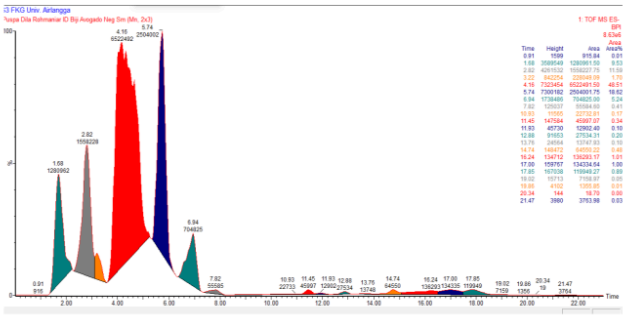


Figure 2. The spectrometry result avocado seeds by ESI negative

The predicted molecular formula is then searched using the ChemSpider web by writing the molecular formula. If the ESI is positive, the number of H molecules should be reduced to match the actual mass because there is an additional hydrogen charge in the positive ESI. After obtaining the name of the compound and structure via ChemSpider, the measured m/z was compared with the calculated m/z. If the difference between the two is 0.0005, the peak belongs to the predicted compound [18,19]. The result of the interpretation of chromatogram data are presented in Table 1.

Table 1 shows that there are three largest compound that were found by adding hydrogen atom detected from LCToFMsMs as Procyanidin at 14.36%. The compound was discovered at 4.95 minutes, the second largest percentage of compounds is glucopyranoside at 12.11%, the compound was discovered at 2.05 minutes, and the third largest is catechine/epicathecin at 5.84% that was found on 5.27 minutes.

Table 1. Compounds detected from LCToFMSMS positive ESI

No.	Real time	mass	calc mass	Element composition	Compound	Area	%
1	4.95	579.1482	579.1503	$C_{30}H_{26}O_{12}$	Procyanidin B2/Proanthocyanidine B2	1410186.5	14.36
2	2.05	381.0793	381.0763	$C_{17}H_{16}O_{10}$	9-Hydroxy-7-oxo-7*H*-furo[3,2-*g*] chromen-4-yl *β*-*D*-glucopyranoside	1189876.88	12.11
3	5.27	291.0882	291.0869	$C_{15}H_{14}O_6$	*D*- (+)-Catechin/Epicatechine	573851.56	5.84
4	3.43	867.2129	867.2123	$C_{45}H_{38}O_{18}$	Procyanidin C1/Proanthocyanidine	572481.69	5.83
5	5.52	307.0934	307.0930	$C_{19}H_{14}O_4$	(±)-Deoxytetrangomycin	117223.63	11.98
6	6.91	515.2234	515.2241	$C_{28}H_{34}O_9$	Nomilin	46444.78	0.47
7	18.72	413.2662	413.2678	$C_{26}H_{37}O_4$	14-Hydroxy-2-(3-methylbutanoyl)-4,4,6-tris(3-methyl-2-buten-1-yl)-3-oxo-1,5-cyclohexadien-1-olate	36459.29	0.37
8	4.29	323.0872	323.0879	$C_{19}H_{14}O_5$	(1*E*,4*E*)-1,5-*Bis*(1,3-benzodioxol-5-yl)-1,4-pentadien-3-one	13919.12	0.14
9	9.72	195.0888	195.0882	$C_7H_{14}O_6$	D-Pinitol	9900.92	0.5
10	0.93	195.0886	195.0882	$C_7H_{14}O_6$	*α*-Methyl *D*-mannoside	1939.16	0.03
11	0.44	195.0857	195.0882	$C_{14}H_{10}O$	Anthrone	589.44	0.01

Table 2. Compounds detected from LCToFMSMS negative ESI

No.	Real time	Mass	Calc Mass	Element composition	Compound	Area	%
1	4.16	353.0871	353.0873	$C_{16}H_{18}O_9$	Chlorogenic Acid	6522491.5	48.51
2	5.74	441.1777	441.1761	$C_{21}H_{30}O_{10}$	Lusitanicoside	2504001.75	18.62
3	2.82	865.1963	865.1980	$C_{45}H_{38}O_{18}$	Procyanidine/ Proanthocyanidine	1558227.75	13.29
4	1.68	191.0566	191.0556	$C_7H_{12}O_6$	Quinic acid	1280961.5	9.53
5	6.94	427.1610	427.1604	$C_{20}H_{28}O_{10}$	Rosavidine	704825	5.24
7	16.24	367.2617	367.2637	$C_{25}H_{36}O_2$	Variecoline	136293.17	1.01
8	14.74	367.2279	367.2273	$C_{24}H_{32}O_3$	Menoctone	64550.22	0.48
9	7.82	507.2435	507.2442	$C_{23}H_{40}O_{12}$	(3a*S*,4*R*,6*S*,7*S*,7a*R*)-6-[(*R*)-	55584.6	0.41

