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RESEARCH ARTICLE

Antibacterial Effect of Semendo Coffee Beans (Coffea Canephora) Extract Against Streptococcus Sanguinis In Vitro Growth

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ABSTRACT

Background: *Streptococcus sanguinis is one of the most dominant bacteria in early colonization of plaque formation. Robusta coffee beans (Coffea canephora) was reported to have antibacterial properties because it contained compounds such as alkaloids, flavonoids, saponins, tannins, and steroids.* ***Purpose:*** *This study aimed to determine the antibacterial effect of Semendo coffee beans extract to inhibit the bacterial growth of S. sanguinis.* ***Material and method:*** *This study was an in vitro experimental laboratory. This study used Robusta coffee beans origins from South Sumatera called Semendo coffee beans. The treatment group used Semendo coffee beans extract with concentrations of 2.5%, 5%, 10%, 20%, and 40%. The positive control used 0,2% chlorhexidine gluconate and the negative control used sterile distilled water. The antibacterial potency test was used dilution method to MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) test, and disk diffusion method to inhibitory zone test.* ***Result:*** *The result showed that the MIC of Semendo coffee beans against S. sanguinis couldn't be determined because it was blocked by the color of the extract while MBC was 5%. The average diameter of the inhibition zone formed on 2.5% Semendo coffee beans extract was 12,0±0,4082 mm and bigger along with higher concentration. These results suggested that the extract of Semendo coffee had an antibacterial effect against the growth of S. sanguinis on 2,5%-40%.*

Keywords: *Semendo coffee beans, Streptococcus sanguinis.*

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INTRODUCTION

Dental plaque is a complex biofilm formed on the tooth surface covered with saliva.[1] Plaque is a local cause of disease in the oral cavity due to the activity of microorganisms contained in plaque.[2] This plaque buildup can cause various diseases, such as caries and periodontal disease.[3] Based on the 2018 Basic Health Research (*RISKESDAS*), the prevalence of dental and oral problems related to caries and periodontal disease in Indonesia reached 57.6%.[4] Plaque formation can depend on the adhesion of bacteria to the salivary components that are absorbed on tooth surface.[1] The process is dominated by *Streptococci* bacteria, and among these bacteria, one of the bacteria that can initiate plaque formation is *Streptococcus sanguinis*.[5]

Streptococcus sanguinis bacteria play a role in early colonization of dental plaque formation. These bacteria have virulence factors in the form of PilA, PilB, PilC, and SsaB (saliva-binding protein) which mediate the attachment of *S. sanguinis* to tooth pellicle by forming protein bonding with the α-amylase saliva.[6] In addition, *S. sanguinis* provides a space for the attachment of other bacteria such as *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*, resulting in the maturation of dental plaque.[6,7]

Plaque can be removed by performing periodontal treatments, such as mechanical therapy, followed by antimicrobial therapy, such as antibiotics prescription.[8] However, antibiotics are often used not in relevance with the rules of their usage, causing bacteria becoming resistant to antibiotics.[9] In addition, mouthwash also contains chemical compounds which often bring side effects. Therefore, to overcome the side effects of these chemicals, compounds derived from natural ingredients such as plants are developed which can be used as medicine. Natural ingredients are generally considered safer compared to chemical ones because they rarely have side effects.[10]

One of the natural ingredients that can be used as an alternative to periodontal disease treatment is coffee.[11] Robusta coffee (*Coffea canephora*), a kind of coffee, can be used for this treatment because this coffee contains several compounds including alkaloids, flavonoids, saponins, tannins, and steroids which are thought playing a role in inhibiting the growth of plaque bacterial colonies.[12] Regarding the role of inhibiting bacteria, one research was conducted comparing the antibacterial properties between Robusta coffee and Arabica coffee against *Lactobacillus acidophilus*. The research showed that Robusta coffee has better bacteriostatic activity in 25% concentration, presumably because there is a big difference in the concentration of active compounds contained in Robusta coffee compared to Arabica coffee.[13] In this study, no testing was carried out at a concentration below 2.5%, because the concentration followed the previous research result.

Indonesia, being based on geographic location, has various types of coffee, one of which is Robusta Semendo originating from South Sumatra. The data for 2014 in South Sumatra, especially Muara Enim Regency, showed that Semendo coffee production reached 136,000 tons per year with a plantation area of 256,000 hectares throughout South Sumatra.[14,15] Semendo coffee is a typical coffee product that is widely consumed by the community and easy to find.[16] However, based on the results of literature reviews, there has never been any research regarding the antibacterial activity of this coffee against plaque bacteria such as *Streptococcus sanguinis.*

Based on the above explanation, the researcher is interested in researching to determine the effectiveness of Semendo coffee bean extract, which is part of the Robusta coffee, against the growth of *Streptococcus sanguinis* bacteria.

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MATERIALS AND METHODS

This research is a laboratory experimental study with a posttest control group design. The research was conducted at the Pharmacy Laboratory of Mathematics and Natural Sciences Faculty, Sriwijaya University; the Microbiology Laboratory of Medicine Faculty and the Pharmacy Biology Laboratory of Pharmacy Faculty, North Sumatra University (*USU*). The research sample was *Streptococcus sanguinis* obtained from Microbiology Laboratory of Medicine Faculty of USU with seven treatment groups; extract concentrations of 2.5%, 5%, 10%, 20%, 40%, chlorhexidine gluconate 0.2% as positive control, and sterile distilled water as negative control with 4 replications of the test.

The inclusion criteria in this research were coffee beans taken from the *Semendo Darat Laut*, Muara Enim, beans from the Robusta type with red fruit and green bean (green coffee). The exclusion criteria were beans from rotten fruit and roasted coffee beans.

The test of Semendo coffee bean extract against *Streptococcus sanguinis* was carried out using the dilution method to obtain the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values, as well as the disc diffusion method to obtain the inhibition zone value. The coffee bean extract was obtained through maceration method using 96% solvent Etanol for 24 hours. The preparation of Semendo coffee bean extract was carried out at the Pharmacy Laboratory of Mathematics and Natural Sciences Faculty, Sriwijaya University by immersing the simplicia of Semendo coffee beans that have been made for 24 hours, occasionally stirred, filtered and evaporated with a rotary evaporator until a thick 100% extract is obtained. Then, the 100% Semendo coffee bean extract was tested with phytochemical test at the Pharmacy Biology Laboratory of Pharmacy Faculty at USU to determine the antibacterial compounds contained in the extract.

The 100% coffee bean extract was first diluted to several concentrations; 2.5%, 5%, 10%, 20%, and 40% using sterile distilled water. After the dilution was carried out, it was continued to the test method to obtain the MIC value by filling the test tube with nutrient broth media and adding a *Streptococcus sanguinis* suspension. Then, all the treatment groups were dropped on each test tube that had been marked and covered with aluminum foil. The test tube was incubated for 24 hours and the turbidity was observed. The MIC value was determined based on the tube with the lowest concentration and no turbidity or clearness was formed when compared to the positive control which showed a bacteriostatic effect. After the MIC test, the MBC test was carried using all treatment groups in the MIC test tube for the subculture test.

The subcultures were carried out by taking bacterial cultures in the MIC test tube using a sterile loop needle which then implanted on the MHA media using the streak technique. Then, the petri dishes were incubated in an incubator (Thermo Scientific IGS 180, USA) at 37ºC for 24 hours. The value of MBC is determined based on the calculation of bacterial colonies growth on petri dishes using conventional method.

The inhibition test was carried out by the disc diffusion method. It was conducted on a petri dish that had been swabbed with *S. sanguinis* bacterial suspension using a sterile cotton swab. Then, the paper discs were placed on each marked petri dish and each was dripped with the seven treatment groups. The inhibition zone can be seen from the presence or absence of the clear zone formed around the disc paper which then measured using a caliper.

The results obtained in the inhibitory test were then analyzed using SPSS (IBM SPSS Statistic version 22, USA). The data analysis begins with a normality test using the Shapiro-Wilk test and homogeneity test using Lavene's test. If the two tests show normal and homogeneous data ($p < 0.05$), the analysis continues to the parametric statistical test with One-Way ANOVA to determine the differences

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between groups, proceeded with the Tukey HSD test to determine the magnitude of the differences between groups.

RESULTS

The phytochemical tests on 100% Semendo coffee bean extract showed that the extract contained alkaloids, flavonoids, saponins, tannins, and steroids. Meanwhile, the results of MIC and MBC tests on the extract against *Streptococcus sanguinis* can be seen in Table 1 and Table 2.

Table 1. The turbidity of *Streptococcus sanguinis* on MIC test.

No	Treatment	Result			
		Repetition I	Repetition II	Repetition III	Repetition IV
1.	2,5%	Turbid	Turbid	Turbid	Turbid
2.	5%	Turbid	Turbid	Turbid	Turbid
3.	10%	Turbid	Turbid	Turbid	Turbid
4.	20%	Turbid	Turbid	Turbid	Turbid
5.	40%	Turbid	Turbid	Turbid	Turbid
6.	Positive control	Clear	Clear	Clear	Clear
7.	Negative control	Turbid	Turbid	Turbid	Turbid

Table 1 shows that the test tubes that experienced turbidity were found in all concentration groups. The clarity and turbidity in this MIC test could not be distinguished due to the influence of coffee bean extract color. Therefore, the MIC value could not be determined. On the other hand, the results of the MBC test are shown in Table 2.

The MBC value was determined based on the calculation of bacterial colonies growth on petri dishes using conventional method (Table 2). It shows that the petri dishes with the lowest concentration and no bacterial growth was found at a concentration of 5%.

Tabel 2. Growth of *Streptococcus sanguinis* on MBC test.

No	Treatment	Total of bacterial colonies (CFU/ml)				Average
		RepetitionI	Repetition II	Repetition III	Repetition IV	
1.	2,5%	97	102	83	116	99,5
2.	5%	0	0	0	0	0
3.	10%	0	0	0	0	0
4.	20%	0	0	0	0	0
5.	40%	0	0	0	0	0
6.	Positive control	0	0	0	0	0
7.	Negative control	>300	>300	>300	>300	>300

The antibacterial test procedure was continued to the inhibition zone test using the disc diffusion method. The results of the inhibition test are shown in Table 3. Table 3 shows that all Semendo coffee bean extracts tested had the ability to inhibit the growth of *Streptococcus sanguinis* bacteria with the largest inhibition zone diameter was found at a concentration of 40% with an average diameter of 26.9 mm.

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Table 3. Inhibition zone diameter of Semendo coffee extract against *S. sanguinis*

Repetition	Inhibition Zone Diameter (mm)						
	Negative control (-) sterile distilled water	Positive control (+) CHX 0,2%	Semendo coffee bean extract 2,5%	Semendo coffee bean extract 5%	Semendo coffee bean extract 10%	Semendo coffee bean extract 20%	Semendo coffee bean extract 40%
1	0	36,0	12,0	15,5	19,5	24,0	27,0
2	0	35,5	11,5	14,5	19,0	23,0	26,5
3	0	36,0	12,5	14,0	19,6	23,5	27,0
4	0	35,0	12,0	14,5	19,5	24,0	27,1
Total	0	142,5	48,0	58,5	77,6	94,5	107,6

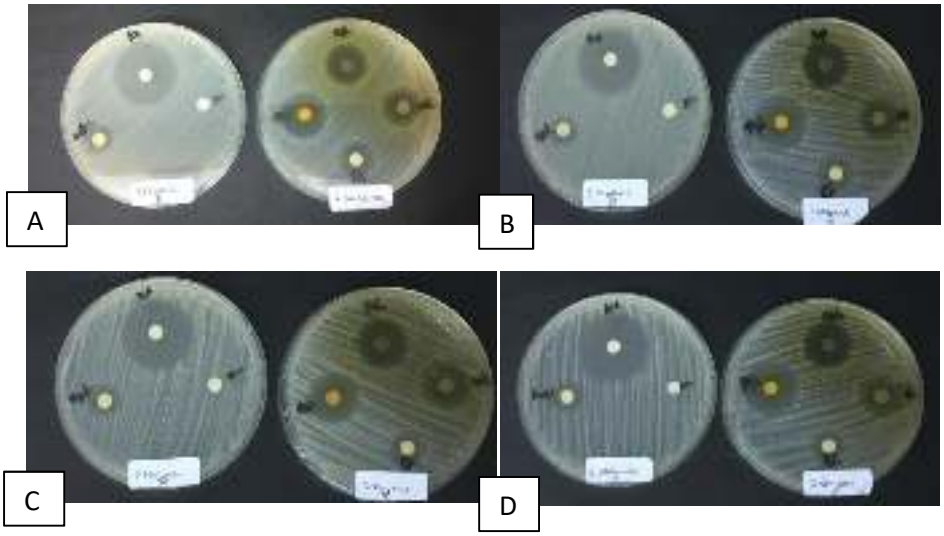
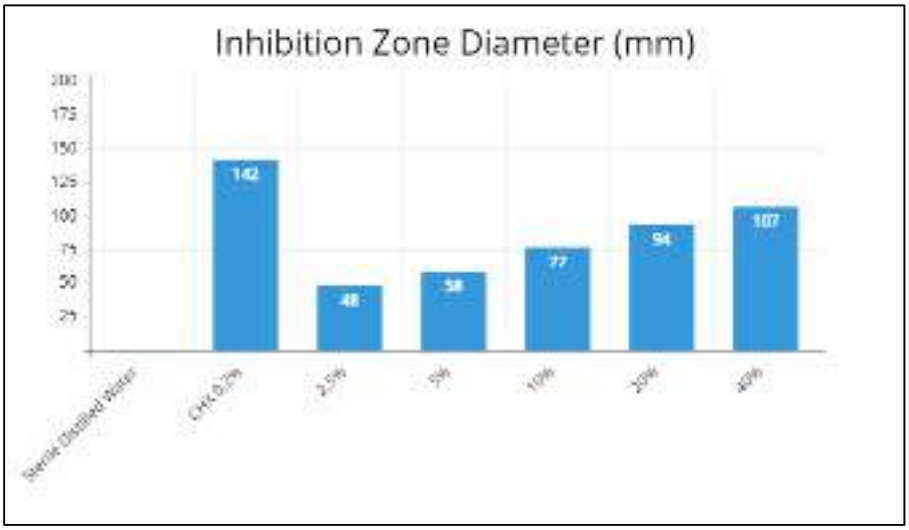


Figure 1. Antibacterial inhibition of the sample group against *S. sanguinis* bacteria
A. 1 repetition, B. 2 repetition, C. 3 repetition, D. 4 repetition
B.



Graphic 1. Inhibiton zone diameter (mm)

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Table 4. Tukey's Post-Hoc analysis test results

Group	Semendo coffee been extract 2,5%	Semendo coffee been extract 5%	Semendo coffe been extract 10%	Semendo coffee been extract 20%	Semendo coffee been extract 40%	CHX 0,2%	Sterile distilled water
Semendo coffee been extract 2,5%		0,000	0,000	0,000	0,000	0,000	0,000
Semendo coffee been extract 5%			0,000	0,000	0,000	0,000	0,000
Semendo coffee been extract 10%				0,000	0,000	0,000	0,000
Semendo coffee been extract 20%					0,000	0,000	0,000
Semendo coffee been extract 40%						0,000	0,000
CHX 0,2%							0,000
Steril distilled water							

The inhibition zone test data analysis was then performed using SPSS. The normality and homogeneity test showed that the data were normally distributed and homogeneous with a significance value of more than 0.05, respectively. Then, the data analysis was continued to the One-Way ANOVA test which showed the results among the treatment groups of Semendo coffee bean extract had a value of p = 0.000. Because the p value <0.05, it means that the values between the treatment groups of Semendo coffee bean extract had significant difference. The analysis was continued to Tukey HSD Post-hoc analysis and it was shown that the results of all treatment groups had a value of p = 0.000. Because the p value <0.05, it can be concluded that the overall test group had significant difference (Table 4).

DISCUSSION

The MIC test results showed that the turbidity and clarity of the test tube could not be determined because the color of the extracts was concentrated, making it difficult to determined the MIC value. This is also in line with a research revealing that the MIC value could not be determined.[17] From the results of the MBC test, it can be concluded that Semendo coffee bean extract (*Coffea canephora*) has the activity of killing *Streptococcus sanguinis* bacteria. It is as shown in Table 2 where the petri dishes in the MBC test had no bacterial colony growth starting from a concentration of 5%. A research showed that Robusta coffee bean extract (*Coffea canephora*) can kill bacterial colony growth starting from a concentration of 3.125%.[8]

The diffusion test showed that the inhibitory response began to appear at a concentration of 2.5% which was indicated by the formation of the inhibition zone diameter on the media. Thus, the diameter had an average of 12.0 mm and it got bigger as the extract concentration value increased. A research concluded that the greater the concentration of the coffee bean extract, the greater the inhibition zone formed.[18] The difference appearing in the inhibition zone is due to differences in the amount of active substances contained in each concentration. The greater a concentration, the greater the components of the active substance

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contained in it, so the inhibition zone formed is also different for each concentration.[19]

The antimicrobial activity against *Streptococcus sanguinis* bacteria can be explained due to the presence of antibacterial compounds as evidenced by the results of phytochemical tests. Based on the results of phytochemical tests, Semendo coffee bean extract *(Coffea canephora)* contains alkaloids, flavonoids, saponins, tannins, and steroids.[12] In addition, it was shown that these compounds were proven to be able to inhibit the growth of the *Streptococcus sanguinis* bacteria.[20]

Some of alkaloid compounds found in coffee are caffeine and trigonelin.[18] Caffeine is an alkaloid compound whose antibacterial ability is due to the presence of alkaline groups containing nitrogen. The presence of this group, when in contact with bacteria, will trigger reaction with amino acid compounds to change their structure and arrangement. This change will affect the balance of genetic and bacterial DNA, causing damage and lysis.[18,21] Meanwhile, trigonelin, which is a pyridine alkaloid, works as an antibacterial by disrupting the stability of the bacterial cytoplasmic membrane which results in an imbalance in the metabolic function of these bacteria, subsequently resulting in inhibition of bacterial growth.[8,22]

The antibacterial activity of flavonoids is carried out by damaging the bacterial cell membrane through differences in polarity between the lipids that make up DNA and the alcohol groups in flavonoid compounds, resulting on damages of which these compounds can enter the nucleus of bacterial cells.[18] Tannins have an antibacterial mechanism by working on polypeptides of the bacterial cell membrane, triggering a chain of process that end up on lysis.[23] Another phenol compound that may also be contained in Semendo coffee beans (*Coffea canephora*) is chlorogenic acid, as mentioned in a research which identified the presence of chlorogenic acid in Robusta coffee beans. Chlorogenic acid acts as an antimicrobial by increasing the permeability of the outer membrane and plasma membrane significantly which causes decreased defense function and leakage from the cell nucleus.[8] However, in this research, the content of chlorogenic acid in Semendo coffee beans was not tested and analyzed.

