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Comparison the Effectiveness of Areca Seed Extract (*Areca catechu L.*) with Betle Leaf Ethanol Extract (*Piper betle L.*) in Shortening Bleeding Time After Tooth Extraction

Bella Fernanda[1*], Welly Effendy Seba[2], Suci Erawati[1]

[1] Magister of Dentistry Program, Faculty of Medicine, Dentistry, and Health Science, Universitas Prima Indonesia, Medan, Indonesia.
[2] Oral Surgery Department, Faculty of Medicine, Dentistry, and Health Science, Universitas Prima Indonesia, Medan, Indonesia.

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Corresponding Author:
Name Bella Fernanda
bellafernanda@unprimedan.ac.id

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Abstract: Tooth extraction is one of the simple procedures commonly found in dentistry. Bleeding is a common complication following this procedure. It requires special equipment and can quickly lead to shock, syncope, and death if not treated promptly. This study aimed to determine the effectiveness of Areca seed or beetle leaf ethanol extract in shortening bleeding time after tooth extraction in a Wistar rat model. Thirty-two male Wistar rats were divided into two treatment groups. The first group was treated with piper beetle leaf ethanol extract, and the second group was treated with areca seed ethanol extract. This extract was directly applied to the tooth socket. Meanwhile, the bleeding time was recorded on a filter paper every half minute using a stopwatch. All data were analyzed by T-Independent Test. The results of this study indicated a significant difference in bleeding time between the first group (betel leaf ethanol extract) and the second group (areca seed ethanol extract) with a p-value < 0.001. The mean bleeding time for the first group (143.19 ± 15.93 seconds) was longer than that for the second group (88.06 ± 23.80 seconds). Overall, it can be concluded that the areca seed ethanol extract significantly shortens bleeding time compared to piper beetle extract.

Keywords: Areca seed; Bleeding time; Ethanol extract; Piper betel leaf; Tooth extraction

Introduction

In dentistry, especially oral surgery, patients are often found with wounds accompanied by bleeding, especially after tooth extraction. Tooth extraction is one of the medical indications if the tooth can no longer survive by removing the tooth from the socket (Passarelli et al., 2020). Bleeding is one of the most common complications during and after tooth extraction (Pedersen, 2012).

The time span from the start of the bleeding point until the formation of a blockage or until the bleeding stops is called bleeding time (Majedi et al., 2013). Bleeding Time is defined as the time interval from the first drop of blood until the blood stops dripping. Bleeding usually occurs shortly after tooth extraction. Normal bleeding after extraction will stop within 3-8 minutes or no more than 10 minutes, but if the bleeding does not stop within 30-60 minutes, then action must be taken to prevent excessive bleeding (Majedi et al., 2013).

Indonesia as a country rich in plants, both plants that have known benefits and those that have not. In Indonesia, there are several medicinal plants as alternative methods that have active substances that can treat diseases, one of which is hemostatic properties (Alang et al., 2022).

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Areca nut is known as an effective medicinal plant in stopping bleeding that contains fat, alkaloids and tannins. Traditionally, areca nut can be used as a hemostatic drug with anti-inflammatory, antibacterial, antifungal, antiviral, and other compounds. The use of herbal plants as medicine in addition to minimal side effects can also utilize plants that exist in Indonesia (Asrianto et al., 2021; Munthe & Ridwanto, 2022).

Due to the above, the author is interested in researching the comparative effectiveness of areca nut extract (*Areca catechu L.*) with ethanol extract of betel leaves (*Piper betle L.*) in shortening bleeding time after tooth extraction in Wistar rats.

Method

This study is an experimental study with Post Test Only Control Group Design conducted on male Wistar rat models that have undergone tooth extraction at the Integrated Laboratory, Universitas Prima Indonesia. The flow of this study can be seen in Figure 1.

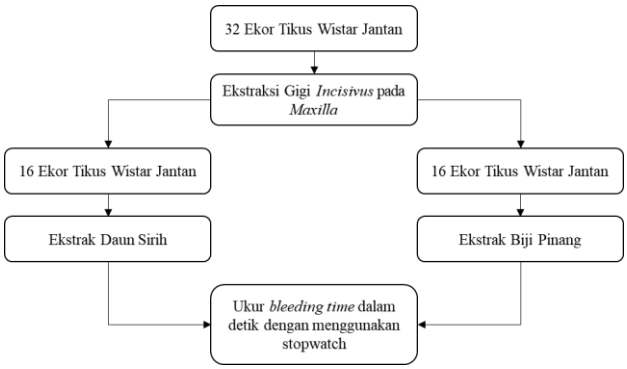

Figure 1. Research flow

This study used materials in the form of 96% ethanol solution, betel leaves, areca nuts, filter paper, ketamine and 70% alcohol solution. Meanwhile, the tools used in this study were Rotary Vacuum Evaporator, Stopwatch, Dropper pipette, diagnostic set, tooth extraction forceps, neirbeken, animal cage, mask and gloves.