No.	Real time	Mass	Calc Mass	Element composition	Compound	Area	%
10	11.45	265.1806	265.1804	$C_{16}H_{26}O_3$	[(4*S*,5*R*)-5-(dimethoxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl]-[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl] methoxy]-4-(hydroxymethyl)-2,2-dimethyl-4,6,7,7*a*-tetrahydro-3*aH*-[1,3] dioxolo[4,5-c]pyran-7-ol methyl (2*E*,6*E*)-9-(3,3-dimethyloxiran-2-yl)-3,7-dimethylnona-2,6-dienoate	45997.07	0.34
11	12.88	397.1917	367.1909	$C_{23}H_{28}O_4$	1-hydroxy-9-methoxy-3-(2-methyloctan-2-yl) benzo[c]chromen-6-one	27534.31	0.20
12	10.93	387.1142	387.1139	$C_{13}H_{24}O_{13}$	formic acid;(2*R*,3*S*,4*S*,5*R*,6*R*)-2-(hydroxymethyl)-6-[(2*R*,3*S*,4*R*,5*R*,6*R*)-4,5,6-trihydroxy-2-(hydroxymethyl) oxan-3-yl] oxyoxane-3,4,5-triol	22732.81	0.17
13	13.76	293.2120	293.2117	$C_{18}H_{30}O_3$	Octoxynol-2	13741.93	0.10
14	11.93	383.1849	383.1858	$C_{23}H_{28}O_5$	Methoxyhalanolide	12902.4	0.10
15	21.47	387.1149	387.1139	$C_{13}H_{24}O_{13}$	formic acid;(2*R*,3*S*,4*S*,5*R*,6*R*)-2-(hydroxymethyl)-6-[(2*R*,3*S*,4*R*,5*R*,6*R*)-4,5,6-trihydroxy-2-(hydroxymethyl) oxan-3-yl] oxyoxane-3,4,5-triol	3763.98	0.03
16	19.85	158.9779	158.9777	CH_4O_9	Trioxidanylperoxyperoxyperoxy methane	1355.85	0.01

Table 2 shows that there are three largest compounds that found by reducing hydrogen atom detected from LCToFMsMs, which are chlorogenic acid at 48.51%. The compound was discovered at 4.16 minutes. The second largest percentage of compounds is Lusitanicoside at 18,62%. The compound was discovered at 5.74 minutes. The third largest is Procyanidine/Proanthocyanidine at 13.29% that was found at 2.82 minutes. Metabolite profiling aims to elucidate the profile of secondary metabolites after a lengthy metabolite isolation process. This method relies on the separation of metabolites through the use of mass spectrometry (MS) in conjunction with gas chromatography (GC) or liquid chromatography (LC) to evaluate metabolite complexes. When it comes to identifying metabolite profiles, Ultra Performance Liquid Chromatography Quadrupole Time of Flight Mass Spectrometry/Mass Spectrometry (UPLC-QToF-MS/MS) is more precise and selective than other methods [4,17]. The results of all of the predictions compound methanol extract of

Avocado seed are not all the same as the results of previous studies; for example, quercetine, which was found in previous studies, was not found in this study. This can be caused by various factors; one of the dominant determinants is external factors in the form of the place of origin of the avocado seeds used [20,21]. After we had determined the compound of the avocado seed, we did *in vivo* research to prove the effect of the avocado seed on woven bone formation. Afterwards, we observed the woven bone area formation by haematoxyline eosine staining. The highest woven bone formation was on the day-14 group treatment. The lowest woven bone formation was on the day-7 group control (Figure 3). The highest density of woven bone shown on Groups treatment on days 14 (KE14). Afterwards, the lowest density woven bone shown on Control Groups on days 7 (K7). Around 21.49% different of woven bone at day 7 control groups and treatment groups. Likewise, around 21.49% difference of woven bone at day 7 control groups and treatment groups and 40.93% different at day 14 control groups and treatment groups (Figure 4, 5).

