Preliminary study on the potential of topical anaesthesia from betel leaf and clove leaf extract

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ABSTRACT

Introduction: Betel leaf (*Piper betle* Linn.) and clove leaf (*Syzygium aromaticum*) are Indonesian herbs which are known to cause a numb sensation. The main ingredients of betel leaf, which act as an anesthetic, are caryophyllene, eugenol, methyl eugenol, eucalyptol (1.8-cineol), linalool, α-Pinene, estragole, while cloves have local anesthetic effects through their ingredients, namely eugenol, and ß-Caryophyllene. Research on the benefits of these two ingredients as topical anesthetics in Indonesia is still rare. This study was aimed to describe the potential of betel leaf and clove leaf extract as a topical anesthetic in Wistar rats. **Methods:** This research was a laboratory experimental research with simple randomized controlled design. The sample consisted of 6 male Wistar rats, each of which was given four treatments, namely extract with a concentration of 50%, 75%, and 100%, and topical anaesthetic benzocaine 20% as a control. Each treatment was tested using an *electric stimulator* and observed onset, duration of action, and depth of topical anaesthesia. The data were processed using descriptive statistics with tables and graphs. **Results:** Betel leaf extract with a concentration of 100% had a better mean onset, duration of work, and depth of anesthesia than the 20% benzocaine control, whereas 75% clove leaf extract had the longest duration of action compared to 20% benzocaine and 100% concentration was the topical anesthetic agent with the highest anesthetic depth. **Conclusion:** Based on the onset, duration of action, and depth of anesthesia, betel leaf, and clove leaf have potential as topical anesthetic agents.

Keywords: Betel; clove; anaesthetic local; herbal medicine; benzocaine

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INTRODUCTION

Pain is the most common experience reported by patients, and patient anxiety is a form of warning signal.[1] The pain can be eliminated by anesthesia which is divided into two types of general anesthesia and local anesthesia. Local anesthetics are the drugs most often used by dentists that can reversibly block nerve conduction, relieving the sensation of pain when applied to certain

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parts of the body without accompanying loss of consciousness.[2] Local anesthesia is applied preventively when the dentist estimates that the treatment being carried out may make the patient feel uncomfortable, painful, or the patient cannot tolerate the pain well.[3] Topical anesthetics change the pain threshold by controlling the sensation of pain through blockade of signals transmitted from peripheral sensory nerve fibers. The use of topical anesthetics in dentistry is very important, especially when extracting teeth, which is the source of the patient's pain.[4]

In Indonesia, betel leaf (*Piper betle* Linn.) is often used as a chewing herb and can cause numbness sensation. While cloves (*Syzygium aromaticum*) is used as a pain reliever during a toothache.[5] The essential oil from betel leaf (*Piper betle* Linn.) has 49 different components after being examined using Gas Chromatography-Mass Spectroscopy (GC-MS). The five main components found in betel leaf essential oil are chavibetol (22.0%), estragole (15.8%), ß-cubebene (13.6%), chavicol (11.8%), and caryophyllene (11.3%). Other components that appear consist of eucalyptol (1.1%), α-cubebene (2.0%), ß-elements (2.7%), γ-muurolene (6.0%), elixene (3.2%), δ-cadinene (2.1%), 4-allyl phenylacetate (1.2%).[6] Based on research by Das et al.[7], betel leaf also contains eugenol.

The main content in the betel leaf (*Piper betle* Linn.) which acts as an anesthetic, namely caryophyllene, eugenol, methyl eugenol, eucalyptol (1,8-cineole), linalool, α-pinene, estragole.[8] Research conducted by Jayasree et al.[9] shows that water extract of betel leaf (*Piper betle* Linn.) at the concentration of 6% and 12% acted as a local anesthetic in the observation of light reflex and corneal reflex to determine changes in rabbit pupil size. Those result had a significant effect compared to xylocaine. As a local anaesthetic, it has the same rapid onset as xylocaine but a shorter duration than xylocaine.[9]