Another content identified in coffee is saponins. Saponins act as antibacterial by hydrolyzing the cell walls. Damage to the cell wall can cause loss of its semi-permeability property so that it cannot select the entering and exiting substances, such as water and enzymes.[20] While steroids work to inhibit bacterial growth by damaging the plasma membrane of bacterial cells, causing the cytoplasm to leak and leave the cell, which in turn causes cell death.[23]

The average diameter of the inhibition zone in the chlorhexidine gluconate control group was 0.2% higher than the Semendo coffee bean extract group, which was 35.6 mm with a statistically significant difference. Chlorhexidine gluconate 0.2% is a gold standard mouthwash that has antibacterial properties that can precipitate cytoplasmic acid proteins, resulting in changes in cell wall permeability and leakage of bacterial cells.[24] Therefore, from the results of this research, it can be seen that although Semendo's coffee bean extract (*Coffea canephora*) has antibacterial properties, its ability has not yet match the 0.2% chlorhexidine gluconate antibacterial power. The limitation of this study is that it cannot determine the MIC value because it is blocked by the dark color of the extract. For further research, MIC testing can be carried out using a lower concentration.

It can be concluded that the Semendo coffee bean extract (*Coffea canephora*) has antibacterial effect to ~~kill~~ *Streptococcus sanguinis* with a MBC value of 5% and an average diameter of the inhibition zone at 12 mm, starting from a concentration of 2.5%.

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LITERATURE REVIEW

Anxiety Management of Patients First Time to The Dentist for Dental Extraction

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ABSTRACT

Background: *Dental anxiety is a problem that often occurs in patients who will undergo dental treatment. Dental anxiety is a complex, multidimensional phenomenon, and there is no single variable that is the main cause. Dental anxiety is known to be a risk factor that causes various conditions such as pain perception, so that patients refuse to do dental work, especially invasive dental treatments such as tooth extraction.* ***Purpose:*** *The aim of this literature study is to provide information and knowledge about the management and treatment of dental anxiety in patients who come to the dentist for the first time for tooth extraction.* ***Literature Study:*** *This literature study was conducted using indexed and accredited national and international journals and provides information about the techniques that make the patient comfortable and reduce patient anxiety, as well as equipment that can be used in the extraction process that is comfortable for the patient.* ***Discussion:*** *Before performing tooth extraction operator needs an in-depth history to find out the patient's medical history and lifestyle. The approach technique used is influenced by the patient's attitude. At the second visit, the patient appeared to be more ready to accept the extraction procedure after providing information and descriptions to be carried out by the operator.* ***Conclusion:*** *Operators need to understand techniques to reduce anxiety levels in patients, especially patients who have never visited a dentist. After tooth extraction is complete, the operator must also pay attention to the consequences arising from the procedure, and pay attention to the post-extraction instructions.*

Keywords: *Dental anxiety, dental extraction, oral surgery, oral maxillofacial*

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INTRODUCTION

Dental anxiety or anxiety of dental treatment is a problem that often occurs in patients who will perform dental and oral care. Dental anxiety is a complex, multidimensional phenomenon, and there is no single variable that is the main cause. Several studies show that the prevalence of anxiety in patients who first visit the dentist reaches 40-60%.[1] Anxiety arises, especially if a tooth extraction is to be performed. Dental anxiety is known to be a risk factor that causes various conditions such as pain perception, so that patients refuse to do dental work, especially invasive dental treatments such as tooth extraction. To measure anxiety and fear of dental care, a questionnaire filling method can be used, the formation of patient behavior and desensitization techniques also can be used to minimize the anxiety of dental.[2]

Tooth extraction is the process of removing a crown including the dental root from the alveolar bone socket, which cannot be treated. Tooth extraction is carried out using extraction equipment. Dental extraction tools used include extraction pliers or dental forceps, beins or elevators, bone cutting forceps and curettes. The extraction forceps used have different shapes and functions according to the tooth to be extracted[2]. The principles of asepsis and surgery need to be applied to the extraction of teeth. The aseptic principle is needed to prevent infection as a complication in post-extraction wound healing. Extraction without pain, minimal trauma to the surrounding periodontal tissues, and complete healing of extraction scars are components of an ideal tooth extraction[3].

Patients who are about to undergo tooth extraction must be ensured that they are in good general health. If the patient has a systemic disease, the extraction procedure must be accompanied by further examination, because if it is forced and using inappropriate tools and techniques it can cause extraction complications. Dentists should have the ability to take a careful history regarding the history of previous tooth extraction, careful clinical examination, and radiographs to predict the difficulty level of tooth extraction.[4]

Indications for tooth extraction include deep tooth caries that cannot be treated, tooth mobility of grades II and III, infection in the periapical area, severe and untreated abrasion / erosion / attrition, impacted teeth causing complaints to the patient, Excess and malposition of teeth that interfere with chewing function, speech, or esthetic needs, orthodontic needs, severe tooth fracture, and teeth that become focal to an infection.[5] Contraindications for tooth extraction include patients with a history of uncontrolled systemic disease, periapical infections in the teeth that are about to be extracted, patients who are pregnant in the first and third trimester, female patients who are menstruating and patients with high risk. as in elderly patients.[6]

The use of anesthetic techniques for posterior mandibular tooth extraction also needs to be considered, according to the patient's condition. Extraction of mandibular teeth requires adequate anesthesia and is generally performed under mandibular block anesthesia for posterior teeth and infiltration techinque for some posterior and anterior teeth, in contrast to maxillary tooth extraction which uses an infiltration technique. the larger needle and syringe size in the mandibular block anesthetic technique makes the patient more anxious and worried, compared to the infiltration technique which can be performed with a smaller needle and syringe or using a cytoject.[2] However, block mandibular anesthesia technique can be an option because it has a wide coverage area and has a long duration, making it easier for the operator to perform the extraction procedure. Another alternative local anesthetic that can be used is the infiltration technique using either a syringe injection or a cytoject with an area that is not as large as the mandibular block technique.[7]

The operator also needs to consider the extraction complications that may occur in the patient. Extraction complications are a specific response from the patient that can occur at the time of the procedure or after the extraction

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procedure. Complications in extraction can include bleeding, fracture, soft tissue injury, nerve injury, edema, drug reactions and dry socket. Extraction complications can be prevented, and their occurrence is minimized with good pre-extraction preparation and in-depth history.[8] In-depth history of the patient can reduce the patient's level of anxiety, especially in patients who are new to a dentist.[9]

Anxiety arising from tooth extraction is not considered a serious health problem but can become a barrier for operators in their efforts to improve oral health. The anxiety experienced by the patient can cause several problems, such as the patient feeling tense, excessive alertness, trembling hands and feet, talking a lot, dilated pupils, giving a picture of uncontrollable fear, excessive sweating of hands and face. Even under certain conditions, patients can experience generalized visceral reactions in respiration, heart, blood vessels, digestive dysfunction, hysteria, and even shock.[1] Anxiety is an affective psychological factor that affects the perception of pain. In many cases of acute pain such as pulpitis, anxiety has a lot to do with an increase in the incidence of pain, i.e., not only decreasing the patient's pain threshold but also in fact resulting in the perception that pain should not be pain, even under different conditions, a patient may show a reaction. different even though the stimulus is the same. Patient anxiety has a negative effect on the treatment procedure to be performed.[10] In this report, to reduce patient anxiety, The authors also list several mandibular block anesthetic techniques that can be used to reduce patient tension and anxiety, and the management of extraction patients who have never been to a dentist before. It is hoped that a comfortable extraction with minimal pain and proper anesthetic technique will reduce the level of anxiety in the patient.

Tooth extraction

Tooth extraction is a process of removing of a tooth that can no longer be maintained from the alveolar bone. Tooth extraction is a minor surgical procedure that involves the soft tissue of the oral cavity, the access is limited by the lips and cheek mucosa which are then joined by movement of the tongue and jaw.[11] A tooth can be extracted if it has a pathological pulp that cannot be treated, either by filling or by root canal procedures. In addition, teeth with severe periodontal disease can also be extracted.[5] There are several steps that need to be considered in carrying out tooth extraction, including good anesthesia procedures, extraction of the tooth along with its roots and control of bleeding during and after the extraction procedure.[12] Tooth extraction can cause complications that should not occur at the time of extraction or after extraction. Complication is a particular response to the patient either during the procedure or after the procedure. Complications due to tooth extraction occur due to various factors. Complications that are often encountered include bleeding, swelling, dry socket, fracture and mandibular dislocation.[13] Post-extraction bleeding often results from excessive trauma, infection and lesions in the vascular tissue. Patients with post-extraction bleeding complications can be treated in the form of spongostan administration in the socket and then suturing.[12] Dry socket is a socket condition that does not form a blood clot. The symptom of a dry socket is localized pain in the area of continuous extraction[14].

Mandibular block anesthesia

Mandibular block anesthesia can be performed if the operator requires a large area of anesthesia, for example in the case of extracting the posterior mandibular teeth or extracting several teeth in the same region with the same innervation.[15] The Gow-Gates anesthetic technique includes the lingual nerve, the buccal nerve, the mylohyoid nerve and the auriculotemporal nerve. The teeth to be anesthetized are the teeth in the mandibular half quadrant area, the buccal mucoperiosteum, mucous membranes in the area of anesthesia, 2/3 anterior of the tongue and floor of the mouth, soft tissue of the lingual part, mandibular body, cheek posterior and temporal.[16]

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The Gow-Gates technique block anesthesia procedure is primarily the operator's position in the 8 o'clock area for the right mandible and the 10 o'clock area for the left mandible. The patient is positioned semi supine and instructed to open the mouth wide.[15] The operator determines the extraoral point, namely the intertragic notch and the corner of the mouth. The operator determines the point of insertion in the mandibular condyle area under the external pterygoid muscle. The syringe is directed at the parallel plane between the corner of the mouth and the intertragic notch. The needle is transverse from the opposite side of the maxillary canine, crossing across the palatal protrusion of the maxillary M2 tooth on the side to be injected. The needle is slowly inserted until it makes contact with the condyle neck bone, to a depth of approximately 25 mm. Once it has contact with the bone, the syringe is aspirated and a slow deposit of 1.8-2 ml can be made.[17]

The akinosi anesthetic technique has the goal nerves of the mandibular half quadrant, mandibular body and lower ramus, buccal mucoperiosteum and mucous membrane in front of the mental foramen, floor of the mouth and two-thirds of the anterior tongue of the tongue, soft tissue and the periosteum of the lingual part of the mandible. This technique is performed with the patient's mouth closed so it is best used in patients who have difficulty or pain when opening the mouth or trismus.[16]

The akinosi block anesthetic procedure is that the patient is first positioned supine or semi supine. Operator position at 8 o'clock for the right and left jaw. The index finger and thumb are placed on the coronoid bulge, the area of the maxillary tuberosity. Then the patient was instructed to perform occlusion. The needle is placed parallel to the maxillary occlusal plane, then inserted at the M3 and M2 mucogingival junctions. Then the needle is deflected toward the ramus. Aspirate and deponit 1.5-18 ml.[17] In Fisher's technique of anesthesia, the patient is first positioned semi supine. The application of antiseptic in the form of povidone iodine in the retromolar trigone area. The index finger is placed behind the last tooth of the mandible, sliding it laterally to feel the external oblique line. Then the index finger is shifted towards the median area to find the internal oblique line, the nail arch tip is in the internal oblique line area and the side surface of the finger is in the occlusal plane of the mandibular teeth. Position I: The needle is inserted in the middle of the nail arch, from the side of the jaw that is not anesthetized, that is, in the premolar region. Position II: The syringe is shifted towards the side to be anesthetized, parallel to the occlusal plane and the needle is inserted 5 mm deep and then aspirated. If the aspiration is negative, 0.5 ml of anesthetic solution is issued for lingual nerve anesthesia. Position III: The syringe is shifted in the direction of position I, but not completely then the needle is inserted while tracing the bone, approximately 10-15 mm deep. Aspiration and if negative the anesthetic solution is issued as much as 1 ml for inferior alveolar nerve anesthesia.[18] In Fisher's modified anesthetic technique, after performing position III, when pulling back the syringe before the needle is released from the mucosa, i.e. right after passing through the internal oblique line, the needle is shifted laterally to the retromolar trigone area, then aspiration and anesthetic solution are removed as much as 0.5 ml Buccal nerve anesthesia. Then the syringe is pulled out again.[19]

Extraction tools

Tooth extraction is carried out using dental extraction tools that have a specific shape and function. Dental extraction tools used include extraction pliers or dental forceps, beins or elevators, bone cutting forceps and curettes. The extraction forceps used have different shapes and functions according to the tooth to be extracted.[2] The maxillary extraction forceps are characterized by an almost straight beak and handle when viewed from the side. Teeth that have a crown use an open beak, while those with a root remaining use a closed beak. The third molars have special pliers that are shaped like a bayonet.[20]

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Management of patient anxiety

Anxiety is a non-specific symptom that is common and is often a normal emotion. Anxiety is the most common and important problem for a dentist, therefore it is best if a dentist should be able to understand the presence of anxiety in patients as early as possible, so as to facilitate identification of patients with a tendency to anxiety. Anxiety in a patient that is left untreated or not understood by the operator can lead to shock and syncope. The approach and way of dealing with anxious patients greatly affects the smoothness and success of the treatment plan that will be carried out. Dealing with a patient who is not cooperative, often makes it difficult for a dentist to perform treatment.[21] Psychodynamic theory explains that anxiety is the result of unconscious psychic conflict. When self-control mechanisms work, anxiety can decrease and a sense of security returns. but when the conflict is continuous and prolonged, then anxiety is at a high level. This self-defense mechanism as a symptom, such as phobia, regression and ritualistic behavior. Behavioral theory states that anxiety comes from a response to a specific stimulus. This anxiety is the result of frustration, so it will interfere with the individual's ability to achieve the desired goals.[22]

Management of anxiety can be done by shaping the patient's behavior towards the ideal. In the field of dentistry, it is said that ideal behavior is shown by patients who maintain excellent oral hygiene, practice dietary management, and are relaxed and cooperative during operative care. If the patient is unable to cooperate, it can be postponed first. In addition, strengthening the patient can be done by giving rewards to patients who can suppress their anxiety.[23] Desensitization is one of the techniques most often used by psychologists to combat fear. This technique includes three stages, firstly training the patient to relax, secondly building a stimulus hierarchy, third introducing each stimulus in the hierarchy to relax the patient, starting with the stimulus that causes the least amount of fear and advancing to the next stage only when the patient is no longer afraid of the stimulus[1]. Cognitive Behavioral Therapy (CBT) or Exposure Therapy through several stages, namely providing information using the "Tell Show Do" method, then relaxing the whole body and muscles. The method often used is to instruct the patient to inhale and exhale slowly while the patient counts to four. Another thing can also be done by distraction by breaking the patient's focus on the anxiety that is being felt by inviting the patient to talk and involving the patient in activities that make thinking, so that they forget about feeling anxious.[11] In addition, video based patient education can also be done in the form of information about the diagnosis of disease, causes of disease, stages of treatment, the healing process and the results, the possibility of recovery (prognosis), complications that may occur after treatment, and the consequences if treatment is not done[24].