This research began with the extraction process of areca nut and betel leaves using the maceration method. A total of 5 kg of young areca nuts were peeled and washed clean. After that, the areca nuts were thinly sliced and then dried at room temperature for 1 week. The areca nuts that were confirmed to be dry were then ground using a blender until they became powder. The areca nuts that had become powder were then extracted using the maceration method accompanied by stirring until homogeneous (Gulo et al., 2021; Suhartomi et al., 2020).

To make betel leaf extract, a similar method is used, where 500 grams of betel leaves are washed and dried at room temperature until dry. After that, all betel leaves are ground into powder. The soaking process is carried out using a maceration vessel or a closed container that is macerated for 72 hours. After that, the soaking is filtered to obtain the macerate, while the residue is re-macerated 3 times in the same way (Chiuman et al., 2023; Mutia et al., 2021).

All macerates from each filtration were collected in a container according to the type of natural material extracted. This macerate was then evaporated with a rotary vacuum evaporator at a temperature of 45 ^{o}C until there was no solvent condensation on the condenser. After evaporation using a rotary vacuum evaporator, evaporation was continued using a water bath at a temperature of 70 degrees for ± 12 hours to obtain a concentrated extract. The betel leaf and areca nut extracts were then stored at refrigerator temperature until the testing time (Chiuman et al., 2023; Kosasih et al., 2019).

Testing the effectiveness of areca nut and betel leaf extracts in shortening bleeding time was conducted on 32 male Wistar rats which were grouped into 2 different groups, namely the areca nut (*Areca catechu L.*) extract group and the betel leaf (*Piper betle L.*) ethanol extract group.

Before treatment was carried out on all male Wistar rats, all male Wistar rats were acclimatized for a week to the environmental conditions and food provided. After the acclimatization process, all rats were anesthetized using ketamine anesthesia (1000 mg/10 ml) at a dose of 20 mg/kgBW injected intraperitoneally. Then, all rats underwent a tooth extraction procedure on the maxillary central incisor. Immediately after tooth extraction, all rats were given extracts according to the treatment group topically on the post-extraction tooth socket (Aslani et al., 2016; Chiuman et al., 2022; Prasetya, 2013; Sing et al., 2020).

Bleeding time is calculated when the tooth is released from the socket until a blood clot forms and is calculated using a stopwatch. The bleeding time begins when the first drop touches the filter paper and is continued by checking the filter paper at 30-second intervals until the bleeding stops, which is indicated by no more blood being absorbed by the filter paper (Jibril & Sanusi, 2021; Pradana et al., 2022).

All bleeding time data from each treatment group were analyzed for data distribution to determine the type of comparative test used. Data distribution analysis was performed using Shapiro-Wilk. If the data is normally distributed, the comparative test used is the T-Independent Test. However, if the data distribution is not normal, then the difference test used is the Mann-Whitney Test (Santoso, 2018, 2019; Widiana, 2015).

Result and Discussion

An overview of the entire research procedure can be seen in Figure 2.

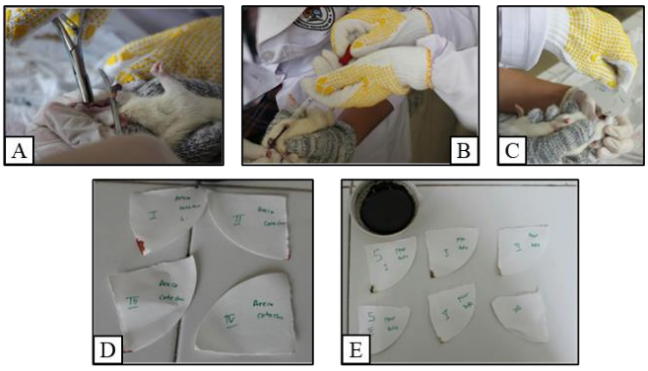

Figure 2. Research procedure. (A) Incisor tooth extraction; (B) Topical application of extract; (C) Bleeding time measurement; (D) Bleeding time measurement results in the areca nut extract group; (E) Bleeding time measurement results in the betel leaf extract group

In Figure 2, we can see a picture of the research process starting from tooth extraction, giving the extract, to measuring the bleeding time and a picture of the results of the bleeding time measurements in the group that received betel leaf and areca nut extracts can be seen in Table 1.