Traditional uses of clove oil include use in dental care as an antiseptic and analgesic. The oil is active against oral bacteria associated with dental caries and periodontal diseases and is effective against a large number of other bacteria: *Escherichia coli*, *Salmonella enteric* and *Staphylococcus aureus*. Clove leaf contain eugenol (75.04-77.54%) and ß-caryophyllene (17.04-19.53%).[5] Cloves have the highest percentage of eugenol in its oil extract, eugenol is also found in allspice, sweet basil, holy basil, bay rum tree, carnation, and cinnamon.[10] ß-caryophyllene, a volatile bicyclic sesquiterpenoid, is a component not only found in betel and cloves, but can also be found in hops, black pepper, oregano, basil, rosemary, and cinnamon.[11]

Other clove essential oil ingredients are vanillin, crategolic acid, tannins, gallotannic acid, methyl salicylate, flavonoids eugenin, kaempferol, rhamnetin, eugenitin, and triterpenoids such as oleanolic acid.[12] In a study conducted by Gaylor et al, the eugenol content in Indonesian clove leaves reached 75.04-77.54% and the highest ß-caryophyllene content was in the leaves of the clove tree, which was 17.04-19.53%.[5] The reason the authors conducted this preliminary research was that there were still few studies testing the effectiveness of betel leaf extract (*Piper betle Linn*) and clove leaves (*Syzygium aromaticum*) as a topical anesthetic, especially in vivo. This study was aimed to describe the potential of betel leaf and clove leaf extract as a topical anesthetic in Wistar rats.

METHODS

This research was laboratory experimental research. Anaesthetics test using betel leaf extract (BLE) and clove leaf extract (CLE) made by maceration method respectively 50%, 75%, and 100% with 96% ethanol solvent compared to 20% benzocaine (BEN) control along with the phytochemical test carried out to determine terpenoid content. The main instrument used for this research is an electric stimulator (induction stimulator) from PT Nasional, tbk, Indonesia. (Figure 1).

The electric stimulator functions to generate an electric voltage that is touched on the skin of the rat. A positive response to the stimulus was seen in the presence of twitching of the rat's skin. The smallest voltage that produces a twitch before the anesthetic is applied is considered the baseline voltage. After the anesthetic is applied, the twitching of the rat's skin will disappear. The voltage was increased progressively until the rat's skin twitched again. This is recorded as the maximum anesthetic depth. The stimulus was

given back by giving a lower electrical voltage and the rat skin showed a negative response. The same amount of electric voltage is given continuously until the rat's skin twitches again. When the skin of the rat has started to twitch, then the electric voltage is reduced again. This is done continuously until the applied voltage is the same as the baseline voltage at the beginning of the experiment. The time taken from the initial stimulus at the baseline voltage to the end of the experiment was recorded as working time. Inclusion criteria in this study were healthy test animals weighing 200-250 grams. The exclusion criteria of tested animals were weight loss and death during treatment.The test animals were male Wistar white rats with the total sample in this study was six rats, each of which was given four treatments on each rat's back, namely a concentration of 50%, 75%, 100%, and 20% BEN control then given a stimulus from the electric stimulator and observed the onset, duration of action, and maximum depth of effect of the topical anesthetics of BLE and EDC. The research was conducted at the Yarsi University Herbal Laboratory and YARSI University Physiology Laboratory in December 2018 and has passed the ethics committee test at YARSI University with approval certificate number of 370/KEP-UY/BIA/XII/2018.

