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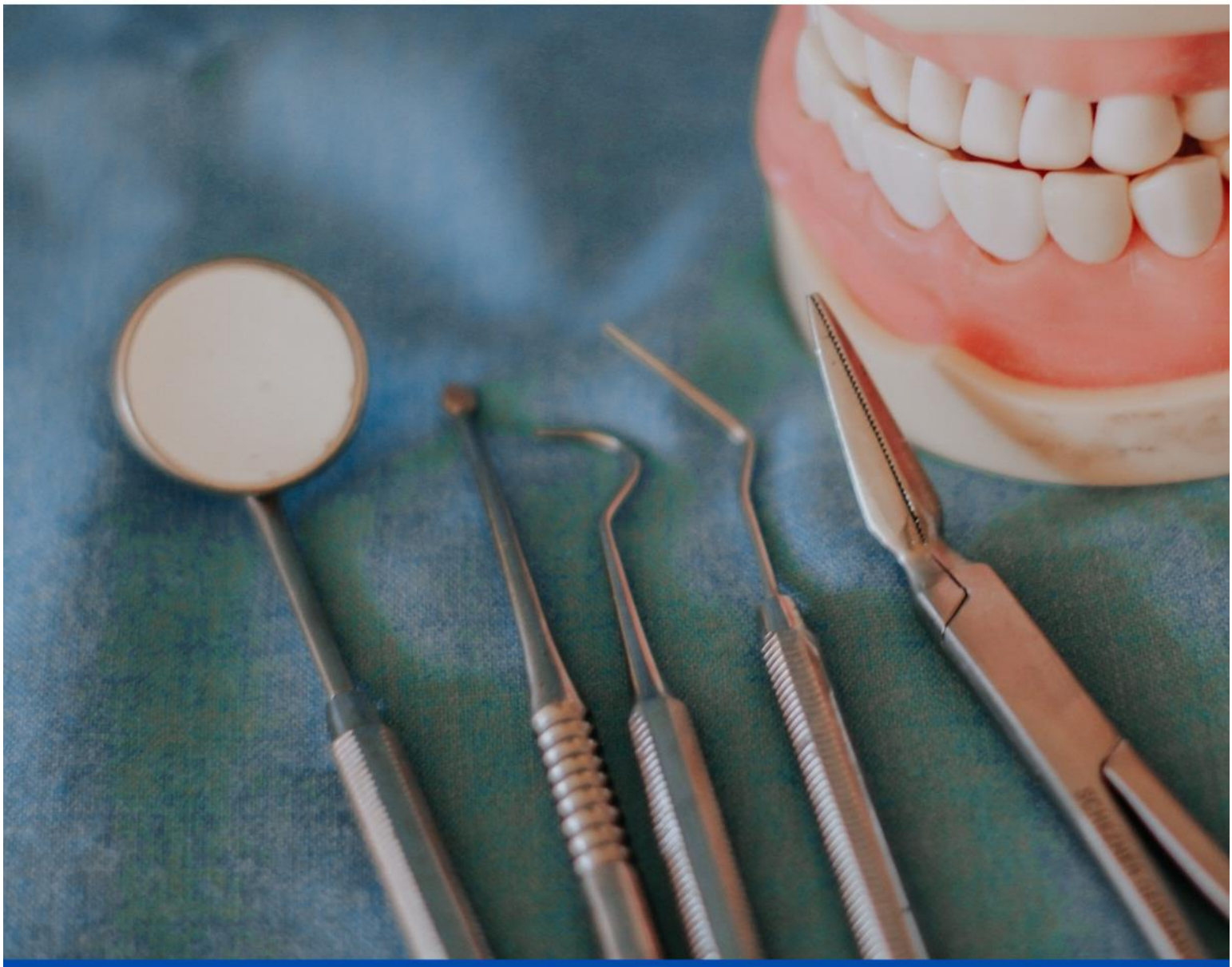

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RESEARCH ARTICLE

An Overview Study of Low Back Pain Event Among the Dentist in Yogyakarta

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ABSTRACT

Introduction: *Low back pain is a pain sensation in spinal and paraspinal structure of lumbosacral region. The prevalence of low back pain event in several country show the significant percentage every year. Dentist is a high-risk profession related to musculoskeletal disorders particularly low back pain. Bad posture, static sitting position and repetition movement are several risks possibility for the occurrence of low back pain.* ***Objective:*** *This research is purposed to evaluate the prevalence overview of low back pain event among the dentist in Yogyakarta city.* ***Methode:*** *This research was designed by the descriptive observational approach using cross sectional research design. The samples were 76 dentists under the "Indonesian Dentist Association of Yogyakarta" region. The research was conducted through questionnaires utilization to measure the level of low back pain from the respondents to the no disabillity, minimal disabillity, moderate disabillity dan severe disability categories.* ***Result:*** *The result showed that among dentist population in Yogyakarta there were 37 dentists (49%)* suffered *low back pain with no disabillity, 35 dentists (46%) low back pain with minimal disabillity and 4 dentists (5%) low back pain with moderate disabillitywhile there were nodentist suffered from low back pain with severe disabillity (0%).* ***Conclusion:*** *The total prevalence of low back pain event among dentist in Yogyakarya city categorized by all the severity level was 39 dentists (or 51%) suffered from a low back pain of musculoskeletal disorder.*

Keywords: *Low back pain, dentist, Yogyakarta, ergonomics.*

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INTRODUCTION

Musculoskeletal disorder (MSDS) is a prolonged pain coming from discomfort sensation in hands, arms, shoulders, neck and spine due to static posture while working. Such pain can vary from mild to severe when the muscles repeatedly receive the static load overa long-period of time (Andyasari and Anorital, 2012)**.** *The World Health Organization* defines MSDS as a "pain in the muscles, tendons, peripheral nerves or vascular system indirectly as a result of an acute or rapid occurrence"

Lowback pain is a pain in the spinal or paraspinal structures of the lumbo-sacred regio in the presence of a stimulus that triggers the afferent sensory nerve, causing muscle contraction and activating the nerve fibers to the inhibition of pain impulses (Ilyas and Dharmaji, 2012)**.** Low back pain will cause pain between the corners ofthe bottom ribs and fold the bottom butt and often spread wider. Some risk factors of low back pain may come from the epidemic information such as age, individual health status and conditions, psychologic and psychosocial problems, drug addiction, smoking, as well as physical factors related to active acitivities such as sitting and driving, sitting or standing in a long hours and the position of staticworking position (Mahadewa and Muliawan, 2009)**.** The symptoms of low back painmay also vary in pain including taste of baal, weakness, stiffness, tingling sensation, pain occurance, which usually occurs as a result of lifting, bending, or straining movements. A number of predisposing factors which were reported to be close related to the low back painare poor posture, standing or bending over a long-period of time, sitting on a chair with noproper back rest, lifting, the valve, pushing the burden too much and the lack of exercise(Bull and Archard, 2007)**.**The problem of musculoskeletal disorder often occurs in a working environment requiring ergonomic principle, a position or movement of the body that is less appropriate can cause fatigue and discomfort. Lower back pain, neck pain, shoulder pain, *carpal tunnel syndrome*, *tendinopathies*, *herniated disks*, *rotator cuff tendinopathies*, and *repetitive strains of injuries* are diseases that can be inflicted from the Musculoskeletal disorder (Chopra, 2014).

A dentist is a very high-level job with the highly-rateof MSDS especially on the neck and the backs. Poor working posture, repetition of work, and prolonged standing canaffect muscle disorders, joints, bones, ligaments, tendons, nerves and blood vessels come to pain, fatigue and a wide range of complaints in the musculoskeletal disturbance. Low back pain is one of the most common complaints which most dentists in the world once had that penduring work (Gaowgzeh *et al.*2015). Physical activities that do not conform to the principle of ergonomic can cause the operator to experience strain on the muscles. it is triggered by an awkward posture due to the lack of ergonomic principle, requires more repeated movements and stronger energy (OSHA, 2000). Dynamic body movements will work optimally if joints and muscles move regularly. Based on a researchin the year 2015 by Faisal rehanet al*,* which came up with 270 dentists in Karachi Pakistan, showed the results that the musculoskeletal disorder is tended to occur on the lower back area, than in other areas such as the neck, wrists, and shoulders. Almost all dentists spend most of their time for working in static positions to perform the precision and extreme procedures in the patient's oral cavity. During maintaining the hand and position of the body to reach out and properly observe working area, 50% of the body muscles contracting to resist gravity. The repetition of this movement results in a bunch of muscles for having an extension and excessive use that leads to muscle fatigue, pain in the back, neck and shoulderareas(Saleem et al*.,*2015)**.**

The results of previous studiesshowed that the prevalence of lowback pain in somecountries continue to present the significant numerical percentage from year to year. Researches which had been done in New

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South Wales 64% (Marshall et al.,1997), Saudi Arabia 52.1% (Abduljabbar, 2005), Nigeria 77.1% (Udoye and Aguwa, 2007), India 79.6% (Shetty et al., 2015) and Pakistan 64.5% (Saleem et al.,2015) of the entire population experienced in lower back pain events.