Table 1. Description of Bleeding Time in All Mice Given Betel Leaf and Areca Nut Extract

Rats	Bleeding Time (Seconds)	
	Betel leaf	Areca Nut
1	121	99
2	115	120
3	128	149
4	127	111
5	150	87
6	160	80
7	151	70
8	155	75
9	152	68
10	160	70
11	158	69
12	157	110
13	129	75
14	124	68
15	151	90
16	153	68

From Table 1, it can be seen that the bleeding time in the group that received betel leaf extract was longer than the group that received areca nut. The bleeding time in the betel leaf group ranged from 115 seconds to 160 seconds, while in the group that received areca nut, the bleeding time ranged from 68 seconds to 149 seconds. The analysis was then continued to compare the average bleeding time between the two groups and the results of the analysis can be seen in the following table.

Table 2. Comparison of Average Bleeding Time in Betel Leaf and Areca Nut Extract Groups

Group	n	Bleeding Time (Second), Mean ± SD	P value
Betel leaf	16	143.19 ± 15.93	< 0.001
Areca Nut	16	88.06 ± 23.80	

The results of the Independent T-Test statistical test showed that the average bleeding time value in the Wistar rats in the treatment group given areca nut extract was 88.06 ± 23.80 seconds and in the Wistar rats in the treatment group given betel leaf ethanol extract was 143.19 ± 15.93 seconds, a significant difference was obtained (P value < 0.001).

Based on the results of this study, it was found that there was a significant difference in the average value of bleeding time in Wistar rats after tooth extraction in the areca nut (*Areca catechu L.*) seed extract treatment group and the betel leaf ethanol extract treatment group (*Piper betle L.*), with a P value < 0.001. Bleeding time was shorter in the areca nut (*Areca catechu L.*) seed extract group, which was 88.06 ± 23.80 seconds, compared to the betel leaf ethanol extract group (*Piper betle L.*) which was 143.19 ± 15.93 seconds.

This is due to the difference in Tannin content in areca nut and betel leaves, where the tannin content is greater in the betel nut extract (*Areca catechu L.*). The tannin content in the areca nut extract using 96% ethanol solvent is 8.53% while the tannin content in the betel leaf ethanol extract is 1-1.3% (Sulastry, 2009).

The selection of betel nut as a wound healing medicine is based on the compound content of betel nut, which is composed of arecoline alkaloids, arecaidine, arecolidine, arecaine, guvakoin, guvasine and isoguvasine, tannin, nicotine, glucide. The compound content in betel nut has astringent, chronic anti-inflammatory, antimicrobial and antioxidant activities (Carolia & Noventi, 2016; Handayani et al., 2016b; Monica & Kurnia, 2019; Telaumbanua & Mayasari, 2021).

Condensed tannins which are classified as flavonoids are effective as antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasodilator agents (Sampepana et al., 2020). In addition, Tannin also has an astringent effect which functions to shrink open skin tissue so that bleeding can stop and wound healing can occur (Handayani et al., 2016a; Jane et al., 2015; Sidrotullah, 2021).

Tannin in the ethanol extract of betel leaves functions as a hemostatic and works as a vasoconstrictor

through its substance effects. Tannin can also increase platelet aggregation, thus forming a platelet plug that functions to stop bleeding (Sutopo et al., 2016). Tannin accelerates the release of proteins from cells and deposits these proteins on the cell surface, also reduces capillary secretion and permeability, contraction of intercellular spaces, hardening of capillary endothelium and then forms a protective layer of skin so that the superficial layer of cells tightens and shrinks (Handayani et al., 2016b; Saxena et al., 2013).

Conclusion

Administration of areca nut extract (*Areca catechu L.*) has better effectiveness in accelerating the hemostasis process of post-extraction wounds compared to ethanol extract of betel leaves (*Piper betle L.*) in male Wistar rats.

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Author Contributions
Conceptualization, B. F., W. E. S., and S. E.; Methodology, B. F., and W. E. S.; Software, B. F.; Validation, B. F., W. E. S., and S. E.; Formal analysis, W. E. S., and S. E.; Investigation, B. F., and W. E. S.; Resources, W. E. S., and S. E.; Data curation, B. F., and W. E. S.; Writing—original draft preparation, B. F.; Writing—review and editing, W. E. S., and S. E.; Visualization, B. F., and W. E. S.; Supervision, W. E. S., and S. E.; Project administration, B. F. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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