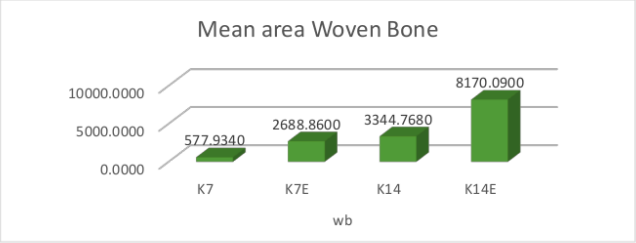
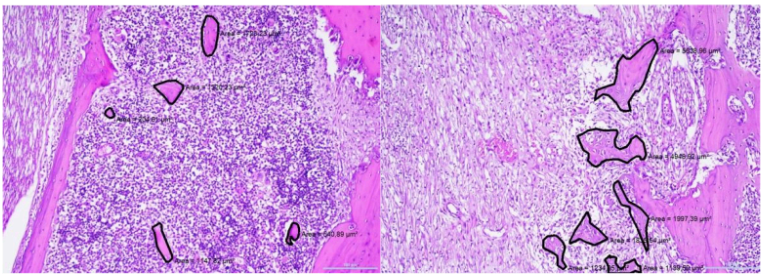
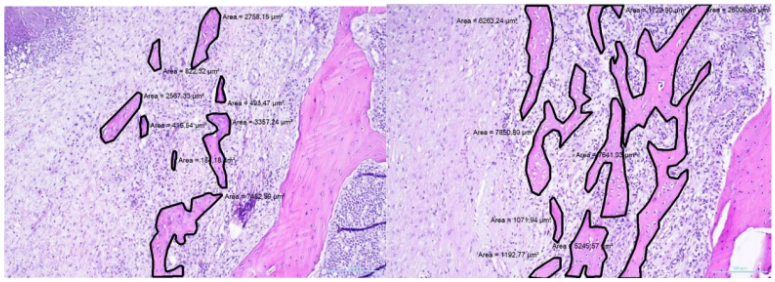


Figure 3. The area of woven bone for control group day 7 (K7), day 14 (K14) and treated group day 7 (K7E) and day 14 (K14E)



K7 KE7

Figure 4. H&E Slide for control group (K7) and treatment group (KE7) on day-7 under light microscope with 100x magnification



K14 KE 14

Figure 5. H&E Slide for control group (K7) and treatment group (KE7) under light microscope with 100x magnification

Table 3. Analysis of Variance using Kolmogorov-Smirnov test

	Woven Bone
Asymp. Sig. (2-tailed)	.085

Kolmogorov-Smirnov test (Table 3) shows that the distribution of data is not normal, so the analysis proceeded with Mann Whitney test to detect any difference. Mann-Whitney (Table 4) test shows that there are significant differences in osteoblast, woven bone formation between the group receiving treatment and control group at day 7th and 14th due to the hypothesis of application of avocado seed gel after tooth extraction.

Table 4. Difference test between control group and treatment group on day 7. Analysis by Mann-Whitney test

	Woven Bone
Mann-Whitney U	0,000
Asymp. Sig. (2-tailed)	0,009

Tables 4 and 5 shows that there are significant differences woven bone on day 7 and 14 between control group and group treatment with avocado seed. Metabolite profiling that we have did show that Avocado seeds contain polyphenol compounds, including procyanidin, chlorogenic acid, and quinic acid. This study is supported by previous research, although not exactly the same compounds contained by avocado seeds [4,22-23].

Table 5. Difference test between control group and treatment group on day 14. Analysis by Mann-Whitney test

	Woven Bone
Mann-Whitney U	0,000
Asymp. Sig. (2-tailed)	0,009

Which are important in inducing the healing process through different pathways and processes. Procyanidin, chlorogenic acid, quinic acid are important in inducing the healing process through different pathways and processes. Previously, Procyanidin could increase osteoblast differentiation [24]. Quinic acid can inhibit the pro-inflammatory and increase osteoblast differentiation [25]. Chlorogenic acid, as an antioxidant, increases osteoblast proliferation and differentiation by repairing oxidative stress damaged by high glucose [26,27]. There are many research before that shown the effect of avocado seeds on bone and healing. Avocado seed extract can increase osteoblast proliferation *in vitro* [1]. It can maintain fibroblast and support the healing