According to the above explanation, it could be interesting to study on the distribution overview of lower back pain "*Low Back pain*" at the dentist in Yogyakarta city. Low back pain has been chosen because it has the highest prevalence rate in almost every case of musculoskeletal disorder in other countries, it is also a great concern particularlyamong the dentists whose mainly work involving a static posture and position. The selection of dentists in Yogyakarta for the subject of research was based on the data obtained from the Ministry of Health in 2015 that the special region of Yogyakarta was ranked second with the most dentist in all of Indonesia (ratio of 8.86 dentists in 100,000 residents) and Yogyakarta City had been the first in the number of dentists compared to the regency of Sleman, Kulon Progo, Bantul, Gunung Kidul (ratio of 23.26 within 100,000 dentist in the population).This research is purposed to understand the ocurrance, characteristic, distribution and basic knowledge of low back pain among the dentist in Yogyakarta city.

MATERIALS AND METHODS

Type of this research was observational descriptive with *cross sectional* research design which was conducted in Yogyakarta special region for approximately 6 months for research completion. The sample population of this study was all dentists who have registered as the member of Indonesian dentist association members in the city of Yogyakarta-branch. Samples were taken by *simple random sampling* technique through specifying the minimum sample that should be included into the study with the calculation of a large sample formula as follows:

$$n = \frac{Z^2_{1-\alpha/2} P\,(1-P)}{d^2}$$

n : large of minimum sample
Z 1-α/2 : standard normal distribution value (Z table) at a certain α
P : price proportion in population
D : error (absolute) that can be tolerated
If:
α : 5% (with confidence level 95 %)
Z 1-α/2 : 1.96 (from table of Z)
P : proportion of the expected population, set by 50% (0.5)
D : degree of desired deviation, in the study was taken 6% (0,06)

The minimum sample size in this study could be determined as:

$$\mathrm{n} = \frac{1{,}96^2 0{,}5\,(1-0{,}5)}{0{,}06^2} = 266{,}7 \approx 267$$

Because the population was known then inserted into the formula as follows:

$$\text{Sample size} = \frac{\text{Minimum sample}}{1 + \frac{\text{minimum sample}-1}{\text{population}}}$$

If:
Minimum sample : 266,7
Achievable sample number : 153 dentists

Then the samples in this study:

$$\text{Sample size} = \frac{266{,}7}{1 + \frac{266{,}7-1}{153}} = 76{,}2 \approx 76$$

So, the required sample size for this study was 76 dentists. Sample for this study has to be dentist who is registered as a Indonesian dentist association of Yogyakarta-branch in active status except for those who do not want to be a subject for this study and do not fill the questionnaire completely.

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Measurement of the low back pain intensity were performed by using questionnaire containing 10 questions. The point or score of each question has a maximum value of 5. A value of 0 is given if giving an answer for the first question at each question point and a value of 5 is given if giving an answer for the last question on each question points. After completing all the 10 questions, then the result is calculated by the following formula:

50 (maximum value of the whole question) x 100 = 500%

In this study used only 9 questions then:

45 (maximum value of question) x 100 = 450%

Result from thecalculation can be categorized according to the following modified interpretation description:

0 % : No disabillity
1 - 20 % : Minimal disability
21 - 40 % : Moderate disability
41 – 60 % : Severe disability
61 – 80 % : Lame
81 – 100 % : Patient just laying down on the bed or actually just pretending

The questionnaire used in this study was modified from the *Oswestry Low Back Pain Disability Questionnaire (ODQ)*(Fairbank et al.,1980)by incorporating a form of dropper subject identity such as age, gender, practical work experience, practice work hours in a day, average number of patients in a week, dominant position during dental procedures, exercise intensity in a week, frequent action on dental procedures (restoration, scaling, orthodontic, and extraction).

RESULTS

Table.1 Characteristics of respondents

Characteristics	Number (n)	Percentage (%)
Age		
- 17 – 25 years	2	3
- 26 – 35 years	34	44
- 36-45 Years	20	27
- 46 – 55 years	8	10
- 56 – 65 years	6	8
- 65 – X Years	6	8
Gender		
- Male	24	32
- Women	52	68
Smoking		
- Yes	0	0
- No	76	100
Intensity of exercise per week	19	25
- Never	45	59
- 1x - 2x	12	16
- > 2x		
Practice experience		
- 1-10 years old	44	58
- 11-20 years old	13	17
- 21-30 years old	8	11
- 31-40 years old	7	9
- 41-50 years old	4	5
Practice hours per day		
- 1 – 5hours	35	46
- 6 – 10hours	38	50
- 11 – 15hours	3	4
Average number of patients per week		
- < 10 Persons	13	17
- 10 – 25 people	33	43
- > 25 Persons	30	40
Dominant position during dental procedures		
- Sitting	64	16
- Standing	12	84
Most common treatment during dental procedures		
- Restoration	47	62
- Orthodontic	13	17
- Extraction	9	12
- Scaling	7	9
Assistant assistance/dental nurses during dental procedures:		
- Yes/Have	66	87
- No/Do not have	10	13
Low Back Pain Degree		
- No disabillity	37	49
- Minimal disabillity	35	46
- Moderate disabillity	4	5
- Severe disabillity	0	0

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The above table shows that the number of respondents comprise of 24 males and 52 females with the range of age between 24 – 77 years with or without smoking habit. The intensity of the sport is divided into unprecedented, 1x to 2x per-week, and more than 2x per-week. The experience of dental practice can be seen from the range of 1 – 50 years, while the practice hours are calculated from 1 – 15 hours per day. The average number of respondents was divided into < 10 people, 10 – 25 people and > 25 people per week. Dental procedures performed by the dentist in a sitting or standing position, with or without assistance from the assistant/dental nurses while the most frequently performed actions include restoration, orthodontic, extraction and scaling. The degree of low back pain in the study is divided into *no disabillity, minimal disabillity, moderate disabillity and severe disabillity*.

Table 2. Characteristics of respondents based on degree of severity *Low Back Pain*

Characteristics	*Low Back Pain* degree			
	No disabillity	*Minimal disabillity*	*Moderate disabillity*	*Severe disabillity*
Age				
- 17 – 25 years	0	2	0	0
- 26 – 35 years	18	14	2	0
- 36-45 Years	4	15	1	0
- 46 – 55 years	6	2	0	0
- 56 – 65 years	3	2	1	0
- 65 – X years	6	0	0	0
Gender				
- Male	12	9	3	0
- Women	25	26	1	0
Smoking				
- Yes	0	0	0	0
- No	37	35	4	0
Intensity of exercise per week				
- Never	10	7	2	0
- 1x - 2x	22	22	1	0
- > 2x	5	6	1	0
Practice experience				
- 1-10 years	19	23	2	0
- 11-20 years	4	8	1	0
- 21-30 years	6	2	0	0
- 31-40 years	4	2	1	0
- 41-50 years	4	0	0	0
Practice hours per day				
- 1 – 5	18	14	3	0
- 6 – 10	18	20	0	0
- 11 - 15	1	1	1	0
Average number of patients per week				
- < 10	8	4	1	0
- 10 – 25	13	19	1	0
- > 25	16	12	2	0
Dominant position in while performing the dental procedures				
- Sitting	31	29	4	0
- Standing	6	6	0	0
Most common precautions during dental procedures				
- Restoration	22	22	3	0
- Orthodontic	5	8	0	0
- Extraction	6	3	0	0
- Scaling	4	2	1	0
Assistance/dental nurses during Dental procedures:				
- Yes/Have	31	31	4	0
- No/Do not have	6	4	0	0

The above table shows the respondents characteristic based on the severity of *low back pain*. The degree of severity of *low back pain* is measured using *oswestry low Back Pain Disability Questionnaire (ODQ)*. The degree of severity of *low back pain* is divided into *no disability, minimal disability, moderate disability,* and *severe disability.* Every aspect of the respondent's characteristics is observed based on the assessment range.