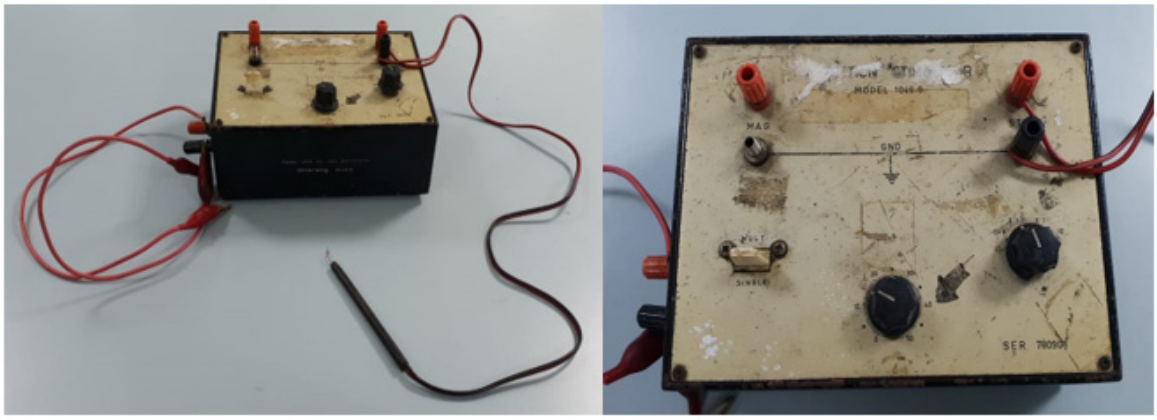

Figure 1. Electric stimulator

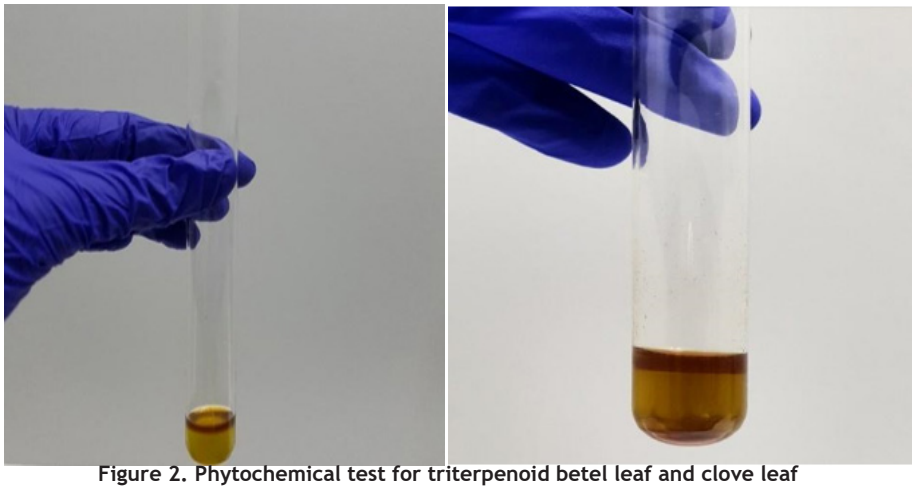

Figure 2. Phytochemical test for triterpenoid betel leaf and clove leaf

RESULTS

The results of the triterpenoid phytochemical test showed that betel leaf and clove extract contained triterpenoid compounds which were indicated by the presence of a brown ring in the extracted sample. The results of this study that BLE and CLE with concentrations of 50%, 75%, and 100% had full duration, mean onset, duration of work and better depth of anesthesia compared to 20% BEN controls (Tables 1 and 2) despite the onset of action of BLE and CLE on the 50% concentration was slightly slower than the control. Examples of graphical illustration of test results of electric *stimulation* can be seen in Figure 3 and Figure 4 below.

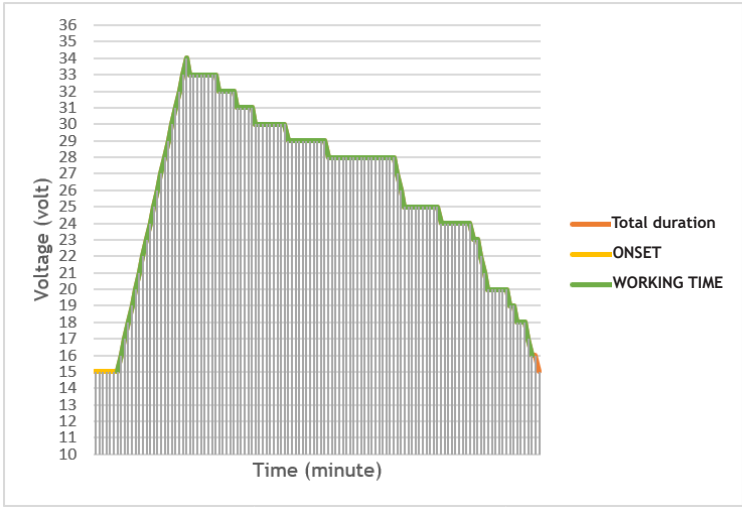

Figure 3. Graph of test results *electric stimulation* Wistar with 100% betel leaf extract (BLE)