process after tooth extraction [28-29]. Increased inflammation, the production of reactive oxygen species (ROS), and advanced glycation end products (AGEs) are all consequences of diabetes mellitus. This results in an increase in osteoclasts, which is confirmed by the rise in the RANKL/OPG ratio. The total result of the depletion in bone formation is due to the bone dysregulation and the decrease in RUNX2, eventually resulting in a decrease in osteoblast proliferation and an increase in apoptosis in osteoblasts [30]. Genes involved in antioxidant activity, such as glutathione synthetase (GSH) and superoxide dismutase (SOD), can be regulated by polyphenol chemicals. RANKL and TNFα, the genes that control osteoclast differentiation and inflammatory pathways, are simultaneously downregulated by polyphenols [5]. The administration of avocado seeds, which contain these compounds, can improve the modulation of osteoblasts on diabetic conditions, resulting in the total elevation of the woven bone formation after tooth extraction.

Conclusion

Avocado seed extract contains bioactive compounds, including catechin, chlorogenic acid, cyanidin, and quinic acid, which have the potential to accelerate bone formation, particularly in diabetic conditions, by enhancing woven bone development. The results of this study indicate that avocado seed extract could serve as a natural alternative for promoting bone healing, especially in patients with diabetes-related complications, where delayed bone regeneration is a common concern. The presence of polyphenols and flavonoids in avocado seed extract suggests possible antioxidant, anti-inflammatory, and osteogenic properties that contribute to its bone-healing potential. These compounds may enhance osteoblast activity, reduce oxidative stress, and regulate inflammatory responses—key factors in improving bone repair, particularly in compromised conditions such as diabetes. While these findings are promising, further research is necessary to fully understand the molecular mechanisms involved and to determine the optimal dosage and delivery methods for clinical applications. Future studies should also explore the long-term effects and potential interactions of avocado seed extract with other therapeutic agents. If validated in clinical trials, this natural approach could provide a cost-effective and sustainable alternative for bone healing, particularly for patients with metabolic disorders that impair bone regeneration.

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Disclosure Statement

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References

[1]. Kim M.J., Im N.K., Yu M.H., Kim H.J., Lee I.S. Effects of extracts from sarcocarp, peels, and seeds of avocado on osteoblast differentiation and osteoclast formation. *Journal of the Korean Society of Food Science and Nutrition*, 2011, **40**:919 [Google Scholar], [Publisher]

[2]. Melgar B., Dias M.I., Ciric A., Sokovic M., Garcia-Castello E.M., Rodriguez-Lopez A.D., Barros L., Ferreira I.C. Bioactive characterization of Persea americana Mill. by-products: A rich source of inherent antioxidants. *Industrial Crops and Products*, 2018, **111**:212 [Crossref], [Google Scholar], [Publisher]

[3]. a) Heidaripour A., Panahi H., Attari G., Jahansooz F. Fabrication of nanocomposite of HAp-SiO_2-Fe_3O_4 suitable for magnetic hyperthermia. *Journal of Medicinal and Nanomaterials Chemistry*, 2024, **6**:251 [Crossref], [Publisher]; b) Li Y., Li L., Li X., Luo B., Ye Q., Wang H., Yang L., Zhu X., Han L., Zhang R. A mechanistic review of chinese medicine polyphenols on bone formation and resorption. *Frontiers in Pharmacology*, 2022, **13**:1017538 [Crossref], [Google Scholar], [Publisher]

[4]. Zhou Q.Y.J., Liao X., Kuang H.M., Li J.Y., Zhang S.H. LC-MS metabolite profiling and the hypoglycemic activity of morus alba L. Extracts. *Molecules*, 2022, **27**:5360 [Crossref], [Google Scholar], [Publisher]

[5]. a) Heidaripour A., Khazaei A. Extraction of nano-hydroxyapatite (Nano-HAp) from sheep molar teeth for nanodentistry. *Journal of Medicinal and Nanomaterials Chemistry*, 2023, **5**:276 [Crossref], [Google Scholar], [Publisher]; b) Valentino A., Di Cristo F., Bosetti M., Amaghnouje A., Bousta D., Conte R., Calarco A. Bioactivity and delivery strategies of phytochemical compounds in bone tissue regeneration. *Applied Sciences*, 2021, **11**:5122 [Crossref], [Google Scholar], [Publisher]