DISCUSSION

In the age-related LBP, it showed that the highest prevalence of LBP was in the age of 36-45 years old. This increasing age of a person may result in the decreasing of physical ability and capacity physiologically which may affect to muscular elasticity (Widjaya et al., 2012). From the weekly patient number-related LBP data showed that the highest LBP

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prevalence occurred in the dentist who having patient 10 to 25 persons weekly, this data was not consistent with the workload due to patient number as reported by Kalantari et al., 2016 since the increased number of patient did not increase the prevalence of LBP in our subjects. By the weekly exercise data showed slightly contradictive information towards the report by Lionel 2014, since the exercised practitioners were complaining LBP more than the unexercised practitioners, this discrepancy could be interfered by the unconditioned exercise conducted by the each respondence. From the experience duration data showed that LBP occurred almost in all categories, this data was corresponding to the previous report by Gaowgzeh et al, 2015 which states that there is no significant link between experience duration and LBP. The dominant position data also interestingly showed in which prior assumption towards sitting position should be better was not so appropriate, this condition suggested that ergonomics position is not just merely static standing or sitting position (Gupta et al., 2008). Our daily practice data showed an in-line findings with the previous research by Maria and Fernandes in 2014, which indicates that the working duration in a day can be supporting evidence for LBP. By the type of dental procedure, our data supported the previous result by Moodley and Naidoo in 2015 with slightly difference, since in our data showed not only restorative but also orthodontic procedure having a higher prevalence.Our data also showed similar condition regarding the prevalence of LBP with the other reports in which mentioned that women tends to be more experienced in LBP (Chou et al., 2013). Interestingly, by the availability of dental assistant, our data showed that almost 50% practitioners still complained about their LBP, but the similar report also have been reported by Singh et al, 2014 that in the presence of dental assistant, some dentist still complain about their LBP.

CONCLUSION

Basically, according to our overview study suggested that the prevalence of Low Back Pain event among the dental practitioner in Yogyakarta city was quite high since it affected in almost half of the population even it showed diversity among predisposing factors. Therefore, this overview study also assumed that LBP was a multifactorial related muscular disturbance.

REFERENCES

1. Abduljabbar, T. A. Musculoskeletal disorders among dentists in saudi arabia. Pakistan Oral & Dental Journal. 2005;28(1):135–144.
2. Alkherayf, F. and Agbi, C. Cigarette smoking and chronic low back pain in the adult population. Clinical& Investigate Medicine Journal (November). 2014. doi: 10.25011/cim.v32i5.6924.
3. Andyasari, L. and Anorital. Musculoskeletal disorders of the dentist's practice and prevention efforts. Medical R & D. 2012;(22):70 – 77.
4. Arya, R. K. Low back pain - signs, symptoms, and management. Journal, Indian Academy of Clinical Medicine. 2014; 15(1):30–41.
5. Asadi, P., Kasmaei, V. M., Mahdi, S., Ziabari, Z. and Zohrevandi, B. The prevalence of low back pain among nurses working in Poursina hospital in Rasht , Iran. Journal of Emergency Practice and Trauma, (April 2012). 2016. doi: 10.15171/jept.2015.01.
6. Bull, E. and Archard, G. Nyeri Punggung. Edited by R. Astikawati and A. Safitri. Jakarta: Erlangga; 2007.
7. CCOHS. Work-related Musculoskeletal disorders; 2014
8. Chopra, A. Musculoskeletal Disorders in Dentistry- A Review, JSM Dentistry. 2014;2(3):2–5.
9. Chou, Y., Shih, C., Lin, J., Chen, T.-L. and Liao, C. Low back Pain Associated With Sociodemographic Factors, Lifestyle and osteoporosis: A Population- Based Study,Journal of Rehabilitative Medicine. 2013:76–80. doi: 10.2340/16501977-1070.

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Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
DOI: 10.30649/denta.v14i1

10. Davidson, M. and L Keating, J. A Comparison of Five Low Back Disability Questionnaires : Reliability and Responsiveness, Journal of Phsyoterapy, (December 2013). 2002. doi: 10.1093/ptj/82.1.8.
11. Fairbank, J. C., Couper, J., Davies, J. B. and O'Brien, J. P. The Oswestry low back pain disability questionnaire. Journal of Physiotherapy, 1980;66(8):271–273. doi: PMID: 6450426.
12. Finkbeiner, B. L. Four-handed dentistry revisited. Journal of Contemporary Dental Practice. 2000:1(4);84–92. doi: 1526-3711-50 [pii].
13. Gaowgzeh, R. A., Chevidikunnan, M. F., Al Saif, A., El-Gendy, S., Karrouf, G. and Al Senany, S. Prevalence of and risk factors for low back pain among dentists', Physical Therapy Journal. 2015:27(9);2803–6. doi: 10.1589/jpts.27.2803.
14. Guo, H.-R. Working hours spent on repeated activities and prevalence of back pain. Occup Environ Med Journal. 2002;680–688.
15. Gupta, A., Bhat, M., MOhammed, T., Bansal, N. and Gupta, G. Ergonomics in Dentistry. Jp-Journal. 2008; 30–34.
16. Harsono. Capita Selekta Neurology. Edition of Kedua, Yogyakarta: Gadjah Mada University Press. 2009.
17. Ilyas, M. and Dharmaji, T. P. Low back pain in dentists of Indonesia. Pakistan Oral & Dental Journal. 2012:32(3); 464–468. Available at: http://www.podj.com.pk/Dec_2012/p-22.pdf.
18. Indriana, T. The Influence of Muscle Fatigue on Work Carefulness. Journal of Dentistry Unej, 7. 2010; 49 – 52.
19. Ishak, N. A., Zahari, Z. and Justine, M. Effectiveness of Strengthening Exercises for the Elderly with Low Back Pain to Improve Symptoms and Functions : A Systematic Review. Scientifica Journal. 2016.
20. Kalantari, R., Arghami, S. and Garosi, E. Relationship between workload and low back pain in assembly line workers Article Info. Journal of Kermansah University of Medical Science (May). 2016.
21. Krause, N., Ragland, D. R., Rugulies, R. and Syme, S. L. Physical workload , ergonomic problems , 7 . 5-year prospective study of San Francisco transit operators. American Journal of Industrial Medicine. 2004;46(6).
22. Lionel, A. K. Risk Factors Forchronic Low Back Pain. Journal of Community Medicine & Health Education. 2013:4(2); 2–5. doi: 10.4172/2161-0711.1000271.
23. T. G. and Muliawan, S. Diagnosis & Treatmentof spinal blood care. Jakarta: CV Sagung Seno. 2009.
24. Maria Santos Pataro, S. and de Cassia Pereira Fernandes, R. Heavy physical work and low back pain : the reality in urban cleaning. Rev Bras Epidemiol Journal. 2014;17–31. doi: 10.1590/1809-4503201400010003ENG.
25. Marshall, E. D., Duncombe, L. M., Robinson, R. Q. and Kilbreath, S. L. Musculoskeletal symptoms in New South Wales dentists. Australian dental journal, 1997: 42(4); 240–246. doi: 10.1111/j.1834-7819.1997.tb00128.x.
26. Moodley, R. and Naidoo, S. The prevalence of musculoskeletal disorders among dentists in KwaZulu – Natal. Journal of South African Dental Association. 2015; 98–103.
27. OSHA. Ergonomics : The Study of Work. 2000. doi: 10.1029/2010RS004575.
28. Paloma, K., Freitas, N., Barros, S. S. De, Oliveira, C. De, Borba, P. and Uchôa, L. Occupational low back pain and the sitting position : effects of labor kinesiotherapy. RevisitaDor Journal. 2011: 12(4); 308–313.
29. Paparang, F., M. L Umboh, J. and A. M Rattu, J. Factors relating to the lower back pain complaint at the Obsgyn resident of Central Hospital Prof. DR. R.D. Kandou. Journal of Community Health. 2017; 18 – 28.
30. Patrianingrum, M., Oktaliansah, E. and Surahman, E. Research articles Journal of perioperative Anaesthesia, 2015:3 (1); 47 – 56. DOI: 10.15851/jap. v3n 1.379.
31. Rehan, F., Leghari, M. A., Memon, M. S., Atif, M., Masood, A., Mohsin, F. and Sheikh, N. Frequency of Musculoskeletal Discomfort Among Dentist in Karachi. Pakistan Female Years in Practice less than 10 years Field of Specialization. Pakistan Oral & Dental Journal. 2015: 35(4); 631–4.
32. Safirin, G. Muscle contractions and fatigue. Ilara, 1.2010; 58 – 60.
33. Saleem, T., Zainab, S. N. and Bhatti, U. D. Prevalence of Causative Factors for Musculoskeletal Disorders and Their Awareness Amongst Dental Surgeons. Pakistan Oral & Dental Journal. 2015: 35(2); 335–338.
34. Shetty, S. M., Shetty, S., Hedge, A. and Babu, N. Prevalence of Neck and Back Pain Among

Denta Jurnal Kedokteran Gigi, Februari 2020; Vol.14 No.1; Hal 1-8
Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
DOI: 10.30649/denta.v14i1

Paediatric Dentists. International Journal of Scientific Study, 2015: 5(1); 1–3.
35. Singh, N., Jain, A., Sinha, N., Chauhan, A. and Rehman, R. Application of Four-Handed Dentistry in Clinical Practice : A Review. International Journal Dental Medicine. 2014: 1(1); 8–13.
36. Sumangando, M., Rottie, J. and Lolong, J. The relationship of nurse workload with the incidence of Low Back Pain (LBP) in the implementing nurse at RS TK. III R. W monginsidi Manado. E Journal Nursing, 5. 2017.
37. Tortora, G. J. and Derrickson, B. Principles of anatomy and physiology. Hoboken: N.J., Wiley. 2011.
38. Udoye, C. . and Aguwa, E. . Musculoskeletal symptoms : A survey among a selected nigeriaan dentist. International Journalof Dental Science. 2007.
39. Wáng, Y. X. J., Wáng, J. and Káplár, Z. Increased low back pain prevalence in females than in males after menopause age : evidences based on synthetic literature review. Quant Imaging Medical Surgery Journal. 2016: 6(2); 199–206. doi: 10.21037/qims.2016.04.06.
40. Whitney, E. N. and Rolfes, S. R. Understanding Nutrition. Food. WILEY-VCH Verlag GmbH, 1994: 38(2); 222–222. doi: 10.1002/food.19940380219.
41. Widjaya, M. P., Aswar, H. and Pala'langan, S. Factors related to the incidence of low back pain on furniture workers. Jurnal Universityhalu Oleo. 2012; 85 – 90.