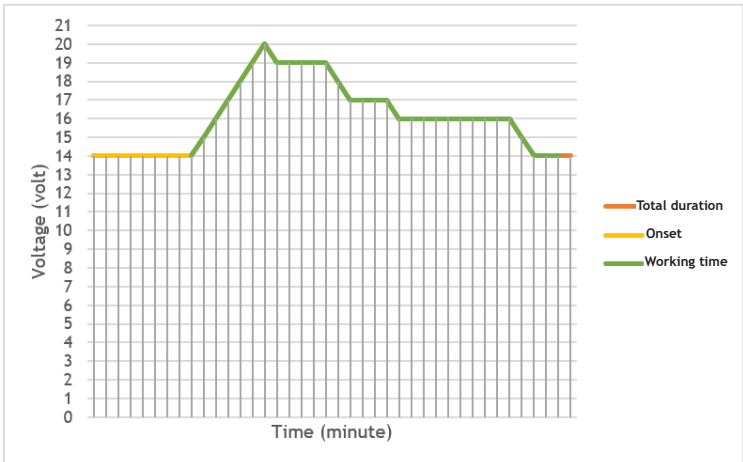

Figure 4. Graph of test results *electric stimulation* Wistar with 20% benzocaine (BEN)

Table 1. Comparison of the subject's mean based on betel leaf extract (BLE) treatments

Subject	Observation result					
	Weight (gram)	Baseline (volts)	Total duration (minutes)	Onset (minutes)	Working Hours (minutes)	Maximum depth (volts)
Rat 1 BLE 50%	230	25	38	4	33.5	38
Rat 2 BLE 50%	210	10	42	6.5	35	25
Rat 3 BLE 50%	210	15	40	4	35.5	27
EDS average 50%	216.67	16.67	40	4.83	35	30
Rat 1 BLE 75%	230	15	73	4	68.5	30
Rat 2 BLE 75%	220	15	100	4	95.5	34
Rat 3 BLE 75%	210	15	71	4	65.5	29
EDS mean 75%	220	15	81.3	4	76.5	31
Rat 1 BLE 100%	230	15	127	4	122.5	34
Rat 2 BLE 100%	230	15	118	2.5	115	32
Rat 3 BLE 100%	210	15	123	4.5	116.5	29
EDS average 100%	223.3	15	122.67	3.7	115	31.67
Rat 1 BEN 20%	220	15	30	4	25.5	22
Rat 2 BEN 20%	210	15	42.5	5	36.5	21
Rat 3 BEN 20%	200	15	30.5	4	26	21
BEN average 20%	210	15	34.3	4.3	29.3	21.3
P-value	-	-	0.024*	0.723	0.024*	0.084

Based on Table 1, the 20% BEN treatment obtained an average weight of 210 grams, a mean baseline of 15 volts, an average total duration of 34.3 minutes, the average onset of 4.3 minutes, an average work duration of 29.3 minutes, and an average maximum depth of 21.3 volts.

In the BLE treatment with a concentration of 50%, the mean weight was 216.67 grams, the mean baseline was 16.67 volts, the average total duration was 40 minutes, the mean onset was 4.83 minutes, the average work duration was 35 minutes and the mean maximum depth was 30 volts. In the BLE treatment with a concentration of 75%, an average weight of 220 grams, a baseline average of 15 volts, an average total duration of 81.3 minutes, the average onset of 4 minutes, an average working duration of 76.5 minutes, and a mean maximum depth of 31 volts. For BLE with a concentration of 100%, the mean weight was 223.3 grams, the mean baseline was 15 volts, the mean total duration was 122.67 minutes, the mean onset was 3.7 minutes.

The average working time was 115 minutes and the mean maximum depth was 31.67 volts. There was a statistically significant difference in each concentration group, namely the variable total duration (p = 0.024) and length of work (p = 0.024).