Stages of care

On the first visit, tools and materials were prepared. Then do an in-depth history of the patient. Furthermore, the patient was inspected and excavated with objective examinations. If the results showed that percussion was (+), it was necessary to give medication before the action and control instructions for 5 days later. Providing information and procedures for actions that will be carried out can reduce the feeling of anxiety in the patient.[25] On the second visit, the dental team should preparing tools and materials in the form of a diagnostic set, handscoon, mask, nurse cap, slaber, suction, mouth rinse, bein, extraction forceps, curette, povidone iodine, syringe, anesthetic solution, tampon and gauze. Inspection and objective examination and evaluation after administration of medication are carried out. If The percussion result is stated (-), the extraction action can be carried out.[10] The patient is given an overview of the tooth extraction to be performed. Providing information and procedures for actions that will be carried out can reduce the feeling of anxiety in the patient. Furthermore, informed consent was given before the extraction action began.[24]

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Disinfection using povidone iodine in the area to be anesthetized. Anesthetize the inferior alveolar and lingual nerves using the Fisher block technique and the long buccal nerve by infiltration. The anesthetic used was pehacain, each ml containing 20 mg of lidocaine HCL and 0.0125 mg of adrenaline with a maximum dose of 7 mg / KgBW.[22] Lift the teeth with the elevator or bein. Extracting the tooth using pliers when the leverage is sufficient and the tooth has been lift up. After the tooth root was completely removed, debridement was carried out in the form of cleaning the wound, smoothing the sharp bone with a bonefile, cleaning the tooth socket from the remaining bone fragments or necrotic tissue using a curette. Irrigate with povidone iodine antiseptic solution. The patient is instructed to bite the tampon for 30-60 minutes. Prompt for regular consumption of antibiotics, anti-inflammatory and analgesics. Post extraction procedure instructions.[22]

DISCUSSION

Before performing the tooth extraction procedure, the operator requires an in-depth history to determine the patient's medical history and lifestyle. In-depth history is in accordance with the literature that a good history will minimize complications after tooth extraction. Tooth extraction is an invasive procedure that can make the patient feel uncomfortable or stressed, so a good history taking approach and eye contact with the operator can assure the patient to feel safe and calm[25]. In addition, a good and targeted anesthetic technique must be recognized by the operator as a successful extraction attempt. To reduce the patient's anxiety when a tooth is extracted under mandibular block anesthesia, a method that is comfortable for the patient can be chosen. In addition, operator skills are also needed in performing mandibular block anesthesia with techniques that minimize pain so that patient anxiety can be reduced. If at the first meeting the patient is not ready for extraction, it is best not to force the tooth to be extracted. Second visit is the right time to carry out tooth extraction for patients with high anxiety levels. The choice of anesthetic technique can also be taken into consideration with the patient's condition to be extracted. For example, Patients who experience high levels of anxiety about injection needles and syringes can be diverted their eyes using visual media or invited to communicate which can reduce patient tension. The dentist can also provide sentences suggesting that the local anesthetic process with mandibular block is carried out comfortably and causes minimal pain. Thus the anesthetic procedure does not interfere with the patient's condition[7]. The use of extraction equipment is adjusted to the location and condition of the patient's teeth. In the extraction of the cases, the operator used tools in the form of beins, forceps, bonefile and curette. This is consistent with the literature that tooth extraction is carried out using dental extraction tools that have specific shapes and functions. Dental extraction tools used include extraction pliers or dental forceps, beins or elevators, bone cutting forceps and curettes. The extraction forceps used have different shapes and functions according to the tooth to be extracted.[2] Post extraction instructions are given by the operator to the patient with the aim of avoiding post-extraction complications and accelerating wound healing. This is in accordance with the literature that post-extraction instructions, both verbal and written, should be done as a form of reducing the percentage of complications after extraction. Information that can be provided includes biting the tampon for 30 minutes or more after extraction, avoiding gargling and spitting too often, consuming soft foods with cold or room temperature temperatures, applying ice cubes to the cheek that has been extracted, maintaining clean cavities mouth, avoiding cigarettes and alcoholic beverages for one week or more after tooth extraction and taking any medications as prescribed.[22]

If The patient has never had his teeth checked or treated by a dentist, the operator should provide an anxiety management

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approach to the patient so that when the extraction is done, the patient feels safe and calm. The management of anxiety is in accordance with the literature described by Mufti.[10] that an approach in managing behavior in patients is important and is an integral part of oral health. Various factors can influence patient behavior in the dental environment. Different approaches are used which are influenced by the patient's attitude. At the second visit, the patient appeared to be more ready to accept the extraction procedure after providing information and descriptions to be carried out by the operator. The operator also reiterates the provision of information so that the patient is truly ready and has complete trust in the operator.[25]

CONCLUSION

Tooth extraction has principles that we must adhere to properly. The various steps carried out in the implementation of tooth extraction pay attention to the position of the operator and the position of the patient's dental unit when carrying out the extraction procedure. Operators need to understand techniques to reduce anxiety levels in patients, especially patients who have never previously visited a dentist. After the tooth extraction is complete, the operator must also pay attention to the consequences of the procedure, and also pay attention to the post-extraction instructions.

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RESEARCH ARTICLE

Beneficial Effect of Arabica Coffee Fruit Skin (*Coffea Arabica*) on Epithelial Thickness after Tooth Extraction

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ABSTRACT

Background: *In Indonesia, tooth extraction is the most common practice in dentistry. A successful indicator of tooth extraction is a perfect healing process. One of the parameters of a perfect healing process is epithelial formation. Based on previous research, arabica coffee fruit skin has proven potential to accelerate the wound healing process. The compounds in the arabica coffee fruit skin are mostly composed of flavonoids, tannins, and chlorogenic acid.* ***Purpose:*** *This research is proposed to understand the beneficial effect of arabica coffee fruit skin (Coffea arabica) towards the increase of epithelial thickness in post tooth extraction socket.* ***Material and Method:*** *The type of this research was laboratory experimental in vivo with post-test only control group design using 24 Wistar rats as a sample. The sample was separated into two groups, control and intervention groups with 12 Wistar rats each. Treatment in both groups was given during 3, 5, and 7 days. Afterward, tissue processing undergoes with buccolingual cutting and HE coloring. Measurements are carried out with the ImageRaster software in the thickest and thinnest part of the epithelium that covers the tooth socket.* ***Result:*** *Epithelial thickness after tooth extraction increased significantly ($p \leq 0.05$) in the intervention group compared to the control group.* ***Conclusion:*** *Arabica coffee fruit skin has the beneficial effect of increasing the epithelial thickness after tooth extraction.*

Keywords: *Tooth extraction, epithelial thickness, arabica coffee fruit skin*

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INTRODUCTION

Tooth extraction is the most common procedure in dentistry. According to RISKESDAS 2018, the amount of tooth extraction is higher (7,8-8%) than restoration (6,6-6.8%).[1] Tooth extraction is usually performed when the conservation treatment fails or is not indicated. Extraction of teeth is successful if it is following by an adequate process of healing.[2]

Sometimes, the healing process causes a problem that leads to failure of wound healing. The most common problem is dry socket,[3] with an incidence of 5% of all failure wound healing cases after tooth extraction.[4] Dry sockets occur as a result due to the disintegration of blood clots by fibrinolysis[4] which causes the healing process to be interrupted. Therefore, the wound healing process needs to be considered to avoid the presence of a dry socket.

There are three stages of the wound healing process after tooth extraction, are inflammation stage, proliferation, and remodeling.[5,6] The proliferation stage is one of the most important stages in the wound healing process. In the proliferation stage, the epithelial tissue damaged will undergo a re-epithelialization process. In the re-epithelialization stage epithelial cells migrate, mitosis, and differentiation. This stage will restore the lost mucosal integrity.[7] The formation of the epithelium is a parameter of the success of wound healing after tooth extraction. When the epithelialization process does not occur, the wound cannot be healed.[8]

The wound healing process can be done, one of which involves administering traditional natural medicines. According to Riskesdas 2018, 31.5% of Indonesia's population uses traditional health treatment to treat their disease disorders. Nationally, the population of East Java is the area that uses medicinal herbs the most (48.3%).[1] Traditional medicine treatments made from nature are preferred because they are considered cheaper with fewer side effects.[9]

One of the compounds from natural ingredients that influence the wound healing process is arabica coffee fruit skin. The arabica coffee fruit skin is the main by-product (waste) generated from coffee processing.[10] Based on the previous research, showed that the content of arabica coffee fruit skin has proven potential in increasing the number of fibroblast cells in wounds after tooth extraction.[11] Fibroblasts are cells that play an important role in wound healing because they are responsible during the tissue reconstruction process.[6] Compounds in the arabica coffee fruit skin are mostly composed of flavonoids, condensed tannins (proanthocyanidin),[12] and chlorogenic acid[13] that play an important role as antioxidants, anti-inflammatory, and antibacterial[14] which can accelerate the wound healing process.

According to the above explanation, it could be interesting to understand the beneficial effect of arabica coffee fruit skin (*Coffea arabica*) towards the increase of epithelial thickness in the post tooth extraction socket of Wistar rat.

MATERIALS AND METHODS

This laboratory experiment *in vivo* with post-test only control group design was approved by the ethical committee of the medical research faculty of dentistry Universitas Jember (No.988/UN25.8/KEPK/DL/2020). The sample of this research was 24 male Wistar rats separated into two groups named control and intervention group. The control group was given a solution of 2 ml water. Meanwhile, the intervention group was given a solution of powdered arabica coffee fruit skin with the dose of 0.117 gr dissolved in 3.6 ml warm water. Both treatments were given intragastrically during 3, 5, and 7 days. Then, Wistar rats decapitated 24 hours after the last treatment on days 4, 6, and 8 to execute tissue processing with buccolingual cutting section and Hematoxylin-Eosin (HE) coloring.

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Next, the epithelium of the tooth socket was observed using OptiLab that was connected with a binocular microscope with a 100x magnifier. Measurements are carried out with the help of the ImageRaster 3.0 software in the thickest and thinnest part of the epithelium that covers the tooth socket. Data then tested using a One-Way ANOVA test with a 95% confidence level. Afterward, tested with post hoc Least Significant Difference test to see differences between each group.

RESULT

Based on the research that has been done, it was found that the average of epithelial thickness in the control and intervention group from days 3, 5 and 7 were increased (Figure 1).

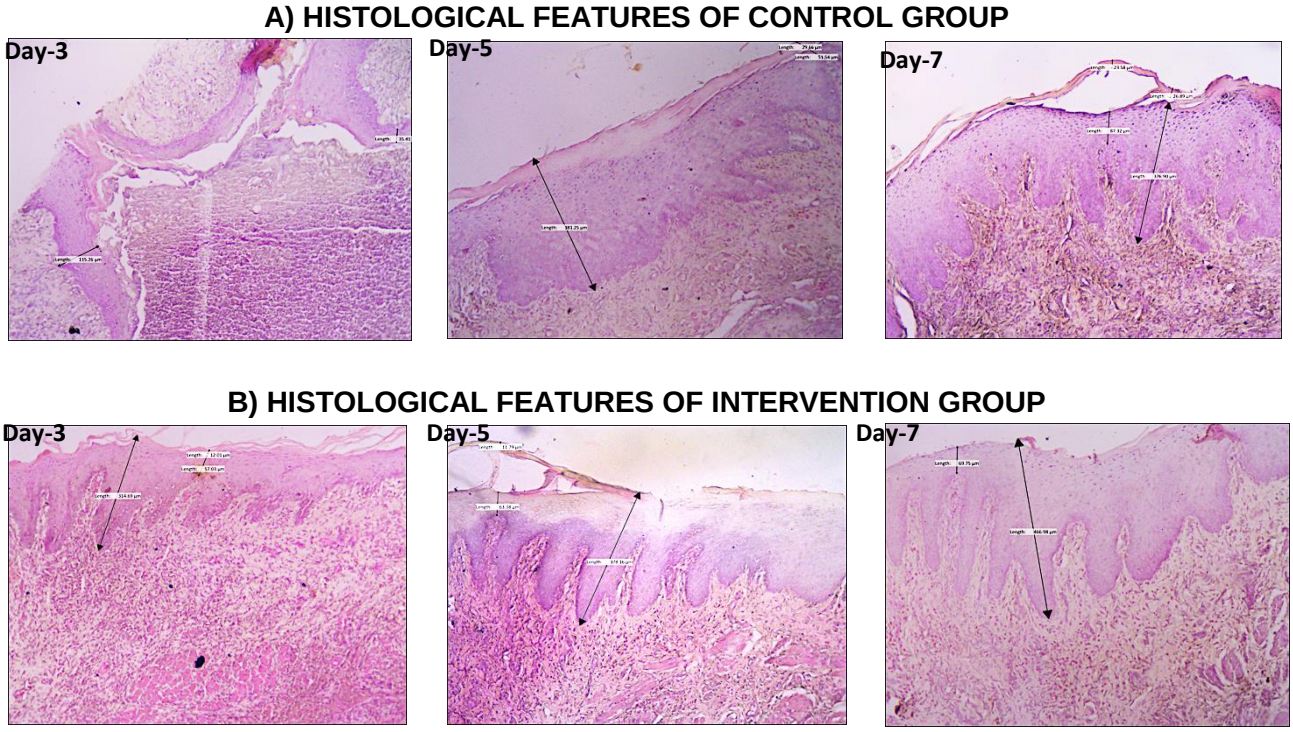

Figure 1. The gingival epithelium covering the tooth socket with 100x magnification. A) Control group show the epithelial formation process that occurs physiologically, starting from the socket which is still open on the 3rd day, until it is closed on the 5th day, and has increased in thickness on the 7th day; B) The intervention group showed a process of increasing the thickness of the epithelium from the 3rd, 5th, and 7th day due to the effect of the powdered arabica coffee fruit skin.

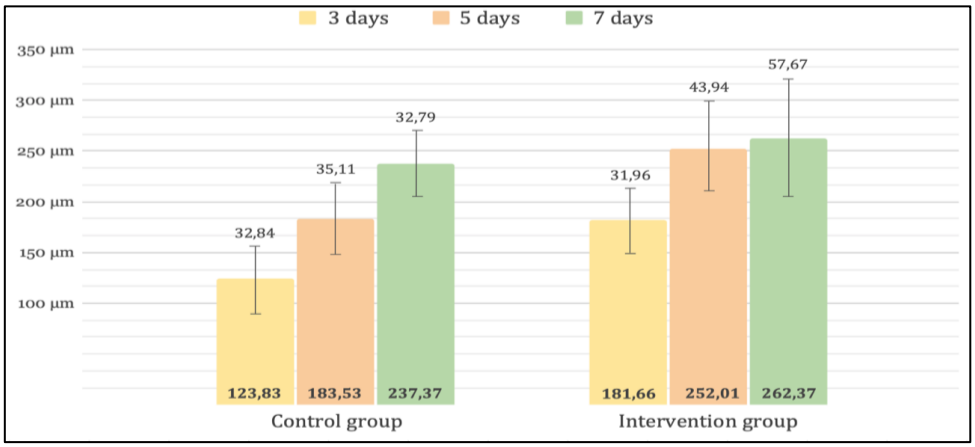

Figure 2. The mean histogram of socket epithelial thickness after tooth extraction of Wistar rats in the control group and the intervention group was accompanied by standard deviations.

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The results showed that the average epithelial thickness after tooth extraction in the control group from the 3rd, 5th, and 7th days is increased. Likewise, the intervention group that was given a solution of powdered arabica coffee fruit skin, from the 3rd, 5th, and 7th days also experienced an increase. The control group experienced a stable increase in epithelial thickness. Meanwhile, in the intervention group, a significant increase in epithelial thickness was only seen between day 3 and day 5, then between day 5 and day 7 did not show a significant increase. When compared, the mean epithelial thickness of the intervention group on day 3 was almost the same as the mean epithelial thickness of the control group on day 5.

One-Way ANOVA test results obtained a significance value of 0.01 ($p \leq 0.05$), which means that there is a significant difference in the average epithelial thickness between all groups. Furthermore, to find out which groups have significant differences in between can be done by LSD Test as summarized in Table 1.

Table 1. Summary of data analysis epithelial thickness after tooth extraction between each group

GROUP	n	Epithelial Thickness (µm)			p
		DAY-3	**DAY-5**	**DAY-7**	
Control	4	123.83 ± 32.84^{aA}	183.53 ± 35.11^{aB}	237.37 ± 32.79^{aB}	0.001*
Intervention	4	181.66 ± 31.96^{aA}	252.01 ± 43.94^{bB}	262.37 ± 57.67^{aB}	0.001*
p		0.057	0.023*	0.390	

The value was expressed as mean ± standard deviation ($\bar{X} \pm SD$)
Explanation:
(*) = there was a significant difference ($p \leq 0.05$)
(^{ab}) = difference superscript in a column shows the significant difference
(^{AB})= difference superscript in a row shows a significant difference

Based on the results of the LSD test, shows that there is a significant difference between each group. In the control and intervention groups, there was a significant difference ($p \leq 0.05$) between day 3 and day 5. Meanwhile, between day 5 and day 7 in both the control and intervention groups, there was no significant difference.

DISCUSSION

Tooth extraction action can cause injury of the tissue around the tooth. According to Ningsih et al.[15], after an injury occurs, the socket will be healing physiologically. This healing process involved soft tissue and hard tissue. The blood clot is formed, which will develop into granulation tissue in soft tissue. Furthermore, the epithelium will be developed to cover the surface of the granulation tissue completely.