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RESEARCH ARTICLE

Correlation Between Age, Gender and Bad Oral Habit of 7-9-year-old Children in Karangjati Primary School, Kasihan, Bantul, Yogyakarta

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ABSTRACT

Introduction: *Bad oral habit is an intraoral abnormal habit, if it continues until school-age children, it needs more attention because of its effects on craniofacial growth. It can be caused by pathological conditions, anxiety, or psychological disorder. The school-age period is a new environment. Children adjust to some conditions that may cause problems but if they cannot, resulting in psychological tension. Girls have a higher level of anxiety than boys. The boys tend to against the advice of their parents, including stopping doing bad oral habits.* ***Purpose****: This study aimed to determine whether there was a correlation between age, gender, and bad oral habit of 7-9-year-old children.* ***Materials and Methods:*** *A cross-sectional study has been done in Karangjati elementary school. The 107 children were examined their oral cavity to observe clinical symptoms that might be lead to bad oral habits. Their parents were asked to fill out the questionnaire to determine whether there were bad oral habits. The data were analyzed by the chi-square.* ***Result:*** *Oral habits were present in 67 from107 children (62.62%) and mostly in 8 years old group (26.17%); more occurred in males (36.45%) than females (26.17%). The highest prevalence was nail-biting (28.97%), followed by lip sucking (23.36%), lip biting (23.36%), thumb/finger sucking (20.5%), bruxism (13.08%) and mouth breathing (8.41%). Chi-square test showed that p-value=0,037 and 0.038 for the correlation between age, gender, and bad oral habit, respectively.* ***Conclusion:*** *There were correlations between age, gender, and bad oral habits of 7-9-year-old children.*

Keywords: *Bad oral habit, gender, age, 7-9-year old children*

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INTRODUCTION

Malocclusion is a significant oral and dental health problem in Indonesia and is ranked third after dental caries and periodontal disease.[1] Bad oral habit is an extrinsic factor that causes malocclusion.[2] Bad oral habits in children can cause serious effects on the growth of the face and teeth. The prevalence of oral habit for the mixed dentition was 54.2%.[3]

Oral habits are classified as physiological and non-physiological oral habits. Physiological oral habit is a normal habit such as chewing, swallowing, and nasal breathing. Non-physiological (abnormal) oral habit is called parafunctional or harmful, also called bad oral habit is the oral habit that causes pressure, persist, and carried out continuously thus affecting craniofacial growth.[4] Bad oral habits inherent in children when emotional distresses are intolerable for children.[5] They can feel safe with these habits, such as thumb/finger sucking, pacifier sucking, lips sucking lip biting, nail-biting, bruxism, mouth breathing, and tongue thrusting. Several effects in the oral cavity essentially depend on the onset and duration.[6]

Children at a school-age period are in a new environment, school environment, and they begin to adjust to social, language, emotional, moral, and motoric developments. Through these developments, sometimes they feel that they have many shortcomings and unable to overcome their problems, resulting in psychological tension that can lead them to have bad oral habit problem.[4]

It is important to examine these oral bad habits because if these habits duration are 6 hours per day and continuous intensity can cause malocclusion.[7] Diagnosis of bad oral habits is very important because it can cause disruption of the normal growth of jaws, development of occlusion and leads to the development of malocclusion.[8]

Research from Garde showed that the prevalence of bad oral habits was more common in females (31%) than males (20.1%).[9] Females tend to be more anxious and sensitive, while males tend to be more active and explorative.[10] However, males tend to against the advice from their families compared to the female, at the time when they asked to stop their bad oral habit.[11] The subjects of this study were children aged 7-9 years because the children were in mixed dentition period and from the previous study it was known that the prevalence of BOH in this period was 54.2%.[3]

MATERIALS AND METHODS

This was an observational study with a cross-sectional design. The population of this study was 7-9-year-old students in Karangjati elementary school, Kasihan, Bantul, Yogyakarta. The subjects were 107 children in 7-9 years old (54 males, 53 females) with valid consent forms signed by their parents. Children's oral cavity was ere examined to observe the clinical symptoms that might be lead to bad oral habits. Their parents were asked to fill out the questionnaire as supporting data to determine whether there was a bad oral habit. The children with craniofacial anomalies, current or previous orthodontic treatment were excluded from this study.

Bad oral habits that were observed were oral habits thats persist until this research was conducted. The questionnaire and the children's data were recorded as well as the presence or absence of bad oral habits such as mouth breathing, thumb/digit sucking, nail-biting, bruxism, and lip sucking/ biting. The presence of bad oral habits was determined by examined clinical signs that lead to bad oral habits, such as thumb/finger sucking can be identified by finger defect, anterior open bite, protrusion of maxillary incisors, high palate, and posterior crossbite. Nail-biting habit can be determined by the presence of inflammation around the nails, crowding or rotation and attrition of incisors, and maxillary incisor protrusion. Lip sucking/biting can be identified by the presence of inflammation around the mouth or vermilion hypertrophy, dry lips, maxillary teeth protrusion and mandibular teeth retrusion; and the clinical

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signs of bruxism are attrition in canines and molars deciduous teeth. Mouth breathing was checked by a mirror test. A double-sided mirror was held between the nose and mouth. Fogging on the nasal side of the mirror indicates nasal breathing while fogging on the oral side indicates mouth breathing. The questionnaire was filled out by their parents as supportive data of this study.

RESULT

This study was conducted in Karangjati Elementary School, Kasihan, Bantul, Yogyakarta, in 107 children aged 7-9 years (54 male and 53 female) who fit the inclusion criteria as the subjects. The highest number of subjects was children in the 8-year-old group, 48 children (44.86%). This result can be seen in Figure 1. This figure also represented that there were more male subjects in this research. The prevalence of bad oral habits based on the age categories can be seen in Figure 2. The most number of bad oral habits was in the 8-year-age groups as many as 28 children (26.17%) and these were more common in the male, 39 children (36.45%) than in the female, 28 children (26.17%) (Figure 3).

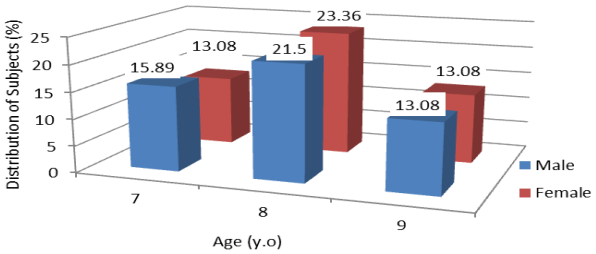

Figure 1. Distribution of subjects

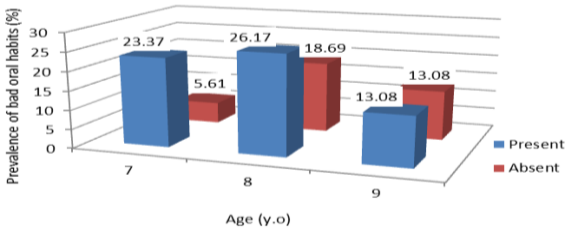

Figure 2. Prevalence of bad oral habits based on age

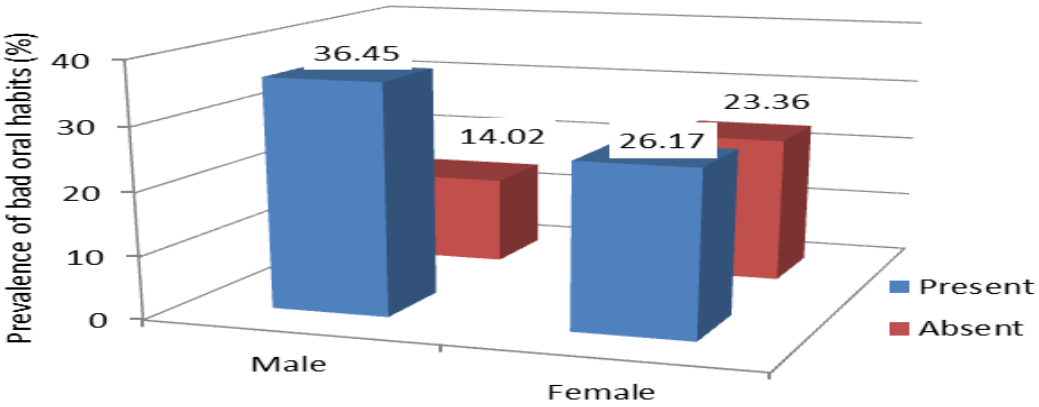

Figure 3. Prevalence of bad oral habits based on gender

Table 1. Prevalence of bad oral habits in 7-9-year-old children based on gender and age