[6]. Kareem R.O., Bulut N., Kaygili O. Hydroxyapatite biomaterials: A comprehensive review of their properties, structures, medical applications, and fabrication methods. *Journal of Chemical Reviews*, 2024, **6**:1 [Crossref], [Google Scholar], [Publisher]

[7]. Sun H., Saeedi P., Karuranga S., Pinkepank M., Ogurtsova K., Duncan B.B., Stein C., Basit A., Chan J.C., Mbanya J.C., Pavkov M.E. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Research and Clinical Practice*, 2022, *183*, 109119 [Crossref], [Google Scholar], [Publisher]

[8]. Imojara A., Ishegbe J.E., Abdullah H., Edet H.O., Gber T.E., Eba M.-B.A., Pembere A.M., Louis H. Phosphorus encapsulated gallium nitride and aluminum nitride nanotubes as nonenzymatic sensors for fructose, glucose, and xylose sugars as biomarkers for diabetes-mellitus: outlook from computational study. *Chemical Physics Impact*, 2023, **7**:100348 [Crossref], [Google Scholar], [Publisher]

[9]. Rohmaniar P.D., Rahayu R.P., Narmada I.B., Sa'adah N., Andriani D. Effect of diabetes mellitus on each phase of tooth extraction socket healing. *Journal of International Dental and Medical Research*, 2024, **17**:429 [Google Scholar], [Publisher]

[10]. Power D.J., Sambrook P.J., Goss A.N. The healing of dental extraction sockets in insulin-dependent diabetic patients: A prospective

controlled observational study. *Australian Dentistry Journal*, 2019, **64**:111 [Crossref], [Google Scholar], [Publisher]

[11]. Zhao M., Xie Y., Gao W., Li C., Ye Q., Li Y. Diabetes mellitus promotes susceptibility to periodontitis—novel insight into the molecular mechanisms. *Frontiers in Endocrinology*, 2023, **14**:1192625 [Crossref], [Google Scholar], [Publisher]

[12]. Blinov A., Rekhman Z., Slyadneva K., Askerova A., Mezentsev S., Lukyanov G., Kirichenko I., Djangishieva S., Gamzatova A., Suptilnaya D., Tashuev A., Nagdalian A. Advanced strategies for the selection and stabilization of osteotropic micronutrients using biopolymers, *Journal of Chemical Reviews*, 2025,**7**:83 [Crossref], [Publisher]

[13]. a) Salahvarzi S., Mohammadpour K., Dadgar Z. The reaction of curcumin-hydrazine and its effect on bone marrow mesenchymal stem cells. *Journal of Medicinal and Nanomaterials Chemistry*, 2021, **3**:227 [Crossref], [Publisher]; b) Di Conza G., Barbaro F., Zini N., Spaletta G., Remaggi G., Elviri L., Mosca S., Caravelli S., Mosca M., Toni R. Woven bone formation and mineralization by rat mesenchymal stromal cells imply increased expression of the intermediate filament desmin. *Frontiers in Endocrinology*, 2023, **14**:1234569 [Crossref], [Google Scholar], [Publisher]

[14]. a) Khatami S.M., Hatamie S., Naderi Sohi A., Parivar K., Soleimani M., Hanaee-Ahvaz H. Reduced graphene oxide/nanohydroxy Apatite-Bismuth nanocomposites for osteogenic differentiation of human mesenchymal stem cells. *Journal of Medicinal and Nanomaterials Chemistry*, 2021, **3**:125 [Crossref], [Google Scholar], [Publisher]; b) Akiva A., Melke J., Ansari S., Liv N., van der Meijden R., van Erp M., Zhao F., Stout M., Nijhuis W.H., de Heus C. An organoid for woven bone. *Advanced Functional Materials*, 2021, **31**:2010524 [Crossref], [Google Scholar], [Publisher]

[15]. Buhari S., Gupta V., Sharma A., Tomar S., Ranjan N., Sharma K., Singh V., Rajeev M., Kumar A., Chalana A. A mini-review of mxenes in biomedicine: From synthesis to revolutionary applications. *Journal of Chemical Reviews*, **7**:108 [Crossref], [Publisher]

[16]. El Baakili S., Semlali A., El Mabrouk K., Bricha M. Synthesis method effect on acellular bioactivity of bioglasses: Structural analysis and solid-state NMR. *Journal of Applied Organometallic Chemistry*, 2023, **3**:268 [Crossref], [Google Scholar], [Publisher]