Epithelialization is an essential component of wound healing used as a defining parameter of successful wound closure.[8] The faster the reepithelialization was done, the faster the wound will be closed, so the faster the wound healing will occur.[16]

Based on the previous study, the mean epithelial thickness in the control group on days 3, 5, and 7 has increased. Based on the theory, the wound healing response occurs physiologically. However, statistically significant differences, based on the post-hoc test with LSD, only existed between day 3 and day 5 ($p \leq 0.05$). This shows that the epithelialization process has increased significantly. This increase can be seen histologically, the state of the socket that is still open to being closed by the epithelium. Smith[17] reported on the 2nd day after tooth extraction in mice, epithelial migration has begun physiologically, and on the 4th day, epithelial migration has increased. This situation causes a significant increase in epithelialization. Next, on observations between days 5 and 7, although there was a stable mean increase in epithelial

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thickness, it was not significant in statistics (p>0.05). These results show, there is no difference between the mean epithelial thickness at day 5 and day 7. Supported by Smith[17], on the 5th day after tooth extraction in mice, the epithelium almost covers the socket and the trabeculae of the cartilage have adhered to the wall at the base of the socket physiologically. Then, on day 8, epithelialization was complete and the 1/3 apical of the socket was filled with cartilage and osteoid. This shows that the physiological proliferation phase of the wound healing process has been completed, and the initial bone formation process begins.

In the intervention group, the mean epithelial thickness on days 3, 5, and 7 also increased. Similar to the control group, there were significant differences, based on the post-hoc test with LSD, which only existed between day 3 and day 5 ($p \leq 0.05$). Meanwhile, between days 5 and 7, the epithelialization process was almost complete because it did not experience a significant increase and almost had the same thickness mean. This was supported statistically, which showed there was no significance (p>0.05). It means, there is no difference between the mean epithelial thickness at day 5 and day 7. It can also be seen histologically that the epithelial thickness at day 5 and day 7 does not appear to have a significant difference in thickness. This may be caused by the epithelialization process which is almost over. It has been known previously that epithelial cell migration will stop if the epithelial cells have made contact with other epithelial cells in all directions (contact inhibition).[15] Thus, it can be concluded that on the 5th and 7th day, by giving a solution of powdered arabica coffee fruit skin, the epithelial cells in the intervention group have reached an almost complete migration pattern. According to Throne[6], after the migration of epithelial cells (keratinocytes) has been completed, the remodeling process in the socket begins.

The mean epithelial thickness of the treatment group on days 3, 5, and 7, when compared, was higher than the mean epithelial thickness of the control group. This shows that, by giving a solution of powdered arabica coffee fruit skin affects increasing the thickness of the epithelium in the wound healing process after tooth extraction of Wistar rats.

The increase in epithelial thickness that occurred in the intervention group was caused by the potential content of chemical compounds in the skin of the Arabica coffee fruit. Arabica coffee fruit skin contains polyphenols which are composed mostly of flavonoids and condensed tannins (proanthocyanidin)[12], and also phenolic acids with the highest content in the form of chlorogenic acid.[13] These three compounds have an important role in accelerating wound healing.

Flavonoids are known to play an important role in wound contraction and increase the rate of re-epithelialization. In a study by Thakur et al.[19], it was explained that flavonoids play a role in the wound healing process because of their astringent and antimicrobial properties which are responsible for wound contraction and an increase in the rate of epithelialization. Flavonoids also possess immunostimulating effects to increase phagocytosis activation by macrophages and increase the inflammatory process. The increase in macrophages affects the production of growth factors that contribute to the proliferation process, thereby helping to accelerate the re-epithelialization process[7]. Also, flavonoids are powerful antioxidants that can reduce lipid peroxidase and increase the speed of epithelialization.[16] The reduction of lipid peroxidase from flavonoids will prevent necrosis and increase the strength of the collagen fiber webbing.[18]

The other compound content is tannins. The active substance of tannins can play a role in increasing wound contractions and the speed of epithelialization.[19] Tannins as an antibacterial can reduce inflammation, increase epithelial formation, and cause a vasoconstrictive effect on blood vessels.[7] Tannins are thought to play a role in regulating transcription and translation of vascular endothelial growth factor (VEGF).[20] VEGF acts paracrine not only on skin vascular endothelial cells but also on keratinocytes and

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immune cells which promote re-epithelialization and at the same time stimulate angiogenesis and restore oxygen perfusion.[8] In addition, tannins have an anti-inflammatory effect that increases the proliferative ability of TGF-ß to increase the epithelialization process. TGF-ß acts as an important regulator in returning activated keratinocytes to the basal cell phenotype. Its expression during injury has been shown to induce granulation tissue formation and myofibroblast differentiation which facilitates contraction of the collagen matrix and wound closure.[8] This was evidenced in the study of Rahayu et al.[21], regarding the effectiveness of proanthocyanidin (condensed tannin) of cocoa pod skin against the socket epithelium after tooth extraction of Wistar rats, which showed an increase in epithelial thickness. Condensed tannins also act as antioxidants which have a role in accelerating the healing process.

The other chemical compound is chlorogenic acid. The amount of chlorogenic acid found in coffee reaches 90% of the total phenol.[22] Chlorogenic acid has strong antioxidant activity as well as anti-inflammatory activity.[10] Duangjai et al.[23] in their research stated that the content of chlorogenic acid and caffeine in the skin of arabica coffee fruit plays a role in antimicrobial activity. Duangjai's research shows that Gram-positive bacteria are more susceptible to the skin of arabica coffee fruit than Gram-negative bacteria. Its effectiveness on Gram positive and negative bacteria is influenced by the structure of the bacterial envelope and active compounds from the arabica coffee fruit skin.

In this study, potential compounds in the arabica coffee fruit skin were shown to increase the thickness of the socket epithelium after tooth extraction. The role of arabica coffee fruit skin compounds in increasing the thickness of the epithelium is seen at the beginning of wound healing. An increase in epithelial thickness in the intervention group was seen on day 3. The mean epithelial thickness in the intervention group on day 3 was almost the same as the mean epithelial thickness in the control group on day 5. This was proven statistically that there was no significance ($p>0.05$). It means, the epithelial thickness of the control group on day 5 was reached in the intervention group on day 3, and the two means were proven to have no significant difference. This shows, by giving a solution of the powdered arabica coffee fruit skin, epithelialization increases in the early stages of wound healing, thus indicating a faster wound healing process. It was also seen that the epithelial thickness in the intervention group on day 3 was almost the same as the epithelial thickness in the control group on day 5 histologically, and the sockets of the two groups had both been closed by the epithelium. Although they had almost the same mean epithelial thickness, the epithelium in the intervention group on day 3 had a clearer rete peg shape than that in the control group on day 5. The existence of a rete peg indicates that the re-epithelialization process goes well informing the epithelial tissue back to normal.[5] So, apart from being able to increase the thickness of the epithelium, giving a solution of powdered arabica coffee fruit skin is proven to produce a better re-epithelialization process.

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that the compound content of arabica coffee fruit skin (Coffea arabica) has the beneficial effect to increase the epithelial thickness of Wistar rat after tooth extraction.

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RESEARCH ARTICLE

Evaluation of Antibacterial Activity of Cymbopogon Nardus L. on The Growth of Enterococcus Faecalis

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ABSTRACT

Background: *Cymbopogon nardus L. known as citronella grass is traditional medicinal plant that contains saponins, polyphenols, alkaloids, flavonoids, and essential oil. Based on the previous study, it is known that it has antibacterial properties.* ***Objective:*** *The aim of this study was to determine antibacterial activity of Cymbopogon nardus L. in inhibiting the growth of Enterococcus faecalis.* ***Methods:*** *The samples were Cymbopogon nardus L. extracts with concentration of 1%, 3%, 5%,7%, 9%, and 5% NaOCl as positive control. Antibacterial activity of citronella extract against E. faecalis was observed with the formation of inhibitory zone diameter in agar. Data were analyzed using SPSS with one-way ANOVA and LSD test.* ***Result:*** *The result showed that Cymbopogon nardus L. was able to inhibit the growth of E. faecalis at concentration 1%,3%,5%,7% and 9%. There was a significant difference in antibacterial activity among all groups. It showed that concentration of 9% showed the highest zone of inhibition, but it was not as effective as NaOCl 5%. NaOCl showed the highest zone of inhibition.* ***Conclusion:*** *Cymbopogon nardus L. had antibacterial activity in inhibiting the growth of E. faecalis. The higher the concentration of citronella extract, the higher the antibacterial activity possessed.*

Keywords: *Antibacterial, Cymbopogon nardus L., Enterococcus faecalis, inhibition zone.*

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INTRODUCTION

Herbal ingredients have been long used in the health for preventive, curative and rehabilitative purposes. The used of medicinal plants in Indonesia is established, in both fields of medicine and dentistry. So that, the medicinal plants need to be explored, especially Indonesian plants which are known for their rich biodiversity. Since long time ago, people have been believed that herbal ingredients are capable of treating various kinds of diseases and rarely cause adverse side effects compared to synthetic drugs.[1] One of Indonesia's native plants that can be used as herbal medicine is citronella grass (*Cymbopogon nardus L.*).[2]

Cymbopogon nardus L. contains saponins, flavonoids, polyphenols, alkaloids, and essential oils.[3] Saponins work as surfactants form in polar which is able to break down the fat layer of bacterial membrane and causes lysis.[4] Antibacterial flavonoids might be connected with their ability in inactivating microbial adhesins, enzymes, and cell envelope transport proteins. Polyphenols have antibacterial activity by mediating hydrogen peroxide generation in inducing oxidative stress in bacteria.[1] Alkaloids are capable of suppressing oral cavity pathogens by inhibiting ATP-dependent transport of compounds across the cell membrane.[5] Essential oils in *Cymbopogon nardus* L. possess antibacterial activity against *E. coli* and *Salmonella thypimurium.*[6] Previous studies reported that *Cymbopogon* has antibacterial properties against *Streptococcus* and *Staphylococcus*. The results showed that the growth of *S. mutans* bacteria began to be inhibited at a concentration of 6% and *S. aureus* began to be stunted at 1.6% concentration of *Cymbopogon nardus* L. [7,8] Besides the several types of bacteria above, *Enterococcus faecalis* is also found as pathogens that cause failed root canal treatment.

Enterococcus faecalis (E. faecalis) is one type of bacteria often found in root canals with periradicular inflammation. It has a good adaptation in unfavorable conditions and commonly found as resistant bacteria that cause endodontic treatment failed. Irrigation solutions commonly used in root canal treatments include sodium hypochlorite, ethylene diamine tetraacetic acid, chlorhexidine, and iodine potassium iodide. *Enterococcus faecalis* is able to suppress lymphocyte action and contribute to the failure of root canal treatment potentially. Cytoplasmic extracts of *E. faecalis* strain ATCC 29212 can suppress human lymphocyte activation to in vitro mitogenic stimulation, and this suppression is concentration-dependent. The component in the bacterial cultures responsible for this effect, and the SDS-PAGE reveals that the active factor of this extract consists of a 60-kDa polypeptide. When lymphocytes were pre-exposed to this bacterial protein, a decreased proliferative response of the lymphocytes was observed compared with the mitogen-activated lymphocyte proliferation. SEF perturbs the lymphocyte proliferation by blocking cell cycle transition from G0/G1 to S phase. The specific mode and site of action of the SEF in cell cycle progression have yet to be determined; however, it is plausible that immunosuppression mediated by *E. faecalis*, like that mediated by a number of other pathogens, involves pertubation of cell cycle regulatory protein.[9] Antibacterial activity of *Cymbopogon nardus* L. against *E. faecalis* has never been done, so the aim of this research was to determine the antibacterial activity of *Cymbopogon nardus* L. in inhibiting the growth of *Enterococcus faecalis*.

MATERIALS AND METHODS

This study has been approved by the Research Ethics Commission of Mohammad Hoesin General Hospital, Palembang and Medical Faculty of Universitas Sriwijaya with certificate No.0098/kepkrsmhfkunsri/2018. This research is an experimental laboratory study. Thirty samples were divided into 6 groups; Group 1,2,3,4,5 consisted of 1%, 3%, 5%, 7%, and 9% citronella extract respectively, and Group 6 was positive control using 5% NaOCl. Samples were *Cymbopogon nardus* L. with age ranging from

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75-90 days because at this age range, *Cymbopogon nardus* L. reaches maximum contents.[10] Replication was carried out 4 times.

Plant Extraction Preparations

Cymbopogon nardus L. was watery washed, rinsed, cut into small pieces, and dried in indirect sunlight until it got simplisia. Simplisia were mashed into fine powder (30 mesh), put into Erlenmeyer flasks and soaked in 70% ethanol with stirring for 48h. The extract was filtered through Whatmann filter paper no. 41 to separate the filtrate and residue. The filtrates were evaporated (DLAB Scientific Co., Ltd, USA) at 40-50^0 C to obtain pure extract of 100%. The extract was dilute to obtain concentrations of 1%, 3%, 5%, 7%, and 9% respectively.

Preparation of *Enterococcus faecalis*

Enterococcus faecalis ATCC 29212 was put into a test tube containing Brain Heart Infusion (BHI) (Merck, Indonesia), screened at a density of 1 x 10^{-3}, then bacteria were taken using 1 cc micropipette and dripped into a petri dish which contained blood agar media, flattened by shaking the petri dish until the liquid was evenly distributed, allowing 30 minutes to freeze.

Determination of the inhibition zone

Determination of inhibitory zone was performed using *Kirby Bauer* disc method. Petri dishes containing *E. faecalis* in the media completed with six-cylinder filter papers were prepared. Filter papers were set ± 20-25 mm in distance. All discs were cultured overnight for 24 hours at 37^{0}C. The antibacterial assay was evaluated the next day. The observation was repeated 4 times.

Data analysis

Data were analyzed using SPSS 22 vs. (IBM® Inc.pvt ltd.) and Microsoft Excel (Microsoft Inc®). Data were subjected to one-way analysis of variance (ANOVA) and differences between samples were determined by Post Hoc LSD. *P* values less than 0.05 were considered statistically significant.

RESULT

The evaluation of inhibitory zone was shown as Figure 1 and the results of antibacterial assay were shown on Figure 2.

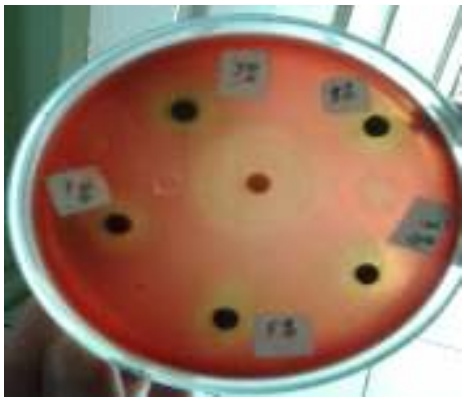

Figure 1. Inhibition zone of 1%, 3%, 5%, 7%, and 9% *Cymbopogon nardus* L. against *E. Faecalis.*

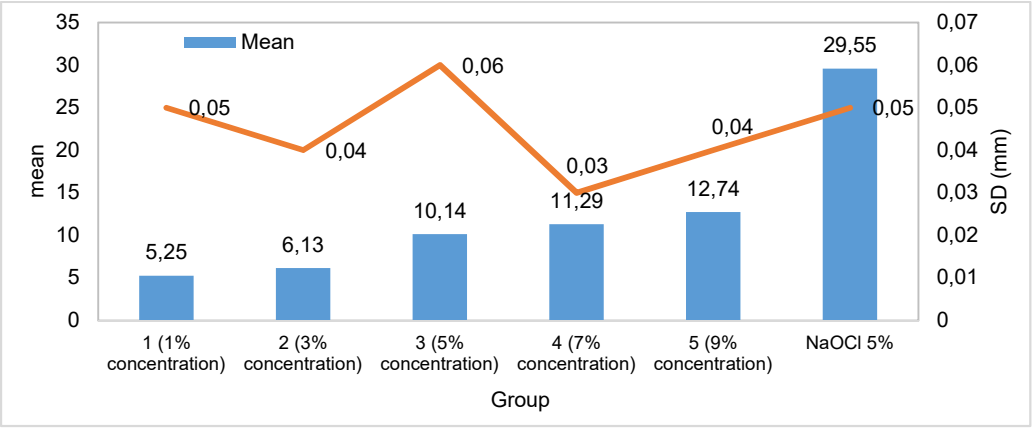

Figure 2. Means of inhibitory zone of *Cymbopogon nardus* L. extract.

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Table 1. Antibacterial effect of *Cymbopogon nardus* L. extracts

	Group 1	Group 2	Group 3	Group 4	Group 5	NaOCl 5%
Group 1		0.00*	0.00*	0.00*	0.00*	0.00*
Group 2			0.00*	0.00*	0.00*	0.00*
Group 3				0.00*	0.00*	0.00*
Group 4					0.00*	0.00*
Group 5						0.00*
NaOCl 5%						

Post Hoc LSD, p<0,05=significant

Figure 2 described that citronella extract with 1% concentration has antibacterial activity. The effect rise by increasing the concentration of *Cymbopogon nardus* L. in which 9% extract had the best effect among other concentrations. NaOCl of 5% had the highest antibacterial effect compared to others.

One-way ANOVA was used to know the difference among all groups, and the result was p<0.00. It meant that there was a significant difference in antibacterial activity of *Cymbopogon nardus* L. against *E. faecalis*. The test was continued with Post Hoc LSD (Table 1).

Table 1 exhibited that there was a significant difference in antibacterial activity among all groups. It showed that concentration of 9% showed the highest zone of inhibition, but it was not as effective as NaOCl 5%.

DISCUSSION

The results showed that *Cymbopogon nardus* L. extract in all concentrations had antibacterial activity. Ukwubile *et al.* [11] reported that Cympogon possessed antibacterial activity with more potency on Gram-positive bacteria than Gram-negative bacteria strains. It was due to the content of the active compounds of *Cymbopogon nardus* L., such as alkaloids, saponins, polyphenols, flavonoids, and essential oils that have antibacterial properties.