Gender	Age (y.o)	Mouth Breathing		Thumb/ Finger Sucking		Nail-biting		Lip sucking		Lip Biting		Bruxism	
		n	%	n	%	n	%	N	%	n	%	n	%
Male	7	2	1,87	6	5,61	4	3,74	6	5,61	6	5,61	4	3,74
	8	2	1,87	7	6,54	7	6,54	5	4,67	8	7,48	1	0,93
	9	2	1,87	2	1,87	5	4,67	6	5,61	4	3,74	3	2,80
Female	7	1	0,93	2	1,87	4	3,74	4	3,74	3	2,80	4	3,74
	8	2	1,87	4	3,74	10	9,35	3	2,80	3	2,80	1	0,93
	9	0	0,00	1	0,93	1	0,93	1	0,93	1	0,93	1	0,93

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Total	9	8.41	22	20.56	31	28.97	25	23.36	25	23.36	14	13.08

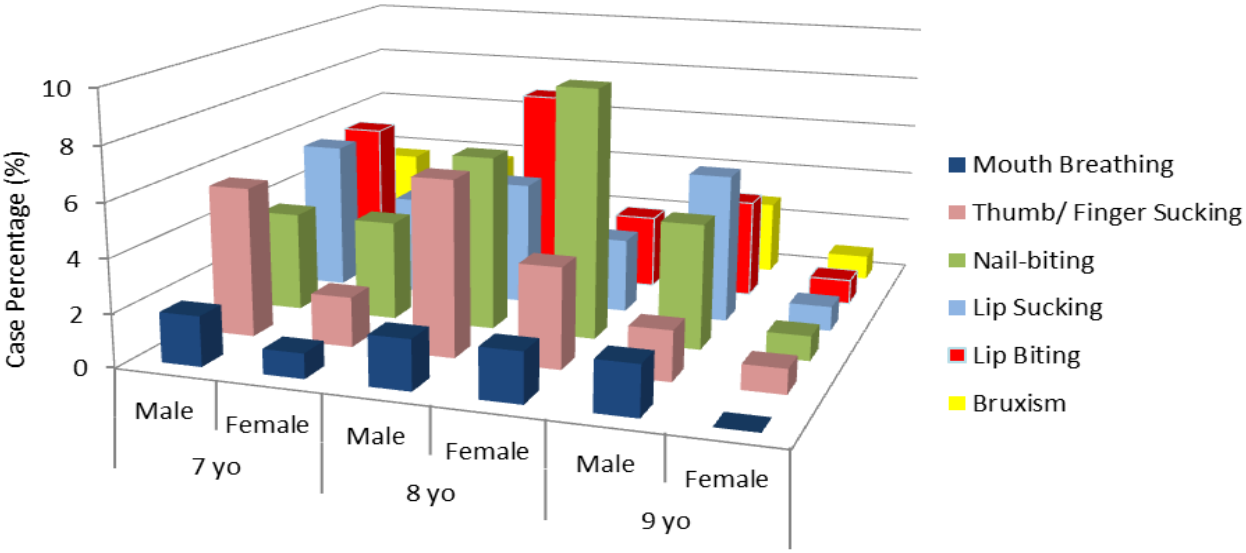

Figure 4. Prevalence of bad oral habits in 7-9-year-old children based on gender and age

Table 1. Chi-square test of gender and bad oral habit

	Value	df	Asymp Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.297[a]	1	.038		
Continuity Correction[b]	3.508	1	0.61		
Likelihood Ratio	4.332	1	0.37		
Fisher's Exact Test				0.47	0.30
Linear-by-Linear Association	4.257	1	0.39		
N of Valid Cases	107				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 19,81.
b. Computed only for a 2x2 table

Table 2. Chi-square test of age and bad oral habit

	Value	df	Asymp Sig. (2-sided)
Pearson Chi-Square	6.585[a]	2	.037
Likelihood Ratio	6.965	2	.031
Linear-by-Linear Association	5.977	1	.014
N of Valid Cases	107		

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 10,47

Examination of individual prevalence of oral habits revealed that 9 children (8.41%) had mouth-breathing habit, 22 children (20.56%) had thumb/finger sucking habit, 22 children (20.56%) had the nail-biting habit, 25 children (23.36 %) had the habit of lip sucking, 25 children (23.36) had lip biting and bruxism was found in 14 children (13.8%) (Table 1).

Chi-square test of gender and bad oral habits using a significance value (p) of 0.05 showed the result that p=0.038. It suggesting that in this study, there was a significant correlation between gender and bad oral habits in 7-9-year-old children (Table 2).

As well as in Table 3 showed that there was a significant correlation between age and

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bad oral habits in 7-9-year-old children (p=0.037).

DISCUSSION

Oral habits have been the topic of interest to the dentist, especially the pediatric dentist. Bad oral habits in children that persisted in the age of more than six years frequently lead to malocclusion and facial deformities.[12,13] This present study evaluates the bad oral habit prevalence and the correlation between age, gender, and bad oral habits. A total of 107 children aged 7-9 years were evaluated. Figure 1 showed the characteristics and distribution of the samples used in this study. It was seen that more males (54=50.47%) than female (53=49.53%) subjects in this study. Oral habits were present in 67 from 107 children (62.62%).

The 8-year-old group of age was the most subject (48 = 44.86%) in this study, and also it was the most age category that having bad oral habits (26.17%) as seen in Figure 2, because at this age children start to make friends, many inter-communications harm children, which can result in psychological disorders that lead to the onset of bad oral habits.[14,15] Actually if it is calculated based on the number of subjects per group of age, the percentage of bad oral habits in the 8-year age group was lower than in the 7-year age group. It was in line with the result of Basra's research. Basra, et al stated that as age increased, bad oral habits decreased.[16] The existence of bad oral habits at the age of 7 years in this study perhaps due to being a new elementary school student where there was a transition from preschool to school period. The presence of children entering a new and more complex school environment which was more complex allowed stressors to them, so they felt uncomfortable, afraid, and anxious. This situation triggered the children to do bad oral habits if they were unable to handle these problems. Emotional and psychological disturbances of the children cannot be separated from bad habits.[17] Oral habits in the infant period and young children were normal things. This habit is considered abnormal when they settled on the age of the child above 3 years old.[18] If the bad oral habit continues after the 6-year-old, it can cause dentofacial abnormalities such as malocclusion and abnormalities in the shape of the face and palate.[17]

Based on gender, the results of this study indicated that bad oral habits occurred more in males (39=36.45%) than in females (28=26.17%). It was illustrated in Figure 3. This is in line with the This is in line with the result of Septuaginta's research which showed that boys have more bad oral habits.[13] The results of this study were also consistent with research conducted by Vishnoi, et al[19] which showed that bad oral habits more common in boys than girls. Bad oral habits that occur in boys due to their attitude that tends to resist demands. Parents who might be mistaken in providing education and supervision of children resulting in children have psychological and mental disorders, thus the psychological disorders that occur can encourage the child to do bad oral habits[13]. Another underlying reason bad oral habits that persist longer in boys than girls because boys are likely to violate the advice of parents, including when they were asked to stop doing bad oral habit.[11]

The most of bad oral habit occurred in this study was nail-biting (31=28.97%), and then followed by lip sucking/lip biting (25=23.36%), thumb/finger sucking (22=20.56%), Bruxism (14=13.08%) and the last is mouth breathing (9=8.41%). These can be seen in Figure 4. Mouth breathing was rarer when compared with other bad oral habits. According to Jefferson, people with mouth breathing habit will have an inhibited maxillary growth, a narrow palate that tends to have an overcrowded of maxillary anterior teeth.[20] The result of other studies indicates that lips sucking habit is rarer when compared with other bad oral habits. The habit of sucking or biting the lips is usually performed on the lower lip. It causes the lower teeth move lingually and the maxillary teeth to move anteriorly. As a result, protrusion of maxillary

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anterior teeth, retrusion of mandibulary anterior teeth, soft tissue inflammation, and anterior open bite.[21] Bruxism can cause damage to the teeth structure causing teeth hypersensitivity in changes of temperature, mobility of the teeth, fractures on the cusps, pulpitis, and pulp necrosis.[22] Nail biting causes crowding, incisal rotation and attrition of the maxillary and mandibular incisors, protrusion and also diastema of the maxillary incisors.[23] The conditions caused by thumb/finger sucking are palatal vaults, inter-canine, and intermolar narrowing arch widths, increasing arch depth, a labial inclination of the maxillary incisors, increasing overjet, the spacing of the maxillary incisors and anterior open bites.[24]