Table 2. Comparison of the subject's mean based on clove leaf extract (CLE) treatment

Code	Observation result					
	Weight (gram)	Baseline (volts)	Total duration (minutes)	Onset (minutes)	Working Hours (minutes)	Maximum depth (volts)
Rat 4 CLE 50%	210	15	31	5	29	23.5
Rat 5 CLE 50%	210	15	34.5	5.5	25	26.5
Rat 6 CLE 50%	210	15	41.5	3.5	29	37.5
EDC average 50%	210	15	35.6	4.6	27.6	29.1
Rat 4 CLE 75%	210	15	55.5	4.5	32	49.5
Rat 5 CLE 75%	210	15	50.5	4.5	31	43.5
Rat 6 CLE 75%	210	15	57	3.5	37	51
EDC Average 75%	210	15	54.3	4.1	33.3	48
Rat 4 CLE 100%	210	15	61.5	3.5	33	57
Rat 5 CLE 100%	220	15	63	4.5	34	57.5
Rat 6 CLE 100%	200	15	63	4.5	29	56
EDC average 100%	210	15	62.5	4.1	32	56.8
Rat 4 BEN 20%	210	14	20	4.5	19	15
Rat 5 BEN 20%	210	15	31	3.5	26	27
Rat 6 BEN 20%	200	15	32.5	3.5	23	28.5
BEN average 20%	206.6	14.6	27.8	3.8	22.6	23.5
P-value	-	-	0.020*	0.537	0.038*	0.084

Based on table 2 on the CLE treatment of 50%, the average weight of rats was 210 gr, the mean baseline was 15 volts, the average total duration was 35.6 minutes, the mean onset was 4.6 minutes, the average working time was 27.6 minutes and the mean maximum depth was 29.1 volts. In the 75% CLE treatment, the average weight of rats was 210 gr, the mean baseline was 15 volts, the mean total duration was 54.3 minutes, the mean onset was 4.1 minutes, the average working time was 33.3 minutes and the mean maximum depth was 48 volts. In the 100% CLE treatment, the mean weight of rats was 210 gr, the mean baseline was 15 volts, the mean total duration was 62.5 minutes, the mean onset was 4.1 minutes, the average working time was 32 minutes and the mean maximum depth was 56.8 volts.

In the 20% BEN control treatment, the average weight of the rats was 206.6 gr, the mean baseline was 14.6 volts, the mean total duration was 27.8 minutes, the mean onset was 3.8 minutes, the average work duration was 22.6 minutes and the mean maximum depth was 23.5 volts. CLE showed a statistically significant difference in the total duration (p = 0.020) and length of work (p = 0.038), while the maximum onset and depth did not show a significant difference.

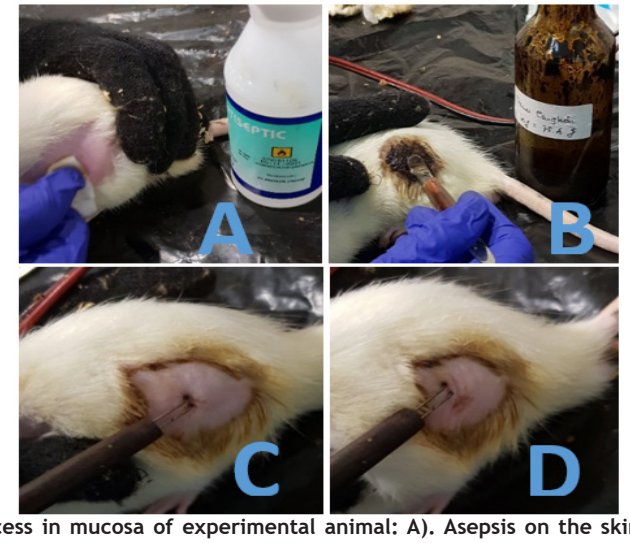

Figure 2. Electric stimuli process in mucosa of experimental animal: A). Asepsis on the skin of experimental animals; B). Application of betel and clove leaf extracts to the skin; C). Pain stimuli showed rat skin without pain reflex (no response); D). Pain stimuli showed rat skin pain reflex (twitching).