Those compounds synergistically lead disruption of membrane integrity, impaired ion balance, membrane leakage, and finally cause cell death.[12] They also have ability to interact with Gram-positive bacterial DNA, damage the double helical structure at the stage replication and transcription, and disrupt bacterial metabolism and growth.[13]

Alkaloids act as antibacterial because of their ability to interact with bacterial DNA, which is to damage the double helix structure at the replication and transcription stages, thereby disrupting metabolism and bacterial growth and finally cell lysis.[14]

Polyphenols are active components that have antibacterial properties. Polyphenols are able to damage bacterial cell walls that have peptidoglycan and inhibit protein synthesis. Flavonoids and essential oils can inhibit bacterial growth by disrupting the permeability of bacterial cell walls.[14]

Manosalva *et al.*[14] found that alkaloids possessed significant antibacterial effects against Gram-positive bacteria strains. Ravi *et al.*[15] reported that saponins were potential as antibacterial activity. Su *et al.*[16] stated that polyphenols contained in some natural plants were capable of killing various types of Gram-positive and Gram-negative bacteria strains, and certain types of bacteria resistant. Alghazeer *et al*.[17] explained that flavonoids were effective as antimicrobials against various microorganisms, because it might have multiple cellular targets, formed complex with proteins, inactivated enzymes and adhesion. Chouhan *et al*.[18] reviewed that essential oil had wide bioactivities as antimicrobial and had been used as alternative medical treatment against multidrug resistance.

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In this study, 9% concentration of *Cymbopogon nardus* L. extract showed the greatest inhibitory zone of *E. faecalis*. The increasing concentration of *Cymbopogon nardus* L. extract showed the higher inhibitory zone of *E. faecalis.* This characteristic is called as dose-dependent effect. The increased concentration resulted in the increased active compounds contained in *Cymbopogon nardus* L. so that the antibacterial activity against *E. faecalis* was also increased.

All concentrations of *Cymbopogon nardus* L. extracts and NaOCl 5% had a significant effect in inhibiting the growth of *E. faecalis*. The difference was because the content of antibacterial substances of 9% was not the same as 5% NaOCl. Zand *et al.*[19] reported that NaOCl was superiorly effective against *E. faecalis* in the root canal system. Sodium hypochlorite (NaOCl) is the most important irrigant in root canal treatment, It is the only presently used solution that can dissolve organic matter in the canal.

Therefore the use of hypochlorite is of utmost importance in removing necrotic tissue remnants as well as biofilm. NaOCl ionises in water into sodium (Na^+) and the hypochlorite ions, OCl^-, and establishes an equilibrium with hypochlorous acid (HOCl). At acidic and neutral pH, most of the chlorine exists as HOCl, whereas at pH of nine and above, OCl^- is most abundant.[20]

Hypochlorous acid has the strongest antibacterial effect while the OCl^- ion is less effective. Hypochloric acid affects directly on the vital functions of the microbial cell, rapidly resulting in cell death.[20] In this study, the concentration of *Cymbopogon nardus* L. extracts used was only 1%, 3%, 5%, 7% and 9%.

Based on the results of this study that the greater the concentration, the antibacterial effect on *E. faecalis* is increasing, so that further research is needed in different concentrations. Further study is needed to find the best concentration in inhibiting the growth of *E. faecalis* effectively.

CONCLUSION

Cymbopogon nardus L. extract had antibacterial activity against *Enterococcus faecalis.* The higher the concentration of *Cymbopogon nardus L*. extract, the higher the antibacterial activity possessed.

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RESEARCH ARTICLE

Impact of the Covid-19 Pandemic on Anxiety Levels in Dental Clinic A.W. Sjahranie General Hospital Samarinda

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ABSTRACT

Background: *The Covid-19 is a viral infection caused by the novel coronavirus and interpersonal transmission occurs mainly via respiratory droplets and contact transmission. In some countries, the recommendations of the dental associations are to interrupt elective dental treatments, so only emergency or urgent cases are allowed. However, many patients are not aware of whether or not to attend their appointments at the dental clinic. Most of the patients are anxious but some patients feel calm to seek dental treatment.* ***Purpose:*** *This research is to find out the impact of the Covid-19 pandemic on anxiety levels and patient's visit in Dental and Oral Clinic, A.W. Sjahranie General Hospital Samarinda.* ***Materials and Method:*** *This research was designed by the descriptive observational approach using a cross-sectional research design. The samples were new patients of child and adult who visited Dental and Oral Clinic, A.W. Sjahranie General Hospital Samarinda. The research was conducted through questionnaires to measure anxiety levels about Covid-19 pandemic conditions and the average anxiety level of patients associated with "stay at home" recommendation and their impact on dental care success.* ***Result:*** *The questionnaires were answered by 55 patients (23 male/42% and 32 female/58%) who visited the Dental and Oral Clinic, A.W. Sjahranie General Hospital Samarinda. The average level of patient anxiety for Covid-19 pandemic conditions is 4.87 for male patients and 4.42 for female patients. The level of anxiety about the impact of the Covid-19 pandemic on dental care is about the same in both men and women.* ***Conclusion:*** *Male patients are more anxious than women about the Covid-19 pandemic. But both showed similar anxiety about how pandemic conditions could affect their dental care.*

Keywords: *Anxiety levels, Covid-19 pandemic, dental treatment.*

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INTRODUCTION

In early 2020, the world was rocked by the presence of a severe infection with an unknown cause. Starting from a report from China to the World Health Organization (WHO), there were 44 severe pneumonia patients in a city area of Wuhan, Hubei Province, China, at the end of 2019.[1] The alleged discovery of the case relates to a wet market selling fish, marine animals and various other animals. On January 10, 2020 the cause began to be identified and results showed the presence of coronavirus infection which is classified as a new type of betacoronavirus.[2] At the end of January 2020, the World Health Organization (WHO) named the new virus Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) and its disease name as Coronavirus disease 2019 (Covid-19) and established the status of a global pandemic given the massive spread and severity in all countries.[3,4] Coronavirus is zoonotic, so it is possible that the virus originates in animals and is transmitted to humans.[5,6] SARS-CoV-2 is currently thought to be strong with evidence from various studies resulting in transmission through aerosols or droplets containing viruses or direct contact with mucous membranes, mouth fluids, instruments and surfaces contaminated with the virus.[7,8]

Dentistry is an action that has the potential to allow the transmission or cross-infection of various diseases because it is always exposed to saliva and blood.[9,10] In some countries, dentists are encouraged to delay elective dental care so that it is prioritized to handle urgent and emergency situations.[11] This began on March 16, 2020, when the American Dental Association (ADA) recommended dentists to delay all elective procedures. The ADA made guidelines on conditions that can be considered as dental emergencies and urgencies.[12] In dental emergencies, the ADA guidelines divide into two, the first being a potentially life-threatening dental emergency and requiring immediate treatment such as stopping ongoing tissue bleeding and reducing severe pain or infection. The second is dental urgencies which is a condition that requires immediate attention to relieve severe pain and or the risk of infection.[13]

In Indonesia, Persatuan Dokter Gigi Indonesia (PDGI) issued a circular letter calling for a temporary halt to dental care practices until the condition recovers. If it is not possible to suspend, then practical activities should only serve dental and oral emergency measures using complete Personal Protection Equipment (PPE following with the recommendations issued by the PDGI. During this situation, the patient is still unsure about whether or not to visit the dentist. This can be seen in the patient's visit to the dentist including to the Dental and Oral Clinic, A.W. Sjahranie General Hospital Samarinda. Patients are still visiting the clinic even though the case is classified as elective cases. For example, the number of Oral Surgery cases with teeth extraction or odontectomy is still relatively high. Most of the patients are anxious and worried if they need dental treatment. However, some patients feel calm and not too disturbed by the news in various media about the risk of transmission of Covid-19 infection. The purpose of this study is to find out the impact of the Covid-19 pandemic on anxiety levels and patient's visits in Dental and Oral Clinic, A.W. Sjahranie General Hospital Samarinda.

MATERIALS AND METHODS

This research has been submitted ethical clearance to the Research Ethics Commission A.W. Sjahranie General Hospital Samarinda. This type of research is descriptive analytics with cross-sectional study design. The subjects of the study were new patients of child and adult who visited Dental and Oral Clinic, A.W. Sjahranie General Hospital Samarinda in the period October - November 2020 (1 month). Sampling techniques using total sampling techniques. This research uses instruments in the form of questionnaires (Figure 1). Anxiety levels about Covid-19 pandemic conditions and the average anxiety level of patients associated with "stay at

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home" recommendation and their impact on dental care success were evaluated on a numerical rating scale, with a score scale of 0 meaning no anxiety and 10 extreme anxious. The data obtained was then analyzed using Chi-square, one-way ANOVA, and Tukey's tests with a $p < 0.05$.

RESULTS

In this study 55 patients visited the Dental and Oral Clinic, A.W. Sjahranie General Hospital Samarinda and filled out a questionnaire within 1 month, with the number of male patients 23 people (42%) and female patients 32 people (58%) (Figure 2). The youngest male patient is 16 years old and the oldest is 64 years old, as well as the youngest female 16 years old and the oldest is 60 years old. The average age of both male and female patients is 36 years and there are 2 male patients (8.7%) who have or are experiencing symptoms of Covid-19.

Question number 5 is the patient's attitude towards "stay at home" recommendation. Results showed there were 8 patients (14.5%) who followed the appeal always at home only, 35 patients (63.6%) who left home for urgent needs, and as well as 12 patients (21.8%) who went outdoors as usual.

Question number 6 is the activity of patients who are studying or working either offline or online. A total of 19 patients (34.5%) went out for school or work, 20 patients (36.4%) school and work online, as well as 16 patients (29.1%) school or work.

Question number 7 is the level of anxiety of patients described with feelings towards "stay at home" recommendation and the condition of the Covid-19 pandemic. Results showed 63.6% of patients felt calm, 21.8% of patients felt anxious, 9.1% of patients were afraid, 1.8% of patients felt panic, and 3.6% of patients did not care about the "stay at home" recommendation and the condition of the Covid-19 pandemic.

Question number 8 is the level of patient anxiety about the Covid-19 pandemic condition which is assessed on a scale of 0 (not anxious) of 10.9%, scale 1 by 10.9%, scale 2 by 3.6%, scale 3 by 5.5%, scale 4 by 5.5%, scale 5 by 32.7%, scale 6 by 3.6%, scale 7 by 10.9%, scale of 8 by 9.1%, scale 9 by 3.6%, scale 10 (very anxious) as much as 3.6% (Figure 5). The average level of patient anxiety for Covid-19 pandemic conditions is 4.87 (SD 2.943) for male patients and 4.42 (SD 2.722) for female patients.

Question number 9 is a type of case in Dental and Oral Clinic, A.W. Sjahranie General Hospital Samarinda. Patients who visited had complaints about tooth extraction (oral surgery) of 30 patients (54.5%), and at least 1 patient (1.8%) who wanted treatment for oral ulcers complaints.

Question number 10 is the patient's willingness to continue dental treatment after being scheduled by the dentist. Results showed there were 45 patients (81.8%) who agreed to come for follow-up, 2 patients (3.6%) who will not come for follow-up care, and 8 patients (14.5%) for follow-up treatment if urgent or emergency only.

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1. How old are you ? ..... years
2. Gender : ( ) Male ( ) Female
3. Where do you live ? City :
4. Have you had or are you experiencing symptoms of **COVID-19** ?
( ) Yes ( ) No
5. How are you complying "STAY AT HOME" recommendation in **COVID-19** pandemic?
( ) I don't leave home for nothing
( ) I leave home as little as necessary to buy food or medicine
( ) I go out normally to do several activities
6. Do you work or study?
( ) Yes, I am going out for work or study
( ) Yes, but I am working or study from home (online)
( ) I do not work or study
7. Considering the general anxiety level, how are you feeling about "STAY AT HOME" recommendation and **COVID-19** pandemic?
( ) Calm
( ) Anxious
( ) Fear
( ) Panic
( ) Indifferent
8. In a scale from 0 to 10, how is your anxiety with **COVID-19** pandemic? (0 = no anxiety, 10 = extreme anxiety)
( ) 0 ( ) 6
( ) 1 ( ) 7
( ) 2 ( ) 8
( ) 3 ( ) 9
( ) 4 ( ) 10
9. What kind of your dental treatment?
( ) I don't know, I was reffered by other clinic (example : radiotherapy, otolaringology, thorax and cardiovascular surgery)
( ) Orthodontic treatment
( ) Restorative treatment (teeth filling)
( ) Endodontic treatment (root canal treatment)
( ) Pedodontic treatment
( ) Oral surgery treatment (teeth extraction, odontectomy)
( ) Oral ulcer treatment
( ) Scalling
10. If you dentist got in touch to schedule an appointment, would you accept/go ?
( ) Yes
( ) Only in case of urgency or emergency
( ) No
11. What is your concern of attending a dental appointment in **COVID-19** pandemic?
( ) The risk of contaminating is high
( ) My treatment is not urgent
( ) No concern
12. What are your concerns about "STAY AT HOME" recommendation can affect ongoing dental treatments?
( ) It will delay the treatment, I was anxious for the result
( ) I am afraid of losing the investment (time or money)
( ) I am not worried
13. In a scale from 0 to 10, how is your anxiety regarding the impact of **COVID-19** pandemic and "STAY AT HOME" recommendation in your treatment ? (0 = no anxiety, 10 = extreme anxiety)
( ) 0 ( ) 6
( ) 1 ( ) 7
( ) 2 ( ) 8
( ) 3 ( ) 9
( ) 4 ( ) 10
( ) 5
14. What do you consider important in Dental and Oral Clinic nowadays?
( ) Personal Protective Equipment for dentists (mask, face shield, and disposable lab coat)
( ) Social distancing in waiting room
( ) Personal Protective Equipment for patients (mask, face shield, and disposable lab coat)
( ) Desinfectant (alcohol gel) in waiting room

Figure 1. Questionnaire applied to patients

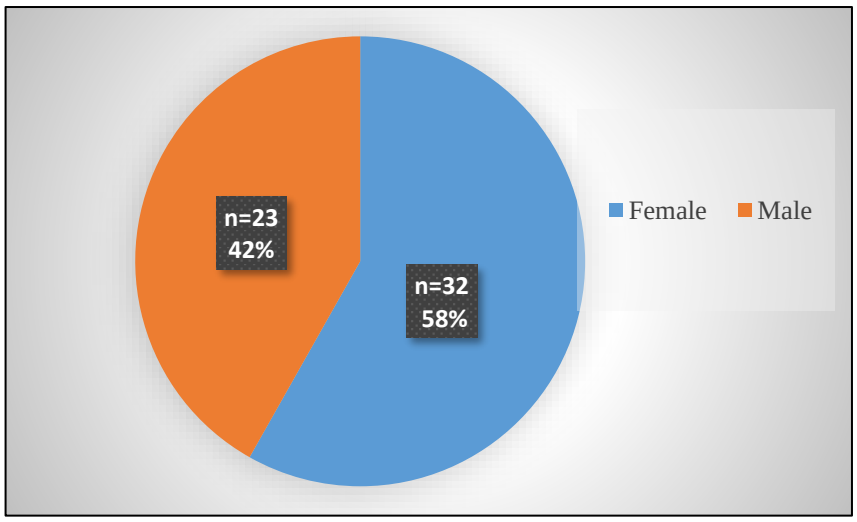

Figure 2. Number of patients

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Question number 11 is a factor that causes patients to come to the Dental and Oral Clinic, A.W. Sjahranie General Hospital Samarinda during the Covid-19 pandemic. There were 23 patients (41.8%) who did not feel worried or anxious at all about his visit to our clinic. The reasons for consideration of the risk of transmission in the clinic are quite high and urgent or emergency dental treatment has a balanced distribution of 16 patients (29.1%).

Question number 12 is a patient's opinion about "stay at home" recommendations that can affect ongoing dental care. Results showed there were 22 patients (40%) expressing fear that the loss of investment in their care or dental condition could worsen, 21 patients (38.2%) feeling "stay at home" may delay dental care so they are more worried if the results are not satisfactory, and 12 patients (21.8%) who are not worried.

Question number 13 is the scale of patient anxiety level against the Covid-19 pandemic that can affect the success of dental treatment. Results showed the presence of 15 patients (27.3%) with moderate anxiety levels (scale 5). The average level of patient anxiety towards the Covid-19 pandemic condition that affects the success of dental treatment is 4.78 (SD 3.074) for male and 4.47 (SD 2.565) for female.

Question number 14 is the opinion of patients about essential points facing the Covid-19 pandemic in Dental and Oral Clinic, A.W. Sjahranie General Hospital Samarinda. There is the presence of Personal Protection Equipment for doctors as many as 26 patients (47.3%), the presence of Personal Protection Equipment for patients as many as 10 patients (18.2%), avoiding crowds with other patients in the waiting room as many as 15 patients (27.3%), and the provision of alcohol disinfectant solutions in the waiting room of clinic as many as 4 patients (7.3%).

Men are more adhering to the "stay at home" recommendation than women. Men and women have a sense of calm towards "stay at home" recommendations and the condition of the Covid-19 pandemic. The level of anxiety about the Covid-19 pandemic is significantly greater in men than women. Male patients are more willing to come on the next visit to the Dental and Oral Clinic. All male and female patients have similar perceptions that "stay at home" can affect dental care, which is worrying about the success of their dental care. The level of anxiety about the impact of the Covid-19 pandemic on dental care is about the same in both men and women (Table 1).