These habits can be caused by environmental factors and psychological factors that can lead to abnormal malocclusion.[21] The presence of bad oral habits on the whole subject of study is quite high, as many as 62.62% (67 children), as illustrated in Figure 3. In different countries, the prevalence of bad oral habits is varied greatly. Urzal, et al., showed the prevalence of high bad oral habits that occurred in the deciduous teeth period amounted to 43.5% and 54.2% in the mixed dentition period.[3]

The result of this study showed that there was a significant correlation between gender and bad oral habits (Table 2). This was in line with research conducted by Motta, et al., which showed that bad oral habit was more common in boys and there was a significant correlation[4], and also consistent with Jajoo research who compared the prevalence of bad oral habits in boys and girls which was showed that statistically significant differences and the prevalence of bad oral habits in boys are more than girls, too.[25] Besides the correlation between gender and oral habits, this study also showed that there was a significant correlation between age and bad oral habit, therefor early prevention of bad oral habits is important for the oral cavity health in children[26] This result was in accordance with Basra's research which states that there was a correlation between bad oral habits and age.[16] Unconsciously, parents as the primary educators for children can determine whether bad oral habits will be permanent in their children or not. Presence or absence of bad oral habits that persist influenced by parent's knowledge and attitudes.[27]

From this study, it can be concluded that there was a relationship between age, gender, and bad oral habits in children among 7-9 years old. There was a high prevalence of bad oral habit and the most common was nail-biting. The most of bad oral habit was in 8 years old group and based on gender, there were more bad oral habits occurred in male compared to female. A holistic approach is needed to replace bad oral habits in children with other good habits, things to do such as counseling for children and parents as well as the use of a special tool to stop bad oral habits.

REFERENCES

1. Badan Penelitian dan Pengembangan Kesehatan. Departemen Kesehatan Republik Indonesia. Laporan Hasil Riset Kesehatan Dasar (Riskesdas) Nasional. Jakarta. 2013. p. 148.
2. Laguhi VA, Anindita PS, Gunawan PN. Gambaran maloklusi dengan menggunakan HMAR pada pasien di Rumah Sakit Gigi dan Mulut Universitas Sam Ratulangi Manado. Jurnal e-GiGi (eG). 2014; 2(2): 1-2.
3. Urzal V, Braga AC, Ferreira AF. Oral Habits as risk factors for anterior open bite in the deciduous and mixed dentition-cross-sectional study, European Journal of Paediatric Dentistry. 2013; 14(4):298-302.
4. Motta LJ, Alfaya TA, Marangoni AF, Mesquita-Ferrari RA, Fernandes KP, Bussadori SK. Gender as a risk factor for mouth breathing and other harmful oral habits in preschoolers. Braz J Oral Sci. 2012; 1 (3):377-80.
5. Dubey R, Kashyap N, Avinash A, Kumar B. Oral Habits in 3 to 12 years of Children : A Burden on Developing Dentition. International Journal of Scientific Research. 2018; 7(4): 36-38.
6. Piteo AM, Kennedy JD, Roberts RM, Martin AJ, Nettelbeck T, Kohler MJ, Lushington K. Snoring and Cognitive Development in Infancy. Sleep Med. 2011; 12: 981-7.

Denta Jurnal Kedokteran Gigi, Februari 2020; Vol.14 No.1; Hal 9-15
Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
DOI: 10.30649/denta.v14i1

7. Hadi R, Adhani R, Widodo. Figures Genesis Central Diastema in Children with Special Needs Accompanied Thumb Sucking Habit. Dentino-Journal of Dentistry. 2016; 1(2): 177-81.
8. Murrieta J, Hernández D, Linares C, González M, Juáre L, Montaño V. Parafunctional Oral Habits and its Relationship with Family Structure in a Mexican preschoolers Group. Journal of Oral Research, 2014; 3(1): 29-36.
9. Garde JB., Suryavanshi RK, Jawale BA, Deshmukh V, Dadhe DP, Suryavanshi MK. An Epidemiological Study to Know the Prevalence of Deleterious Oral Habits Among 6 to 12-Year-Old Children. International Journal of Oral Health. 2014; 6(1): 39-43.
10. Saputra DNA, Widayanti CG. Academic Anxiety Differences Seen from Gender in class X SMA Negeri 2 Ungaran. Empathy. 2014; 3(3): 42-51.
11. Sharma S, Bansal A, Asopa K. Prevalence of Oral Habits among Eleven to Thirteen Years Old Children in Jaipur. International Journal of Clinical Pediatric Dentistry. 2015; 8(3): 208-10.
12. Bareto BL, Souza RA, Freitas RM, Pithon MM. Association between Breastfeeding and Deleterious Oral Habits with Development of Malocclusions in Pediatric Dental Patients. International Journal of Experimental Dental Science. 2012; 1(2):61-66.
13. Septuagint AA, Kepel BJ, Anindita PS. Oral Habit picture on Catholic Elementary Students II St. Antonius Palu. Journal e-GiGi. 2013; 1(1), 18-27.
14. Laksmiastuti RS. Pemakaian lip bumper pada anak-anak dengan kebiasaan jelek menggigit bibir bawah dan menghisap ibu jari. Denta J. 2007; 1(2):90-91,93-94.
15. Nirwana AB. Psikologi bayi, balita, dan anak. Yogyakarta: Nuha Medika, 2011; p.116-128.
16. Basra US, Kaur N, Singh A, Singh K, Singh KP. Deleterious Oral Habits among School Going Children - A Cross-Sectional Study. Journal of Interdisciplinary Medicine and Dental Science.2017; 4(5): 1-4.
17. Omer MI, Abuaffan AH.Prevalence of Oral Habits and its Effect in Primary dentition among Preschool Children in Sudanese Khartoum City. Indian Journal of Dental Education. 2015; 8 (2): 57-62.
18. Gairuboyina S, Chandra P, Anandkrishna L, Kamath PS, Shetty AK, Ramya M. Non-nutritive sucking habits : a review. Journal of Dental & Oro-facial Research. 2014; 10 (2): 22-27.
19. Vishnoi P, Kambalyal P, Shyagali TR, Bhayya DP, Trivedi R, Jingar J. Age-wise and gender-wise prevalence of oral habits in 7-16-year-old school children of Mewar ethnicity, India. Indian Journal of Dental Science. 2017; 9 (3): 184-8.
20. Jefferson Y. Mouth Breathing: Adverse effect on facial growth, health, academics, and behavior. Academy of General Dentistry. 2010; 58(1): 18-25.
21. Joelijanto R. Oral habits that cause malocclusion problems. IDJ. 2012; 1(2): 82-88.
22. Alfaya, TA, Tannure PN, Barcelos R, Castro E, Uemoto L, and Gouvêa CVD. Clinical management of childhood bruxism RGO - Revista Gaúcha de Odontologia 2015; 63 (2): 207-12.
23. Herdiyati Y, and Marhani ND. A description of nail biting habit in elementary school children. OnLine Journal of Biological Science. 2017; 17(2): 66-69.
24. Gairuboyina S, Chandra P, Anandkrishna L, Kamath PS, Shetty AK, Ramya M. Non-nutritive sucking habits: a review. Journal of Dental & Oro-facial Research. 2014; 10(2): 22-27.
25. Jajoo S, Chunawala Y, Bijle MN, Shah R, Kamble A, Gaonkar NK Oral Habits in School Going Children of Pune: A Prevalence Study. International Journal of Oral Health. 2015; 7(10): 96-101.
26. Shah AF, Batra M, Sudeep CB, Gupta M, Kadambariamblidhok, Kumar R. Oral Habits and Their Implications. Annals Medicus. 2014; 1(4):179-86.
27. Barboza N, Santana N, Lunardi N, Boeck EM, Pizzol KEDC. Evaluation of Knowledge on Oral Health, malocclusions, and Non-Nutritive Habits of Daycare Educators. Journal of Research in Dentistry. 2014; 2(4): 286-97.