[17]. Liu J., Zhong X., Jiang Y., Yu L., Huang X., Dong Z., Yang S., He W., Zeng J., Qing Z. Systematic identification metabolites of Hemerocallis citrina Borani by high-performance liquid chromatography/quadrupole-time-of-flight mass spectrometry combined with a screening method. *Journal of Pharmaceutical and Biomedical Analysis*, 2020, **186**:113314 [Crossref], [Google Scholar], [Publisher]

[18]. Brenton A.G., Godfrey A.R. Accurate mass measurement: terminology and treatment of data. *Journal of the American Society for Mass Spectrometry*, 2010, **21**:1821 [Crossref], [Google Scholar], [Publisher]

[19]. Pitt J.J. Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. *The Clinical Biochemist Reviews*, 2009, **30**:19 [Google Scholar], [Publisher]

[20]. Setyawan H., Sukardi S., Puriwangi C. Phytochemicals properties of avocado seed: A review. *IOP Conference Series: Earth and Environmental Science: IOP Publishing*, 2021, 012090 [Crossref], [Google Scholar], [Publisher]

[21]. Bangar S.P., Dunno K., Dhull S.B., Siroha A.K., Changan S., Maqsood S., Rusu A.V. Avocado seed discoveries: Chemical composition, biological properties, and industrial food applications. *Food Chemistry: X*, 2022, **16**:100507 [Crossref], [Google Scholar], [Publisher]

[22]. Bangar S.P., Dunno K., Dhull S.B., Siroha A.K., Changan S., Maqsood S., Rusu A.V. Avocado seed discoveries: Chemical composition, biological properties, and industrial food applications. *Food Chemistry: X*, 2022, **16**:100507 [Crossref], [Google Scholar], [Publisher]
[23]. Folasade O.A., Olaide R.A., Olufemi T.A. Antioxidant properties of Persea americana M. seed as affected by different extraction solvent. *Journal of Advances in Food Science & Technology*, 2016, **3**:101 [Google Scholar]
[24]. Hu B., Chen L., Chen Y., Zhang Z., Wang X., Zhou B. Cyanidin-3-glucoside regulates osteoblast differentiation via the ERK1/2 signaling pathway. *ACS Omega*, 2021, **6**:4759 [Crossref], [Google Scholar], [Publisher]
[25]. Benali T., Bakrim S., Ghchime R., Benkhaira N., El Omari N., Balahbib A., Taha D., Zengin G., Hasan M.M., Bibi S. Pharmacological insights into the multifaceted biological properties of quinic acid. *Biotechnology and Genetic Engineering Reviews*, 2024, **40**:3408 [Crossref], [Google Scholar], [Publisher]
[26]. Huang J., Xie M., He L., Song X., Cao T. Chlorogenic acid: A review on its mechanisms of anti-inflammation, disease treatment, and related delivery systems. *Frontiers in Pharmacology*, 2023, **14**:1218015 [Crossref], [Google Scholar], [Publisher]
[27]. Tian X., Zhang J., Guo Q., Wang G., Wang H., Li Z., Dong J. Chlorogenic acid improved high glucose induced apoptosis and osteogenic differentiation in mc3t3-e1 cells through eliminating oxidative stress. *Acta Poloniae Pharmaceutica*, 2020, **77** [Google Scholar]
[28]. Rohmaniar D. Effectiveness of avocado seed extract (persea americana mill) in maintaining fibroblast cell viability of avulsed teeth in male wistar rats. *Jurnal Wiyata: Penelitian Sains Dan Kesehatan*, 2023, **10**:184 [Crossref], [Google Scholar], [Publisher]
[29]. Ariesanti Y., Rasad I.S.S.P., Nimas M., Syabilla N. The effect of Persea americana Mill. seed extract on inflammatory cells and fibroblast formation in tooth extraction socket healing. *Dentistry Journal*, 2021, **54**:190 [Crossref], [Google Scholar], [Publisher]
[30]. Jiao H., Xiao E., Graves D.T. Diabetes and its effect on bone and fracture healing. *Current Osteoporosis Reports*, 2015, **13**:327 [Crossref], [Google Scholar], [Publisher]

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11

PAGE 12

PAGE 13

PAGE 14