Patients who have feelings of calm, anxiety, fear, and indifferent about the "stay at home" recommendation and the covid-19 pandemic condition want to come to the next dental care. Patients who agreed to come to the next dental care control whether it was an emergency or not showed a higher anxiety score than patients who did not want to come under control (Table 2).

Table 1. Comparison between men and women

Question	Male (N = 23)	Female (N = 32)	*P*
5.How are you complying with the "STAY AT HOME" recommendation in **COVID-19** pandemic?			
( ) I don't leave home for nothing ( ) I leave home as little as necessary to buy food or medicine ( ) I go out normally to do several activities	2 (8.7%) 17 (73.9%) 4 (17.4%)	6 (18.75%) 18 (56.25%) 8 (25%)	*P* = .00 2[a,*]
7. Considering the general anxiety level, how are you feeling about the "STAY AT HOME" recommendation and **COVID-19** pandemic?			
( ) Calm ( ) Anxious ( ) Fear ( ) Panic ( ) Indifferent	13 (56.6%) 5 (21.7%) 3 (13%) 0 (0%) 2 (8.7%)	22 (68.7%) 7 (21.9%) 2 (6.3%) 1 (3.1%) 0 (0%)	*P* = .01 2[a,*]
8. On a scale from 0 to 10, how is your anxiety with **COVID-19** pandemic?			
	Mean (SD) 4.87 (2.943)	Mean (SD) 4.42 (2.722)	*P* = .01 1[T,*]
10. If your dentist got in touch to schedule an appointment, would you accept/go ?			
( ) Yes	21 (91.3%) 2 (8.7%)	24 (75%) 6 (18.75%)	

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( ) Only in case of urgency or emergency ( ) No	0 (0%)	2 (6.25%)	*P* = .031[a,*]
12. What are your concerns about whether the "STAY AT HOME" recommendation can affect ongoing dental treatments?			
( ) It will delay the treatment, I was anxious for the result ( ) I am afraid of losing the investment (time or money) ( ) I am not worried	8 (34.8%) 10 (43.5%) 5 (21.7%)	13 (40.6%) 12 (37.5%) 7 (21.9%)	*P* = .027[a,*]
13. On a scale from 0 to 10, how is your anxiety regarding the impact of **COVID-19** pandemic and "STAY AT HOME" recommendation in your treatment?			
	Mean (SD) 4.78 (3.074)	Mean (SD) 4.47 (2.565)	*P* = .008[T,*]
14. What do you consider important in Dental and Oral Clinic nowadays?			
( ) Personal Protective Equipment for dentists (mask, face shield, and disposable lab coat) ( ) Social distancing in waiting room ( ) Personal Protective Equipment for patients (mask, face shield, and disposable lab coat) ( ) Desinfectant (alcohol gel) in waiting room	13 (56.5%) 6 (26.1%) 4 (17.4%) 0 (0%)	13 (40.6%) 9 (28.1%) 6 (18.8%) 4 (12.5)	*P* = .005[a,*]

[a]Chi-square test
[T]Independent T-Test
[*] Statistically significant for *P* < 0.05

Patients obeying the "stay at home" recommendation showed much lower levels of anxiety about the covid-19 pandemic condition and its impact on the success of dental treatment (Table 3).

Pearson's Correlation value of -0.235 indicates weak correlation strength and negative correlation. This indicates an inversely proportional correlation, meaning that the lower the age, the higher the anxiety level for Covid-19 (P = .002). Pearson's Correlation value of -0.256 indicates weak correlation strength and negative correlation. This indicates an inversely proportional correlation, meaning that the lower the age, the higher the level of anxiety that affects dental treatment during the Covid-19 pandemic (P = .002) (Table 4).

Table 2. Comparison between patient willingness to come care control and feelings towards Covid-19 pandemic conditions

Answers	Yes N (%)	No N (%)	Yes, if emergency N (%)	*P*
Calm Anxious Fear Panic Indifferent	27 (60%) 12 (26.67%) 4 (8.89%) 0 (0%) 2 (4.44%)	1 (50%) 0 (0%) 0 (0%) 1 (50%) 0 (0%)	7 (87.75%) 0 (0%) 1 (12.5%) 0 (0%) 0 (0%)	*P* = .000[*,**]
Level of anxiety/ Covid-19	Mean (SD) 4.73 (2.864)[a]	Mean (SD) 2.50 (3.536)[a]	Mean (SD) 4.38 (2.387)[b]	*P* = .000[*,T]
Level of anxiety/ impact on dental treatment	Mean (SD) 4.69 (2.827)[a]	Mean (SD) 2.00 (3.142)[a]	Mean (SD) 4.75 (2.605)[b]	*P* = .000[*,T]

Note : Different lowercase letters in the same row indicate the presence of a statistically significant difference by Tukey's test.
*Statistically significant for P < .05.
**Chi-square test.
†One-way ANOVA test.

Table 3. Comparison of the observance of "stay at home" recommendation and anxiety about the condition of the Covid-19 pandemic and its impact on the success of dental treatment

Levels of anxiety	Do not leave home N (%)	Stay home as much as possible N (%)	Go out normally N (%)	*P*
Level of anxiety/ Covid-19	3.25 (11.6)[a]	4.66 (63.6)[a]	5.33 (21.8)[b]	*P* = .000[*]
Level of anxiety/ impact on dental treatment	2.75 (21.8)[a]	5.09 (63.6)[a]	4.42 (11.6)[b]	*P* = .000[*]

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Note : Different lowercase letters in the same row indicate the presence of a statistically significant difference.
*Statistically significant for P < .05

Table 4. Correlation between patient age and anxiety levels about Covid-19 pandemic conditions and their impact on success of dental treatment

Correlations	r	*P*
Age x Level of anxiety/ Covid-19	- .235	*P* = .002
Age x Level of anxiety/ impact on dental treatment	- .256	*P* = .002

DISCUSSION

All countries including Indonesia have been affected by the Covid-19 pandemic which has lasted almost 1 year since the emergence of its first case in Wuhan, China. This research was conducted after 10 months of the Covid-19 pandemic. Patients visiting Dental and Oral Clinic, A.W. Sjahranie General Hospital Samarinda seems to comply with the social distancing appeals and recommendations that have been set by the government as one of the efforts to prevent the spread of Covid-19. This can be shown with as many as 63.6% of patients leaving home only when they are urgent because they have to work, buy food, or seek treatment.

The internet, mass, and social media are currently considered a tool of finding health education and information. However, social media also can be spreading rumors and misinformation, causing people to panic and confuse [14]. The public can access many pieces of information about the current global situation including the number of deaths due to the Covid-19 outbreak [15]. Related to the condition, there will be a close relationship between the use of mass and social media with anxiety [16]. In this study, as many as 63,6 % of patients feel calm during this pandemic situation. They seemed calm, but the level of anxiety of men was even a little higher against the Covid-19 pandemic condition. This condition is different from Brazil and China in which is male patients appear calmer and not worried about the Covid-19 pandemic condition.[17] In our research, the average patient's anxiety level for the Covid-19 pandemic is about scale of 5, which indicates moderate anxiety levels.

As recommended by the World Health Organization and Persatuan Dokter Gigi Indonesia (PDGI), all elective dental care for all individuals should be deferred during the pandemic crisis. But dentists must be available to their patients for urgent and emergency care. This research found that dental caries and third molar impaction (oral surgery cases) was the most common dental problem during the Covid-19 pandemic in A.W. Sjahranie General Hospital Samarinda. Most patients (81.8%) states will come for follow-up care. Male patients have more cooperativeness to come to Dental and Oral Clinic than female patients, perhaps because men spend most of their time working either offline or online so when there is time to continue dental care it will actually take the time to seek treatment. Concerns about the success of dental care in men and women are not much different: they have the same fear that the results are unsatisfactory, indicated by a very small scale (Table 1). Patients who follow the "stay at home" recommendation have much lower levels of anxiety about the Covid-19 pandemic and their impact on the success of dental care. It can be explained that they are only at home so they feel safe and not too worried about the Covid-19 pandemic condition and so does the impact on the success of dental care that has been done.

Patients are more worried about delays in completing treatment because they expect the shortest duration of dental care possible. Patients also feel they have sacrificed time and money for dental care so that they can be cooperative to continue their dental care. Moreover, the presence of complaints especially tooth extractions or dental surgery is quite high resulting in the patient's willingness to carry out dental care. Inability to withstand dental pain outsized by anxiety over the pandemic situation

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itself so that patients ventured to come for dental treatment. The use of Personal Protection Equipment for dentists such as surgical masks, face shields, and surgical gowns and avoiding crowds with other patients in the waiting room is the most important precaution considered by patients to avoid contamination of Covid-19 in Dental and Oral Clinic, A.W. Sjahranie General Hospital Samarinda.

This research also obtained data that higher the level of anxiety about the Covid-19 pandemic in younger people compared to older ages. Older people tend to be less socially mobile than younger ones, especially those under 25 years of age, who are known to spend more time on social media and other news outlets.[18,19] Young participants may watch and listen to more negative news which will then intensify their feelings of anxiety and depression in times of crisis.[16] The level of adolescent anxiety during the Covid-19 pandemic is in a high category. This is in line with several previous studies.[20,21] It is necessary to conduct similar research with a greater number of samples.

CONCLUSIONS

"Stay at home" recommendation as a form of effort to prevent the spread of Covid-19 infection has an impact on the level of anxiety and patient's visiting who require dental and oral treatment. Patients who are willing to come or will only come in an emergency at advanced care show higher levels of anxiety than patients who do not want to come. Male patients are more anxious than women about the Covid-19 pandemic. But both showed similar anxiety about how pandemic conditions could affect their dental care. The increase in the number of confirmed cases of Covid-19 patients is increasing so that there needs to be innovations inpatient services that require initial treatment of patient complaints with teledentistry.

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CASE REPORT

Multidisciplinary Management of Recurrent Aphthous Stomatitis Triggered by Severe Depression

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ABSTRACT

Background: *Recurrent Aphthous Stomatitis (RAS) is an inflammation of the oral mucosa in the form of a single or more ulcer that occurs repeatedly. One of the predisposing factors that contribute to the onset of RAS is depression. Depression is a mood that is not happy or sadness that is experienced as part of the pattern of life.* ***Objective:*** *Discuss multidisciplinary management in cases of 65-year-old women with large multiple ulcers with depression-triggering factors.* ***Case:*** *Women aged 65 years complained of recurrent canker sores. The frequency of RAS increased by 10 years due to economic and household problems. It has been treated with topical non-steroidal anti-inflammatory drugs but does not heal. Does not have a history of systemic diseases.* ***Case Management:*** *Do investigations are complete peripheral blood examination, Depression Anxiety Stress Scale (DASS), Refer patients to the psychiatric specialist. Multidisciplinary treatment is performed by a psychiatric specialist. Treatment of oral mucosa with topical anti-inflammatory steroid drugs.* ***Conclusions:*** *in accordance with the treatment that has been given, this case has recovered after day 14. So it can be concluded that proper multidisciplinary management and elimination of triggering factors are the main roles to prevent recurrence and reduce the frequency of recurrent aphthous stomatitis.*

Keywords: *Recurrent Aphthous Stomatitis (RAS), Ulcer, Depression, Depression Anxiety Stress Scale (DASS)*

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INTRODUCTION

Recurrent aphthous stomatitis (RAS) is an inflammation of the oral mucosa that occurs repeatedly. RAS is one of the most common oral diseases. RAS is a reserve in the oral mucosa with a worldwide prevalence of 5-25%. RAS mostly occurs at the age of 10-40 years and many occur in women. The main characteristics of RAS are the presence of painful and reoccurring ulcers on the oral mucosa.[1,2,3]

RAS is divided into three types, minor, mayor and herpetiform. Minor RAS has characteristics such as small ulcers with a diameter of less than 4mm. This type of RAS has a healing process that lasts about 7-14 days without scarring. While Major RAS is a large ulcer with a diameter of more than 10 mm, and the healing process requires period of 2-12 weeks accompanied by scarring.The Herpetiform RAS is multiple ulcers numbering 10-100 ulcers with a diameter of about 1-2 mm.[4,5] The clinical appearance of RAS consists of round or oval, single or multiple, ulcers, yellowish- white understood as halo erythematous, painful and recurred.[6 i]

Until now the etiology of the RAS is unclear. The emergence of RAS is related to several triggering factors such as hematinic deficiency (iron, folate, and vitamin B12), hormonal, age, sex, hypersensitivity to food and medicine, family, trauma, nutrition, hormones, and stress.[4]Several studies have revealed that stress has a close relationship with RAS, where there is a relationship with cortisol and reactive oxygen species (ROS). The RAS also opposes changes in the immune system, namely modifications that affect many immune systems such as the distribution, proliferation and activity of lymphocytes and natural supporting cells, phagocytosis, and the production of cytokines and antibodies.[4,7] The purpose of this paper is to describe a case report about the management of recurrent Aphthous Stomatitis that is triggered by stress.

CASE

A 65-year-old woman came to the Dental Clinic of Universitas Airlangga Surabaya with chief complaints of sore mouth on the buccal mucosa referred from a poly prosthodontist. From the history, it is known that sore mouth appeared 3 days ago after being impress using alginate ingredients. Patients have a history of recurrent sore mouth since young, increasing in the last 10 years or so. The patient treats sore mouth by gargling warm salt water. The patient loses all of the maxillary and mandibular teeth beginning with cavities until the extraction of all her teeth. Have used dentures since 7 years ago but are loose and uncomfortable. The patient is a housewife who also works in a canteen. Patients feel they do not have allergies. The family has no history of allergies. The patient has no history of systemic disease.

The general condition of the patient looks thin and weak. Examination of vital signs of tension, pulse, respiration, and temperature normal. Extra-oral examination of the right submandibular gland feels soft and painless. The left submandibular gland is not palpable. There were no lesions or complaints in the eyes, skin, and genitals. Intra-oral examination of the buccal mucosa dextra appears ulcer, single, oval-shaped, size 3x9mm, central yellowish-white, clear borders, irregular edges, normal surrounding area, and pain. Based on the history and clinical examination, the working diagnosis of this case is Recurrent Aphthous Stomatitis triggered by trauma.

Figure 1. Single ulcer in the right buccal mucosa

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Patients were given symptomatic therapy containing steroids with topical cream prescription applied 4x per day (Triamcinolone Acetonide 0.1% in ora base). Information and Education Communication to patients to improve oral hygiene, instructions to use medication appropriately and regularly, patient instructions carefully with the location of the lesion, patient instructions to improve high-calorie and high-protein soft food diets, increase vegetable and fruit intake Get enough rest and rehydration. Patients were scheduled for control 7 days later.

In the first control, a sore mouth on the right inner cheek has slightly disappeared and not too painful. Two days ago a new sore mouth appeared on the upper inner cheek, appearing suddenly. The patient's claimed stress in economic matters and the husband who had an affair. Patients use oral medication regularly. Patient's diet is irregular, only 1 time a day, and rarely drinks water. The patient's rest is still lacking, the patient claimed to have difficulty sleeping.

Extra-oral examinations normal. Intra-oral examination showed the presence of ulcers, multiple, in the buccal mucosa dextra oval, with sizes 3x9 mm and 2x7mm, the central yellowish-white, clear boundary, irregular edges, the area around normal, pain. in the edentulous ridge anterior of the mandible there is an ulcer, round shape, measuring 5mm, yellowish-white, irregular edges, the area around normal, pain.

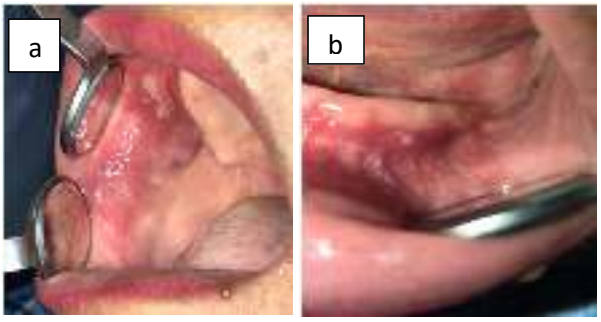

Figure 2. a. Multiple ulcers in the buccal mucosa dextra. b. Single ulcer in the lower jaw gingiva

For diagnosis, the patient is instructed to conduct a supporting examination, namely a complete peripheral blood examination, blood sugar examination during, examination of HBA1c, SGOT, SGPT, and Ig E Total. The patient was examined for Depression Anxiety Stress Scale (DASS).The results of blood tests showed high blood sedimentation rates, eosin, lymphocytes, total IgE, and HBA1c. While the segment, MCH, and MCHC are low. Other blood tests appear normal. Based on the results of the DASS scoring examination the patient is in a state of severe depression, very severe anxiety and, severe stress. Based on the history, clinical examination, and supporting examination to eat a working diagnosis of this case is Recurrent Aphthous Stomatitis that is triggered by stress.

Patients were given symptomatic therapy containing steroids with topical cream prescription applied 4x per day (Triamcinolone Acetonide 0.1% in ora base). Patients are also prescribed multivitamins once a day. Information and Education Communication to increase oral hygiene, instructions to use medication appropriately and regularly, patient instructions to avoid contact with lesion locations, patient instructions to improve high-calorie and high-protein soft food diets, increase vegetable and fruit intake, eat slowly, adequate rest, and rehydration. Calms the patient regarding the problem being faced. Patients were referred for prick tests and referred to a medical specialist (Psychiatrist). Patients were scheduled for control 7 days later.