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RESEARCH ARTICLE

Estimation of Biological Ages with Kvaal Method Using Panoramic Radiography in Semarang City

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ABSTRACT

Introduction: *Forensic odontology is a branch of dentistry that has disciplines learn about the examination of evidence derived from teeth, and how to deal with the evidence for legal concerns.* ***Purpose:*** *The aim of the study was to describe biological age estimates by using Kvaal method in Semarang, and the difference between biological and chronological age by using the Kvaal method in Semarang. This study was descriptive with crosssectional design.* ***Materials and Methods:*** *One of the methods in determining the estimated age by using teeth is the Kvaal method. Kvaal method determines the estimated age based on the pulp size using radiography.* ***Result:*** *The results showed the difference between biological and chronological age was ±4,57 years. This result is lower than Kvaal's previous study result which was ±9,5 years.* ***Conclusion:*** *Based in the findings above, it can be concluded that the difference of chronological and biological age using Kvaal method in Semarang was ±4,57 years within 15-60 years old individuals in Semarang.*

Keywords: *Kvaal method, biological age, panoramic radiograph.*

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INTRODUCTION

Radiography is a radiographic images that use non-ionizing and ionizing energy. In the field of therapy and diagnostics, which includes non-ionizing energy are radio waves, microwaves, infrared rays and ultraviolet rays. Neutrons, alpha particles, beta particles, gamma rays and X rays are ionizing energies.[1] The use of X-rays in medical field is very helpful in establishing diagnosis, prognosis and determining treatment plans.

Forensic identification is an attempt by medical personnel with the aim of helping investigators to determine a person's identity by comparing data when the person's still alive (ante mortem) and after death data (post mortem). Forensic identification results can be obtained information about sex, race, age, height and blood type. The method commonly used in forensic science is a primary examination that has very individualistic characteristics, namely the need for fingerprint data, tooth data and DNA.[2]

Forensic odontology identification examination is the main means of the body being destroyed and unknown, both due to accidents, fire, explosion, etc.[3] Teeth are one part of the body that is very important in the identification of forensic odontology process. This is due to the nature of the teeth that have the fewest biological changes so that they can be used even if other parts of the body are destroyed, burned, decomposed or become skeletal remains. So that teeth can be used as great and precise indicator to determine the estimated age.

One of the method in determining the estimated age is the Kvaal method. Kvaal et al., stated that this method can be used to estimate a person's biological age based on pulp size using radiography. Kvaal et al conducted a study of the relationship between age and dental pulp size in individuals aged over 20 years using periapical radiographs.[5] Age changes result in changes in the pulp due to continuous deposition of dentinal tissue during pulp life and dentin deposition secondary to stimuli resulting in pulp chamber size, root canal size and pulp volume shrinking.[6]

The aim of this study was to determine the difference between biological age and chronological age using the Kvaal method in Semarang. The benefits of this study are expected to be able to find out the difference in the Kvaal method in determining the difference in chronological age and biological age in Semarang City.

MATERIALS AND METHODS

128 panoramic radiographs from patients aged 15-60 years (according to the age range that can be estimated using the Kvaal method) and radiograph quality is excellent or diagnostically acceptable, in the other word, canines or mandibular first premolars to be measured must be in a well-read condition.from 3 hospitals in Semarang (with the code Hospital X, Y, Z) were used in this study. All procedures were approved by the Health and Medical Research Ethics Commission of the Faculty of Dentistry Sultan Agung Islamic University Semarang (No. 058/B.1-KEPK/SA-FKG/XI/2018) and the ethics committee at Hospital X, Y, Z. Measurement of age estimation using direct digital panoramic radiographs data meeting the inclusion criteria was done using graphic application of RadiANT DICOM and CorelDraw X7. Radiographic data measurements were performed on the mandibular canines by measuring pulp-tooth (P), pulp-root length (R), tooth-root length (T), pulp-root width in CEJ (A), pulp-root width at the midpoint between C and A (B), and the pulp-root width in the middle root (C), which is the width of the pulp at the root of the tooth in the middle of the root, and average the value of the whole ratio (M).

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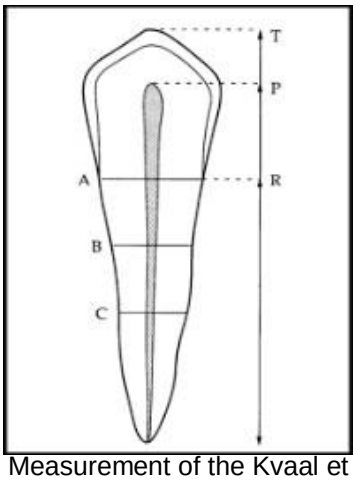

Figure 1. Measurement of the Kvaal et al., (1995)

Explanation :
P : pulp length / root length
R : pulp length / tooth length
a : pulp-root width at CEJ
b : pulp-root width at the midpoint between c and a (b)
c : pulp-root width in the middle of the root

The measurement results were calculated using the Kvaal method formula :

$$Age = 158,8 - (255,7 \times M)$$

Explanation :
M : (P+R+a+b+c)/5

Chronological age recording was done by 2 observer according to the age at the time of making radiographic photographs. Data was presented in descriptive statistics using SPSS v. 20

RESEARCH RESULT

The results of measurements of biological age and chronological age are as follows

Table 1. Descriptive statistical test results of biological age and chronological age

	Mean	SD
Biological Age	37,54	±18,79
Chronological Age	32,97	±10,92

It can be determined that using the Kvaal method there is an age difference of 4.57 years (Table 1). Furthermore chronological age is grouped into 3 groups, namely group I with ages 15-30 years, group II with ages 31-45 years and group III with ages 46-60 years.

Table 2. Descriptive statistical test results for age groups I, II and III

	Chronological Age		Biological Age	
	Mean	SD	Mean	SD
Age 15 - 30	23,85	±3,70	32,24	±12,00
Age 31 – 45	36,44	±4,34	36,9	±17,29
Age 46 – 60	52,83	±3,96	54,32	±29,68

Group I with 48 panoramic radiographs, there was an age difference of 8.39 years. Age group II with 54 panoramic radiographs, obtained an age difference of 0.46 years. Age group III with the number of panoramic radiographs of 18, obtained an age difference of 1.49 years. Next, grouping by sex was carried out on 128 panoramic radiographic photo samples (Table 2).

Table 3. Results of descriptive statistics grouping gender test

	Chronological Age		Biological Age	
	Mean	SD	Mean	SD
Male	34,08	±10,44	37,94	±17,29
Female	32,3	±10,27	35,11	±12,86

Based on table 3, in the male sex group with 51 panoramic radiograph samples, an age difference of 3.86 years was obtained. The age difference in the female sex group was 2.81 years with 77 panoramic radiograph samples. In previous studies with populations in India and showed an age difference of ± 6.4-7.8 years with modified calculations and measurements on 6 teeth. The difference can be influenced by several factors such as diet, lifestyle and race.

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In certain races found a narrow jaw bone condition causes smaller tooth size, so that the structure of the pulp is also smaller and affects the calculation due to the narrowed pulp space. Pulpal reaction that occurs when in contact with antagonistic teeth during the mastication process also affects the formation of secondary dentin deposition caused by first forming of this type of dentin when the erupted tooth completely and the mastication process.[8]In the study conducted, the number of male samples was 51 and 77 women. There were differences in biological and chronological age, namely in men by 3.86 years and the age difference in women by 2.81 years. This is not in accordance with the Saxena study which conducted research on 60 men and 60 women with populations in India. The results of these studies in the male and female groups did not show differences between biological age and chronological age. The difference in results is due to calculations using different regression formulas because the characteristics of the Kvaal method have been modified the calculations on the different races and teeth used are different. The teeth used in this study were mandibular canines, but if the mandibular canines cannot be read properly they were transferred to the mandibular first premolar. In the Saxena study only used mandibular canines.[9]

The results of this study in general can be concluded that the Kvaal method can be applied effectively in Indonesia. This is based on the difference between the biological and chronological age in this study conducted in the city of Semarang of ± 4.57 years. The difference is smaller than Kvaal's research with the population in the Caucasoid race (population in Norway) with an age gap of ± 9.5 years. This might be because in this study using digital panoramic radiographs which have advantages that can be used for evaluation of all teeth and alveolar bones in both jaws, and also digital panoramic can be obtained using standard techniques with high reproducibility.[10] In addition the radiation dose of the patient can also be minimized.[11]

However, according to Landa, et al,[12] if the radiographic data is in the form of digital data, determining the reference point on the monitor screen will reduce the accuracy so that a good resolution is needed. The patient's unfavorable position when taking a radiograph also affects the calculation of the estimated age, due to distortion and sharpness from the results of the poor photograph. This can be overcome by the selection of panoramic digital radiographs with good resolution.

CONCLUSION

The average of discrepancy between chronological age and biological age using the Kvaal method in Semarang City is ± 4.57 years in individuals aged 15-60 years and the average was 37.54 years, and the average difference between chronological age and biological age using the Kvaal method in Semarang City was ± 4.57 years in individuals aged 15-60 years.