DISCUSSION

Both betel and clove contain eugenol and carryoplene which can function as local anesthetics. Eugenol is a member of the phenylpropanoids, a large family of organic chemicals that contain a phenyl ring and a C_3 side chain. When tested in dental primary afferent neurons and dorsal root ganglion neurons of rats, eugenol successfully inhibited voltage-gated sodium channels. It is suggesting that eugenol might block action potentials in both nociceptive and non-nociceptive afferent fibers. In addition to these voltage-gated channels, voltage-gated potassium channels are also inhibited by eugenol. However, voltage-gated potassium channels play roles in repolarization of cell membrane after action potential firing. Therefore, inhibition of voltage-gated potassium by eugenol might result in enhanced neuronal activity.[10]

SS-caryophyllene has local anesthetic-like activity, which could protect the nervous system from oxidative stress and inflammation and can act as an immunomodulatory agent. Most neurological activities have been linked with the cannabinoid receptors (CBRs), especially CB2R. Possible pain-killing effect of SS-caryophyllene is through stimulation of CB2R, or p38 MAPK/NF-κB system. SS-caryophyllene can down-regulate the microglia activation, leading to down-regulation of C-C chemokine receptor type 2 (CCR2) and iNOS, which will eventually reduce the neuropathic pain.[13]

The fastest onset was obtained from subjects with 100% BLE treatment with a time of 2.5 minutes with a mean of 3.7 minutes. While the longest onset was obtained with BLE 50% took 6.5 minutes with a mean of 4.83 minutes. In a previous study conducted by T. Jayasree on topical anesthesia in rabbit pupils, betel leaf extract with a concentration of 12% took 5 minutes to reach the onset time.[9] The onset of work in this study was slightly faster when compared to the results of previous studies possibly because in this study a larger percentage of the extract was used. The mean onset of BLE is also almost the same as the mean onset of BEN, meaning that BLE has a good onset of anesthesia. The CLE treatment also showed that the onset of action was not much different from that of the control, namely the fastest mean onset was 4.1 minutes, while in the BEN control it was 3.8 minutes.

The onset of anesthesia is dependent on several factors, including lipid solubility and the relative concentration of the un-ionized lipid form (B) and the water-soluble ionic form (BH+) expressed via $pK._a$. The pK_a is pH when the ionized and nonionized fractions of the drug are the same, but it also depends on protein binding and the drug-specific local vasodilating effect.[3,14] In this study, BEN 20% which was used as a control was made from a gel preparation. The extracts used in this study were made by the maceration method. In the variable length of work in this study, BLE with a concentration of 100% had a longer average working duration of 115 minutes compared to the

20% BEN control for 29.3 minutes. In a previous study conducted by T. Jayasree, betel leaf extract with a concentration of 12% applied to rabbit pupils only had a 17-minute working duration.[9] Whereas in this study with BLE concentrations of 50%, 75%, and 100%, the average length of work was 35 minutes, 76.5 minutes, and 115 minutes, respectively.

The longest mean duration of action of topical anesthetics in CLE treatment was 75% with a duration of 33.3 minutes compared to 20% BEN controls with a duration of 22.6 minutes. The results of measuring the working duration of CLE in this study were different from those that were done by Ghelardini, where the length of work obtained was 15 minutes.

This is influenced by the bonds between materials with different affinities to proteins. Like most drugs, local anesthetics bind to plasma proteins as they circulate in the bloodstream. It is expressed as a percentage of the drug that binds to protein and has been correlated with anesthetic affinity in protein and sodium channels. The greater the tendency for protein binding, the longer the anesthetic will maintain nerve blockade.[15]

In the variable depth of anesthesia in this study, BLE with a concentration of 100% had the highest mean depth of anesthesia, namely 31.67 volts compared to 20% BEN control with a mean of 21.3 volts. The mean maximal depth of topical anesthetic on CLE treatment is the highest with concentration 100%, namely with a voltage of 56.8 volts compared to 20% BEN control, namely with a voltage of 23.5 volts. The higher drug concentration, the higher penetration rate into the skin.[14]

CONCLUSION

BLE with a concentration of 100% had the potential as a topical anesthetic agent with a better mean onset, duration of action, and depth of anesthesia as compared to the 20% BEN control. Further research needs to be carried out with more experimental samples, changing the method of pain assessment, changing the test animals, testing the toxicity of the extraction results, and preparing suitably for the active substance in betel.

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