The second control (14 days), sore mouth the edentulous ridge and buccal mucosa has healed. Oral ointments are used routinely 3 times a day. But because it was cured the patient stopped using the drug himself. The patient had gone to a psychiatrist, the patient was diagnosed with a Severe Depression Episode and was prescribed Fluoxetine, Zipren and Clobazam. Patients consume drugs according to the rules and regularly. Extra-oral examinations appear normal, body temperature normal, palpation of the left and right submandibular glands appear

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normal. The normal intra-oral examination has no lesions.

Figure 3. a Mucosa Right buccal & b. Lower anterior gingival normal

Based on the history, clinical examination, supporting examination and inter-clinical integration, the final diagnosis of this case is Aphthous Recurrent Stomatitis triggered by severe depression. Information and Education Communication to patients to increase oral Hygiene by cleaning the tongue, instructing patients to improve high-calorie and high-protein soft food diets, increasing vegetable and fruit intake, eating slowly, getting enough rest, and rehydration. Patients are encouraged to remain in control of a psychiatrist. If there are complaints in the oral cavity the patient is recommended to control again.

DISCUSSION

Stress can be considered as a psychophysiological process that is a product of evaluating certain situations to assess potential difficulties and the ability to overcome potentially harmful situations. Events that have the potential to be a threat are called stressors. The situation is obtained from experiences in daily life, including daily busyness. Stressors often occur from interaction with family, school environment or workplace. Stress can be in the form of mental/ psychological stress and physical stress, in this patient psychological stress occurs, and is supported by physical stress.[6] Based on duration, stress can occur acutely (minutes to hours and days), subacute (duration less than one month), or chronic (months to years). based on intensity, acute stress that has a high intensity can have longer lasting effects.[7,8,9] Based on the duration and intensity of stress experienced by the patient is recurring chronic stress.

Depression Anxiaty Stress scale (DASS) is a question designed to measure the level of negative emotions of depression, anxiety, and stress. The stress scale in the depression anxiety stress scale consists of 14 questions and is very sensitive to the level of non-specific chronic causes. The stress scale is used to measure the level of relaxation, the emergence of nervous disorders, the level of anger, excessive reaction, and the level of patience. The results of respondents answers based on stress felt by individuals that cause efforts to react to the stress they experience. Stress is a manifestation of the relationship between environmental events or conditions with an individual's cognitive assessment of the level, type of challenge, difficulty, loss, and threat. The reaction is an activity to adjust to certain stimulant situations, which if it cannot be done properly will cause physical and psychological disorders. In these patients, the DASS results were depression 21 (severe), anxiety 27 (very severe), and stress 23 (severe).[10,11]

In stressful conditions, the adrenal cortex will secrete cortisol which will cause a decrease in IFN-produksi production and increase IL-10 and IL-4 production which will trigger a change in the balance of pro-inflammatory and anti-inflammatory cytokines more towards the proinflammatory response. However, recent research states that it is this dysregulation of cytokine balance that plays an important role in linking the effects of stress on the immune system. There are several immune responses that occur in RAS, namely a decrease in the number of CD4 lymphocytes and changes in the ratio of CD4: CD8, decrease in regulation of CDreg CD4 Tregs, increase in B lymphocyte counts, increase in T cell counts, decrease HSP expression, increase in complement system, increase in NK cell counts , reactivation and hyperreactivity of neutrophils, decreased expression of anti-inflammatory cytokines produced by Th2 and TGFβ, increased

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expression of pro-inflammatory cytokines produced by Th1 and IL-2, IL-12, IFN-gamma and TNFα.[12,13]

In the humoral response, levels of salivary IgA in patients with RAS show an increase in the acute period and decrease in the period of regression and healing. In addition to IgA, there is an increase in IgG and IgM levels. IgG and complement work together to help each other as opsonin in antigen destruction. IgG has effective opsonin properties because phagocyte, monocyte and macrophage cells have receptors for the Fc fraction of IgG so that they can strengthen the relationship between phagocytes and target cells. These changes can lead to pathological conditions in the oral mucosal epithelial cells, so that epithelial cells are more sensitive to stimulation.[12]

The impact of stress on the immune system through the relationship between the brain and the immune system involves two main pathways, namely: Automone nervous system (ANS) and the-hyphophyseal-pituitary-adrenal (HPA) axis. Perception of stress causes activation of the HPA system which starts with secretion of corticotrophin releasing hormone (CRH). CRH induces secretion of adrenocortictrophic hormone (ACTH) by the anterior pituitary lobe. ACTH activates cortisol secretion by the adrenal cortex and catecholamines (adrenaline and noradrenaline) by the adrenal medulla. Catecholamines and cortisol suppress the production of IL-12 by antigen-presenting cells which are the main induction stimulus for cytokine TH1. Cortisol can also have a direct effect on TH2 cells thereby increasing IL-4, IL-10 and IL-13 production. The end result is an imbalance between TH1 and TH2. This condition is dominated by TH2 cells which will support the inflammatory response to release.[7,9,13]

Based on the history, clinical examination and supporting examination, the final diagnosis in this case is Stomatitis Aphtous Recurrent triggered by stress. The non-pharmacologic management in this case is referring the patient to a psychiatric specialist to be given therapy which is expected to reduce the level of depression as a triggering factor. In addition, topical oral drug therapy containing corticosteroids was given, namely triamcinolone acetonide. Acetonide is a moderate to high potency corticosteroid, a fluorinated prednisolone derivative and is considered a glucocorticoid with intermediate-actiglucocorticoids having anti-inflammatory effects. Glucocorticoids decrease the production of cytokines, chemokines, and increase the production of macrophage migration inhibitory factors. As an anti-inflammatory, glucocorticosteroids will pass through the membrane by diffusion. In cell membranes, glucocorticosteroids will be captured by their receptors (GR) and assisted by heat shock protein-90 (hsp90). As anti-inflammatory glucocorticosteroids can be through 2 pathways. The first pathway, glucocorticosteroids with receptors will directly activate anti-inflammatory proteins. the second path, will enter the nucleus. In the nucleus, it inhibits the transcription of NF-kB in producing inflammatory proteins. this is the reason we give Triamcinolone acetonide to suppress the inflammatory process and accelerate the healing process of the lesion3.[3,14,15]

CONCLUSION

RAS is an inflammation of the oral cavity that often occurs. RAS can be triggered by several factors, one of which is psychological stress which can alter the immune system and body tissues and reducing mucosal resistance. The main therapy is to eliminate the causative factors / predisposing factors and followed by the therapy needed to relieve pain and speed healing.

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RESEARCH ARTICLE

The Inhibition Effect of Green Microalga Ethanol Extract (Nannochloropsis Oculata) on The Number of Bacteria Streptococcus Mutans (In Vitro)

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ABSTRACT

Background: *Antibacterial is a substance produced by a microorganism, which has the ability to inhibit growth or kill other microorganisms. Antibacterial activity is measured in vitro to determine the potency of an antibacterial agent in a solution, its concentration in body fluids or tissues, and the susceptibility of certain microorganisms to certain concentrations of drugs.* ***Purpose:*** *The purpose of this research was to find the power of extract ethanol Nannocholoropsis oculata as an alternative antibacterial causes of the growth of the caries which is Streptococcus mutans.* ***Material and methods:*** *extract ethanol. Nannocholoropsis oculate divided into three parts of concentration 1,5%, 2% and 2,5%, Chlorhexidine 0,12% as a control, culture bacteri Streptococcus mutans with McFarland 0.5 standart. The metode used in this study was One way analysis of varians (ANOVA), but first it had to be a homogenius test and a normalitas test.* ***Result:*** *The result of this study showed that no barrier power at concentration 1,5%, 2% and 2,5%.* ***Conclucion:*** *The result showed no inhibition in the three observed groups of 1.5%, 2%, and 2.5% of extract ethanol Nannochloropsis oculate. This meant that this article cannot prove the inhibition characteristic of Nannochloropsis oculate. This may be because the concentration of the extract Nannochloropsis oculata used in this study was too small. Henceforth, it is necessary to conduct research with a higher concentration of extract. On the other hand, the positive control group, using 0.12% Chlorhexidine solution, showed an inhibition characteristic while the negative control group, using 1 % of DMSO showed no inhibition.*

Keywords: *Antibacterial, nannochloropsis oculata, streptococcus mutans*

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INTRODUCTION

Dental caries is an infectious disease that involves four main factors, namely host, substrate, microorganisms, and time. This disease occurs due to the continuous demineralization process of hard tooth tissue by organic acids that come from foods containing sugar and fermented by bacteria. The demineralization process takes place when the pH of saliva is below 5.5 or acidic. In this state, bacteria acidogenic causes of dental caries, such as Streptoccocus mutans and Lactobacillus reproduce rapidly.[1]

The pH value of saliva is inversely proportional, where the lower the pH value the more acid in the solution, conversely, the higher the pH value means the increase in base in the solution. At pH 7, there is no acidity or basicity of the solution, and this is called neutral. Bacterial growth occurs at the optimum salivary pH ranging from 6.5-7.5 and if the oral cavity pH of saliva is low (4.5-5.5) it will facilitate the growth of acidogenic germs such as Streptococcus mutans and Lactobacillus.[2]

The bacteria that can cause dental caries can be gram positive and gram negative both in the form of cocci and rods. Bacteria that play an important role in the formation of dental plaque and dental caries are bacteria from the genus Streptococcus, namely Streptococcus mutans bacteria.[3]

Streptococcus mutans is a cariogenic gram-positive bacterium because it is able to immediately form acids from distributed carbohydrates. These bacteria can thrive in an acidic environment and can stick to the tooth surface because of their ability to form extra cell polysaccharides. These extra cell polysaccharides mainly consist of glucose polymers which cause the plaque matrix to have a gelatin-like consistency, as a result of which the bacteria adhere to the teeth and stick to each other. Plaque gets thicker over time, so it will inhibit the function of saliva to carry out antibacterial activity.[4]

One of the efforts that can be done to prevent caries is a plaque control, especially in children, this is intended to minimize the incidence of dental caries in children. Plaque control can be done in two ways, namely by mechanical and chemical means. Plaque control by mechanical means, one example is by brushing teeth properly regularly every day. Meanwhile, chemical plaque control can be done by using mouthwash or toothpaste with antibacterial agents, because the occurrence of caries is related to the presence of bacteria.[5]

Antibacterial activity is measured in vitro to determine the potency of the antibacterial agent in solution, its concentration in body fluids or tissues, and the susceptibility of certain micro-organisms to certain concentrations of drugs. There are several factors that influence in vitro antimicrobial activity, namely environmental pH, medium components, drug stability, inoculum size, incubation time, and metabolic activity of microorganisms.[6]

Nannochloropsis oculata is a green single-celled (unicellular) microalgae that has a very simple morphology.[7] It is a non motile, non flagellated, and spherical, with a diameter of 2-4 µm.[8] It has the potential due to high growth rates and ease of cultivation even in unfavorable environmental conditions.[9]

The results showed that the Nannochloropsis oculata extract contained a compound derived from fat oxidation called oxylipin. Through these compounds various types of secondary metabolite compounds are produced including terpenoids, alkaloids, and flavonoids. These various compounds are antibacterial.[10] In the research of Yanuhar et al in 2011, it was stated that Nannochloropsis oculata extract was able to inhibit the growth of Vibrio alginolitycus bacteria at concentrations of 20%, 25%, 30% and 35% and had the ability to kill bacteria at a concentration of 40% of the colony.[11] In a study by Kafaie et al (2012), it was shown that Nannochloropsis oculata did not have acute and subchronic toxicity effects on plasma cells in mice at low to high doses.[12]

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MATERIALS AND METHODS

The material used in this study was Nannochloropsis oculata. N. oculata was obtained in the form of dry powder from Balai Perikanan Budidaya Air Payau, Situbondo, JawaTimur, Indonesia.[13]

Nannochloropsis oculata extracted by maceration method, then made into several concentrations, namely 1.5%, 2%, 2.5%. Chlorhexidine 0.12% as positive control.

In this study, samples of Streptococcus mutans bacteria were made from stock derived from culture by making Streptococcus mutans colony suspensions using Brain Hearth Infusion Broth (BHIB) in a test tube, incubated at 37 ° C for 24 hours in an anaerobic atmosphere and standardized equally. with a standard of 0.5 Mc Farland.

This type of research is true experimental laboratories. Because in this study, control was carried out on all variables that could affect the research process and results. In addition, the sampling in this study was carried out randomly.[14]

The research design in this study was the post test only control group design. The research object was divided into 4 groups. Group 1 was the positive control group, group 2,3, and 4 were the treatment groups that were given extracts of green microalgae (Nannochloropsis oculata) with concentrations of 1.5%, 2% and 2.5%.

RESULTS

Observation data in the clear zone against Streptococcus mutans bacteria colonies using calipers. Analysis of the data obtained was then tested using statistical tests with a significance level of 95% (p = 0.05) and then processed by the SPSS version 23 program. The data from the research results were analyzed descriptively.

Table 1. Table of Inhibitory Power Examination Results of the inhibition zone diameter of Nannochloropsis Oculata extract against the growth of Streptococcus mutans bacteria.

Sample	K+	K-	P1 (1,5%)	P2 (2%)	P3 (2,5%)
1	22,45	6,00	6,00	6,00	6,01
2	30,43	6,00	6,01	6,00	6,00
3	31,35	6,01	6,00	6,00	6,00
4	31,01	6,00	6,00	6,00	6,00

Table 2. The mean diameter of the inhibition zone of Nannochloropsis Oculata extract against the growth of Streptococcus mutans bacteria.

Group	N	Mean	Std. Deviation
K-	4	6.0025	0.005
K+	4	28.81	4.25698
P1	4	6.0025	0.005
P2	4	6	0
P3	4	6.0025	0.005
Total	20	10.5635	9.51188

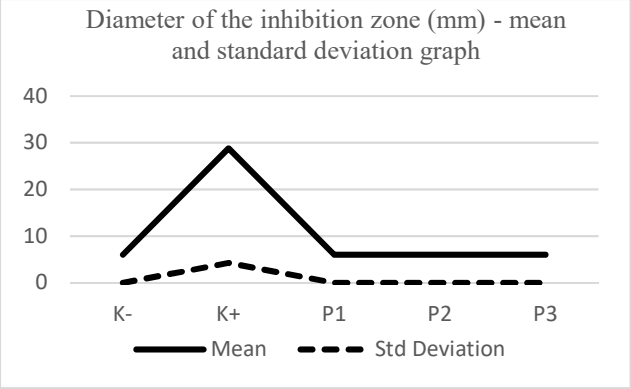

Figure 1. Diameter of the inhibition zone (mm) - mean and standard deviation graph.
K + (positive group / Chlorhexidin 0.12% solution); K- (negative group / DMSO 1% solution); P1 (1.5% extract group); P2 (2% extract group); P3 (2.5% extract group).

Based on Figure 1 and Table 2, the research data showed an inhibition zone of green microalgae extract Nannochloropsis Oculata against the growth of Streptococcus mutans bacteria in the K + group, while the K-, P1, P2 and P3 groups did not show any inhibition zones. The treatment was then tested for significance with an error rate of 5% (p = 0.05).

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Furthermore, the results of the study were tested for the normality of the distribution using Shapiro-Wilk, because the number of research subjects was less than 50. This test aims to determine whether the data obtained is normally distributed or not with a significance level of 0.05 (p = 0.05). If the data is normally distributed (p> 0.05) it can be continued with a homogeneity test and if the data is not normally distributed (p <0.05), then the data transformation is carried out.

Table 3. Shapiro-Wilk test results

	Shapiro-Wilk		
	Statistic	**Df**	**Sig.**
VAR00001	.710	4	.015
VAR00002	.632	4	.001
VAR00003	.630	4	.001
VAR00004	.	4	.
VAR00005	.630	4	.001

After the normality test was arried out and it was found that the data was not homogeneous, then the Kruskal-Wallis non-parametric hypothesis test was carried out. The results of the Kruskall-Wallis test were used to determine whether there was a significant difference in inhibition of the inhibition zone extract of the green microalgae extract of Nannochloropsis Oculata on the growth of Streptococcus mutans bacteria.

Table 4. Kruskal-Wallis test for data variables

Independent-Samples Kruskal-Wallis Test Summary	
Total N	20
Test Statistic	13.114[a]
Degree Of Freedom	4
Asymptotic Sig.(2-sided test)	.011

Remarks * there is a significant difference

Table 4 shows a significance value of 0.011 (p <0.05). This indicates that there is a difference in meaning between the positive control and each treatment group that has different concentrations. Based on this, the Mann-Whitney test was continued.

Table 5. Mann-Whitney test results

	K+	K-	P1	P2	P3
K+		0.18*	1.00*	0.317*	1.00*
K-			0.18	0.18	0.18
P1				0.317	1.00
P2					0.317
P3					

Based on the results of the Mann-Whitney test, there was no significant difference in the inhibition of Streptococcus mutans bacteria (p <0.05) in the K + group with the K-, P1, P2 and P3 groups, P1 groups with K- groups, P2 groups with K- groups. and P1, as well as the P3 group with the K-, P1 and P2 groups because the significant value is greater than 0.05 (p> 0.05).

DISCUSSION

In this study, Nannochloropsis Oculata extract was used because this species is known to have fast and easy growth in culture. The selection of concentrations of 1.5%, 2%, and 2.5% is based on research by Revianti and Parihsini (2013), proving that Nannochloropsis oculata extract is not toxic in fibroblast stem cell culture with a concentration threshold of 2.5%, whereas above that concentration is toxic to fibroblast stem cells.[15]

In making the extract of *Nannochloropsis Oculata*, researchers used ethanol solvent with a concentration of 96% because it can produce easier separation of the content. Ethanol is a universal polar compound that can dissolve polar compounds and few non-polar compounds. Ethanol can generally extract components from the glycoside and essential oil groups. The test results of thecontent *triterpene glycoside* in the ethanol extract were greater than thecontent *triterpene glycoside* in the hexane extract.[16] *Triterpene glycoside* is thefor *termholothurin* which is a saponin compound in

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sea cucumbers and functions as an antibacterial.[17] The concentration of ethanol used as the solvent in this study was 96% because it resulted in an easier separation of the content.[18]

Researchers used Streptococcus mutans bacteria because these bacteria are the most dominant cause of human dental caries.[19] The metabolic acid produced by Streptococcus mutans can cause demineralization of the tooth surface and play a role in dental caries. The glucosyltransferase enzymes produced by these bacteria are the key to this process. These bacteria use sucrose as a substrate to synthesize soluble and insoluble glucans. Kinetic studies with divalent metal ions have shown the strength of the affinity bond for glucosyltransferase. The metal ions also exhibit the inhibitory power of the enzyme.[20]

The positive control used 0.12% Chlorhexidine solution because the components consisted of two parts of para-chlorophenol and three parts of camphor. Its disinfectant irritant properties are less than formocresol, has a broad antibacterial spectrum and is effective against fungi. Chlorhexidine 0.12% is able to destroy various microorganisms in the root canal. Kamfer as a means of diluting and reducing the irritation properties of pure para-chlorophenol. Apart from that it extends the effect of the antimicrobial properties and has also been compared with the antimicrobial effect of other root canal sterilizers. Menthol reduces the irritating properties of chlorophenol and relieves pain. This material can be used in the treatment of root canals that have apical disorders.[21]

This study has divided bacteria into five groups. The K + group as a positive control was given 0.12% Chlorhexidine solution. The administration of DMSO 1% in the K- group (negative control), 1.5% Nannochloropsis Oculata extract as the first treatment group (P1), 2% Nannochloropsis Oculata extract as the second treatment group (P2), and 2.5% Nannochloropsis Oculata extract as the third treatment group (P3). The results showed that the inhibition zone of Nannochloropsis oculata extract against the growth of Streptococcus mutans bacteria in the K + group, while the K-, P1, P2 and P3 groups did not show any inhibition zones.

The negative control used is DMSO 1% because it does not have antibacterial properties that will affect the inhibition of bacteria and the extract tested is a natural material. In addition, DMSO solution functions as a solvent that quickly penetrates into epithelial extracts without damaging cells and is often used in medicine and health.[22]

Based on the results of the Kruskal-Wallis test, it was obtained p = 0.011 (p <0.05) which indicated a significant difference in all groups, followed by the Mann-Whitney test to see the significance of the two groups' data. There was no significant difference in the inhibition of Streptococcus mutans bacteria (p <0.05) in the K + group with the K-, P1, P2 and P3 groups, P1 groups with K- groups, P2 groups with K- and P1 groups, and P3 groups. with groups K-, P1 and P2 because the significance value is greater than 0.05 (p> 0.05). The mean of the antimicrobial zone at a concentration of 1.5% (6.01 mm), 2% (6.01mm), 2.5% (6.00mm), DMSO negative control 1% (6.00 mm), and control Chlorhexidine positive 0.12% (28.81 mm).

This research is still qualitative in nature, which is to show differences in the inhibition of Nannochloropsis oculata extract against the growth of Streptococcus mutans bacteria with concentrations of 1.5%, 2%, and 2.5%, and is a preliminary research.

CONCLUSION

The results obtained showed that there was no inhibition at concentrations of 1.5%, 2%, and 2.5%. This may be because the concentration of the Nannochloropsis oculata extract used in this study was too small, whereas based on previous research by Fadhilah (2013), showed the effectiveness of the inhibition of Nannochloropsis oculata extract on bacterial

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growth in root canals at a concentration of 80% .[10] Because it is suggested for further research, research can be carried out with a higher concentration of Nannochloropsis oculata extract.

Henceforth, it is necessary to conduct quantitative research to determine the decrease in the number of bacterial colonies.

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RESEARCH ARTICLE

The Reasons for Primary Tooth Extraction in Primary School-age Children in RSGM Baiturrahmah

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ABSTRACT

Background: *Tooth extraction is an important measure in determining oral health status because it generally reflects untreated periodontal tissue disease and caries. Tooth extraction can also be used as a measure of people's knowledge and motivation in maintaining their teeth. The higher the revocation rate, the lower the community's knowledge and motivation.* ***Purpose:*** *The aim of this study is to see the main reasons for tooth extraction in children who are in the pedodontics of the RSGM Baiturrahmah.* ***Material and Methods:*** *This type of research is quantitative with analytic descriptive methods. The target population of this study were students of coass Lab Paedodonti RSGM Baiturrahmah in October-December 2019, with a sample of 76 people. The research was conducted at the Paedodonti Lab RSGM Baiturrahmah in October-December 2019 using a questionnaire. Data analysis using SPSS program with chi square analysis.* ***Results:*** *The distribution of reasons for deciduous tooth extraction is presented, it is found that most of the reasons for extracting deciduous teeth in primary school children are physiological mobility, as many as 53 people (69.7%). In addition, there were 19 people (25%) of the reasons for removal because of excessive retention of 1 person (1.3%), and because of the request of patients / parents as many as 3 people (3.9%).* ***Conclusion:*** *The main reason for the extraction of deciduous teeth in elementary school children at the Lab Paedodonti RSGM Baiturrahmah, was physiological mobility, with the teeth that were often extracted were the mandibular incisors in the 7 year age group.*

Keywords: *Tooth extraction, tooth caries, primary teeth*

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INTRODUCTION

Primary teeth take on different and important tasks, such as chewing, speaking, space maintenance, and providing aesthetics,[1] and early loss of them may lead to malocclusion, speech pathology, and functional and aesthetic problems.[2] Therefore, reporting the reasons for extraction of primary teeth is crucial for communities to take necessary steps to eliminate those unfavorable results while developing and implementing their own health policies. Additionally, periodic reports about the reasons for primary teeth extraction may help update oral health programs, thereby effectively preventing the loss of primary teeth.[2,3] Recent studies have indicated that tooth caries is the most important reason for extraction of primary teeth in children.[4-7] In addition to the occurrence of caries, dental trauma, periodontal disease, and orthodontic and physiological factors have also been identified as reasons for extraction of primary teeth.[2,3,5,8,9]

Many factors make it important to identify the main reasons for primary tooth extraction in primary school-age children. One of these factors is being far from their parent's observance in school time. Parents play a crucial role in helping their children maintain good oral hygiene by controlling their children's diet and requiring periodic tooth brushing.[10] However, it is more difficult to ensure these children adhere to these factors during the hours in which they are in school; thus, the decreasing level of oral hygiene may lead to caries.[11] In addition to oral hygiene and diet control, parental observance can protect their children against certain dental traumas; however, it is almost impossible to be diligent about this when their children are in school. Thus, it is well known that school-age children are the target group that is most at risk for dental trauma.[12] Additionally, since schoolage children are in mixed dentition period, it is more difficult to provide good oral hygiene. Furthermore, malocclusion may become intensified due to caries or tooth loss, so these children should be checked regularly.[13,15] Based on these explanations, it is thought that the loss of primary teeth in primary school-age children may occur for a variety of reasons.

The present study aimed to determine the main reasons for extraction of primary teeth in children who are in primary school and living in Zonguldak, Turkey. The study also sought to identify which of these reasons was most prominent in this group of children.

MATERIALS AND METHODS

This type of research is quantitative with analytic descriptive method. The target population of this study were students of coass Lab Paedodonti RSGM Baiturrahmah in October-December 2019. Sample in this study used the total sampling method, which means that all members of the population were used as samples. The total sample in this study was the total coass students who had performed dental extraction at the Paedodonti Lab, Baiturrahmah Hospital, as many as 76 people.

Sampling Method

The sampling technique used was total sampling technique. With the research object of elementary school children and young dentists through distributing questionnaires via google form.

Research procedure

Ethic consent. Licensing for Baiturrahmah Hospital. Subjects who meet the inclusion criteria are given an explanation of the research to be carried out. Subjects signed informed consent. Subjects are given a questionnaire that asks questions related to this research, as for the types of questions asked can be seen in the questionnaire (attached). The questionnaires that have been filled in by the subject are collected, then data are grouped, data processing and analyzed.

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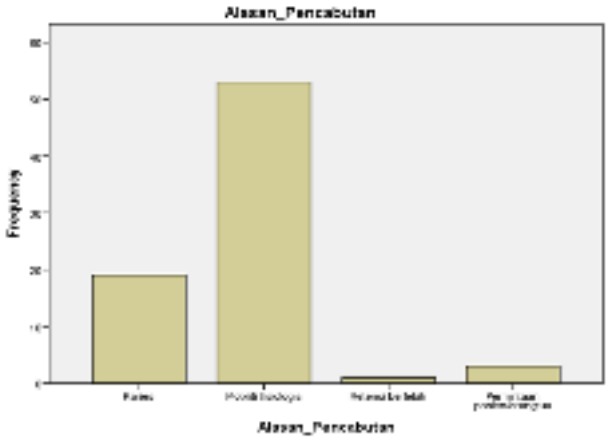
RESULTS

The research on the reasons for the extraction of decidui teeth in elementary school age children in the pedodontics lab of the Baiturrahmah Hospital was conducted at the Paedodonti Lab, Baiturrahmah Hospital, Padang. The sample in this study were 76 students of the 2018 Coass RSGM Baiturrahmah. The distribution of reasons for deciduous tooth extraction is presented in Table 2. Based on the table below, it is found that most of the reasons for extracting deciduous teeth in primary school children are physiological mobility, as many as 53 people (69.7%). In addition, there were 19 people (25%) of the reasons for removal because of excessive retention of 1 person (1.3%), and because of the request of patients / parents as many as 3 people (3.9%). For more details, see the bar chart below.

Table 1. Reasons for tooth extraction in primary age children at the pedodontics lab at the RSGM Baiturrahmah.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Tooth caries	19	25.0	25.0	25.0
	Physiological mobility	53	69.7	69.7	94.7
	Over retention	1	1.3	1.3	96.1
	Patient/parent requests	3	3.9	3.9	100.0
	Total	76	100.0	100.0	

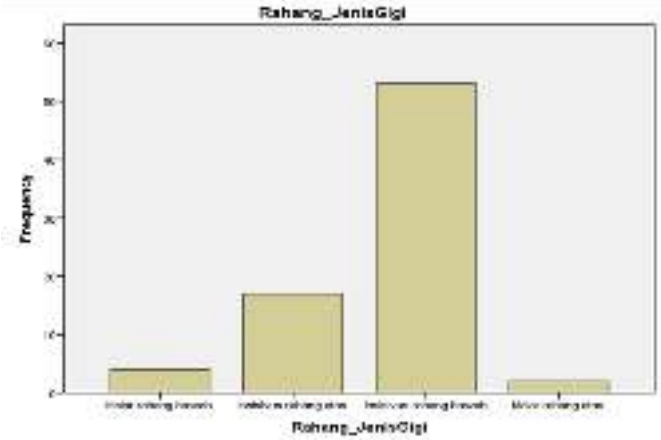


Graph 1. Reasons for extraction of deciduous teeth in primary age children at the pedodontics laboratory of the RSGM Baiturrahmah.

Based on the table below, it was found that most of the jaw and types of deciduous teeth in primary school children extracted were mandibular incisors, namely as many as 53 people (69.7%). In addition, the jaw and types of deciduous teeth extracted were 4 mandibular molars (5.3%), 17 maxillary incisors (22.4%), and 2 maxillary molars (2.6%). For more details, see the bar chart below.

Table 2. Commonly extracted deciduous teeth based on jaw and tooth type.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Molar mandibula	4	5.3	5.3	5.3
	Insisivus maksila	17	22.4	22.4	27.6
	Insisivus mandibula	53	69.7	69.7	97.4
	Molar maksila	2	2.6	2.6	100.0
	Total	76	100.0	100.0	



Graph 2. Often extracted deciduous teeth based on jaw and tooth type

Based on the table below, it is found that most of the ages of deciduous teeth in primary school children extracted were 7 years old, as many as 51 people (67.1%). In addition, there were 20 people aged 8 years (26.3%), 9 years old as many as 3 people (3.9%), and 10 and 11 years old as many as 1 person (1.3%). For more details, see the bar chart below.

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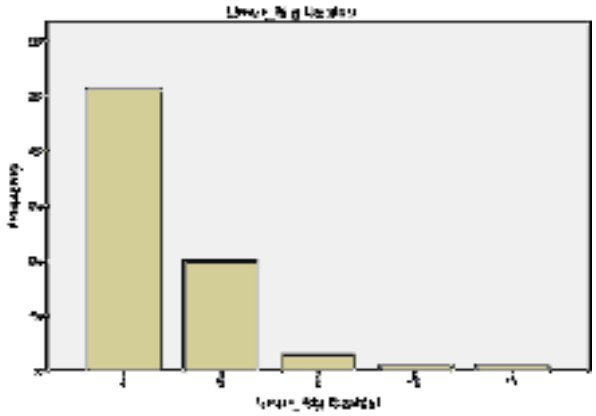
Table 3. Commonly extracted deciduous teeth by age group

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	7	51	67.1	67.1	67.1
	8	20	26.3	26.3	93.4
	9	3	3.9	3.9	97.4
	10	1	1.3	1.3	98.7
	11	1	1.3	1.3	100.0
	Total	76	100.0	100.0	



Graph 3. The most frequently extracted deciduous teeth by age group

DISCUSSION

Determining the percentages of and reasons for tooth mortality in a population provides information about the dental disease prevalence, the availability of dental treatments, and measures that can be taken to prevent tooth loss.[2,3] In order to develop and implement a plan to reduce tooth mortality, it is very important to clarify the main reasons for this type of loss.[2,15] Although tooth extraction is a common intervention to provide oral health among children attending dental clinics in developing countries,[14] data about the reasons for tooth loss in primary dentition are limited.[2,3] In addition, studies that focus on groups of primary school-age children are also limited, even though schools are accepted as favorable places to provide oral health education and efficient and effective preventive services.[16] Moreover, school-based oral health programs have been reported to be effective in providing preventive and curative services than community-based approaches.[17] Therefore, in the present study, primary school-age children were chosen as the population to be investigated.

When the results of the study were obtained, it was found that most of the deciduous teeth extractions were caused by physiological mobility (69.7%) in primary school-age children in the pedodontic laboratory of the Baiturrahmah Hospital (69.7%). age who frequently extracted deciduous teeth was 7 years old (67.1). These results differ from previous studies in Turkey which reported that the main reason for the extraction of deciduous teeth was caries, the type of tooth that is often extracted from deciduous molars.

If the extraction of a deciduous tooth is done prematurely and no treatment is done such as a space maintainer, it will cause other problems, such as decreased arch length, migration of adjacent or opposite teeth, rotation, and crowding of the affected tooth and the remaining deciduous teeth without eruption.[19] Despite the negative results, most parents do not pay attention to the function of the deciduous teeth and they do not contact their dentist unless their children experience pain or other problems with eating.[19]

Research conducted by Mansour also shows that tooth mobility and persistence are also one of the causes of tooth extraction in children. In the study conducted by Mansour (2010) it was found that 20 out of 130 teeth extracted were caused by tooth mobility (12.3%) while there were 14 teeth (8.6%) for tooth persistence.[4] The study conducted by Mansour was also not much different, where 29 teeth (12%) were removed due to tooth mobility and 20 teeth (8%) due to tooth persistence.[4] Mobility here is a normal tooth unsteadiness that occurs in children, namely tooth unsteadiness that occurs because the tooth has fallen out of time. Tooth mobility here is due to the resorption of the roots which is a normal physiological process of deciduous teeth. Tooth persistence is also a cause of tooth extraction in children. Parents will usually come to the dentist if they see a replacement tooth has appeared. Even parents who are usually “brave” to pull out their own

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children's teeth will be confused and even afraid to do the extraction of their own teeth because usually the replacement teeth will erupt from the lingual or palatal direction so that they are considered the replacement teeth to be disturbing or not in normal circumstances. In these circumstances, parents usually go to the doctor to perform tooth extraction on their child.[19]

CONCLUSION

The rate of loss of deciduous teeth associated with physiological mobility is very high. This suggests that deciduous tooth extraction due to physiological mobility is still a health problem today. In the programs that need to be developed and implemented to reduce this, it is necessary to provide information to school-age children about the importance of deciduous teeth and what can happen if they are removed. In addition, teachers and parents should be trained in oral hygiene, parents should be informed about the importance of consulting a dentist for deciduous teeth requiring treatment, and should be told that they should not be late in ensuring that their children receive treatment. Childhood includes various periods during which different factors can play a major role in the growth of primary teeth. Further research is needed to evaluate the main reasons for the extraction of deciduous teeth in certain age groups of children.

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