REFERENCES

1. Anonim. Pedoman Keselamatan dan Proteksi Radiasi Kawasan Nuklir Serpong, Serpong: PUSPIPTEK, BATAN; 2011. p. 43-63.
2. Bansode SC, Kulkarni MM. Importance of palatal rugae in individual identification. J Forensic Dent Sci. 2009;1:77-81.
3. Harlan J. Dentin Hypersensitivity. J Dentistry. 2016; 12: 220-8.
4. Panchbhai, A. S. Radiographic Evaluation of Developmental Stages of Third Molar in Relation to Chronological Age as Applicability in Forensic Age Estimation, Dentistry. 2012. S1:002.1–7.
5. Kvaal SI., Kolltveit KM., Thomsen IO., Solheim T. Age Estimation of Adults From Dental Radiographs. Forensic Sci Int. 1995; 74(3):175- 85.
6. Murray EP, Matthews JB, Sloan AJ, Smith AJ. Analysis of Incisor Pulp Cell Populations in Wistar rats of Different Ages. Arch Oral Biol. 2002:709-15.

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Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
DOI: 10.30649/denta.v14i1

7. Rajpal, P. S., & Krishnamurthy, V. Age estimation using intraoral periapical radiographs, J Forensic Dent Sci. 1995;8:56-7, 2–7.
8. Bhakhar V., Ghanchi M., Saxena M. Secondary dentin as an age indicator parameter, Int J Ant For Od. 2016;1(2): 20-22.
9. Saxena, S. Age Estimation of Indian Adults FromOrthopantomographs, Braz Oral Res. 2011;25(3):225-9. doi: 10.1590/s1806-83242011005000009.
10. Chandramala, R., Sharma, R., Khan, M., & Srivastava, A. Application of Kvaal ' s Technique of Age Estimation on Digital Panoramic, J Dentistry. 2012; 2:142.
11. Murat, O. Application of Kvaal et al's age estimation method to panoramic radiographs from Turkish individuals, J. Forsciint. 2012: 219(1-3):141–6.
12. Landa, M. I., Garamendi. P.M., Botella, M. C., & Aleman, I. Application of the method of Kvaal Et Al To Digital Orthopantomograms. Int J Legal Med. 2009: 123(2):123-8.

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RESEARCH ARTICLE

Kaempferol and Quercetin Isolated from The Leaves of Atingia Excelsa to Arrest Cell Cycle in G0/G1 Phase Human Tongue Cancer SP-C1 Cell Lines

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ABSTRACT

The leaves of Altingia excelsa were found to strongly inhibit SP-C1 human tongue cancer cell lines. This study was focused on identifying the antiproliferative compound found in A. excelsa leaves and assessing its mechanism of action. The active compound was isolated using column chromatography and identified by the spectroscopic method and was also tested for its anti-proliferative properties and the cell cycle analysis in SP-C1 cells by flowcytometry analysis. This work resulted in the isolation of a flavonoid, which was identified to be kaempferol and quercetin. The compounds inhibited SP-C1 cell proliferation in a time- and dose-dependent manner with IC_{50} values of 0.72 µg/mL and 0. 70 µg/mL for the 24 hours treatments, respectively. Furthermore, the flowcytometry analysis suggested that the compounds exerted its anticancer activities by inhibiting cell cycle. These results suggested that compounds found in A. excelsa provides a basis for its potential use in cancer disease management.

Keywords: *Altingia excelsa, cancer, cell cycle, kaempferol, quercetin.*

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INTRODUCTION

Approximately 300.373 new cases of oral squamous cell carcinoma (OSCC) are annually reported around the world, what makes oral cancer the sixth most common cancer worldwide. The term oral cancer is referred to as a subgroup of head and neck malignant neoplasms affecting the lips, the anterior two-thirds of tongue, the salivary glands, the gingiva, the floor of the mouth, the oral mucosal surface and the palate, with the tongue being the most common location.[1] Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of this anatomic site, and in approximately 80% of cases, it is associated with extrinsic factors such as the use of tobacco, alcohol or both.[2] OSCC is a very difficult disease to treat because of multidisciplinary and diverse treatment strategies and the varied natural behavior of the cancer. Local invasion and frequent regional lymph node metastases together with relative resistance to chemotherapeutic. Management of OSCC varies consider-ably; small cancers of the oral cavity are usually managed by surgery alone, whereas advanced oral cancers are usu-ally treated with primary radical surgery followed by ra-diation or chemoradiation and targeted therapy.[3] The poor outcome of chemotherapy to OSCC contributes to the poor prognosis for OSCC.[4] Therefore, novel, effective therapy for OSCC treatment is still needed. Due to this high incidence, the identification of novel compounds that inhibit cancer development has become a crucial objective for scientists. Of the hundreds of chemicals that have been and are being evaluated for their anti-cancer activities, natural products derived from medicinal plants rank among the most promising.[5] To identify novel agents that may inhibit cancer development, we have focused our investigations on discovering bioactive compounds from high plants level. [6]

In our previous study, we found that the leaves of the Hammamelidaceae family, demonstrated anti-tumor properties.[7,8] These preliminary studies suggest that *A. excelsa* may be further developed as a source of anti-cancer agents. Thus, in this study, we investigated and characterized the inhibited cell cycle activities of *A. excelsa* leaf extracts.

MATERIALS AND METHODS

Plant materials. A. Excelsa leaves were gatted from Wayang Windu Mountain, Pangalengan, West Java, Indonesia. The plant species were identified by plant taxonomy laboratorium of Biology Departement, FMIPA, Padjadjaran University, Indonesia.

Extraction and isolation. Simplicia leaves of *A. excelsa* (2.5 kg) were extracted with methanol (3x24 h) at 20-25^{0}C (Room temperature). The solvent was subsequently evaporated under reduced pressure at 50˚C to produce a concentrated extract. 280 g of methanol extract was fractionated by *n*-hexane and water to gained an 86 g *n*-hexane extract and the layer of water. The water layer then extracted with ethyl acetate to gained an 120 g ethyl acetate fraction and 90 g water fraction. Fractions cytotoxicity was assessed on SP-C1 tongue cancer cells using methyl thiazolyl tetrazolium (MTT) assay. Ethyl acetate fraction, which was the most active fraction, was chromatographed by Wakogel C_{200} (Wako Pure Chemical, Japan) with a mixture of *n*-hexane, ethyl acetate and methanol with raised the polarity. Main compounds were then isolated and purified by silica G_{60} with sulfuric acid-ethanol (1:9) and were identified by spectroscopic methods consist of mass spectroscopy (MS), ultraviolet (UV), infrared spectrometry (IR), and nuclear magnetic resonance (NMR).[9]

Cell culture and treatment. SP-C1 human tongue cancer cell line was used in this research were used RPMI-1640 as cultured medium (Sigma, St. Louis, MO, USA) added with 10% FBS and antibiotics among them 100 U/mL penicillin and 100 µg/mL streptomycin. Cell's treatments, several concentrations of the sample were added to the cell culture medium. After 24 h, the cells were released from treatment, the medium was replaced, and cells were subsequently gathered at the indicated times.[10]

Cell cycle analysis using flow cytometry. The cells were grown in 24-well plates at 37ºC with CO_2 from 5% until 80% encounter was

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reached. Afterwards, medium was changed, and flavonoids were added to the indicated concentrations. Furthermore, cells were incubated for 48 h at 37ºC. After incubation, the cells were harvested and rinsed three times with ice-cold PBS (pH 7.4). The supernatant was eliminated, and the cells were rinsed with 1 mL of PBS and centrifuged at 4 °C. Finally, the supernatant was eliminated, and 200 µL of 70% ice-cold ethanol and 200µL of PBS was added to the cells and stored at –20 ºC for further use. Utilization in the flow cytometry experiments, the cell pellet was rinsed twice with PBS. Cell pellet was suspended in 0.5 mL of staining reagent (50µg/mL PI, 50 U/mL RNase, 0.1 mM EDTA,0.1% Triton X-100, and PBS) and incubated for 30 min at 37 ºC in the dark. Measuring DNA fluorescence was used Becton Dickinson (Franklin Lakes, USA) FACScanto II flow cytometer with 488 nm excitation wavelength and 585 nm emission wavelength. Pulse width area signals were used to discriminate between G2 cells and cell doublets. Data analyzed was used FACSDiva Software (Beckton Dickinson). Relative distribution of 10^4 events for each sample was analyzed for background aggregates and debris, apoptosis indicator and the G0/G1-, S-, and G2/M-phases of the cell cycle. Control treatments consisted of a culture medium supplemented with FBS. Serum-deprivation treatment was used as an inducer of G0/G1 cell cycle arrest.[11]

RESULTS

Fraction of Ethyl acetate *A. excelsa* leaves inhibits SP-C1 cell proliferation. Treatment MTT assay shown that *A. excelsa* methanol extract was inhibit the proliferation of SP-C1 human tongue cancer cells (IC_{50} 75.41 µg/mL) (Fig. 1). Methanol extract then fractionated based on polarity, using *n*-hexane, ethyl acetate and water. Afterward the fractions individually applied to SP-C1 cells and inhibit cell proliferation with an IC_{50} value of 44.85 µg/mL for the *n*-hexane fraction, 12.85 µg/mL for the ethyl acetate fraction and 18.02 µg/mL for the water fraction. Due to its low IC_{50} value, we then explored the ethyl acetate fraction for its anti-cancer potential.

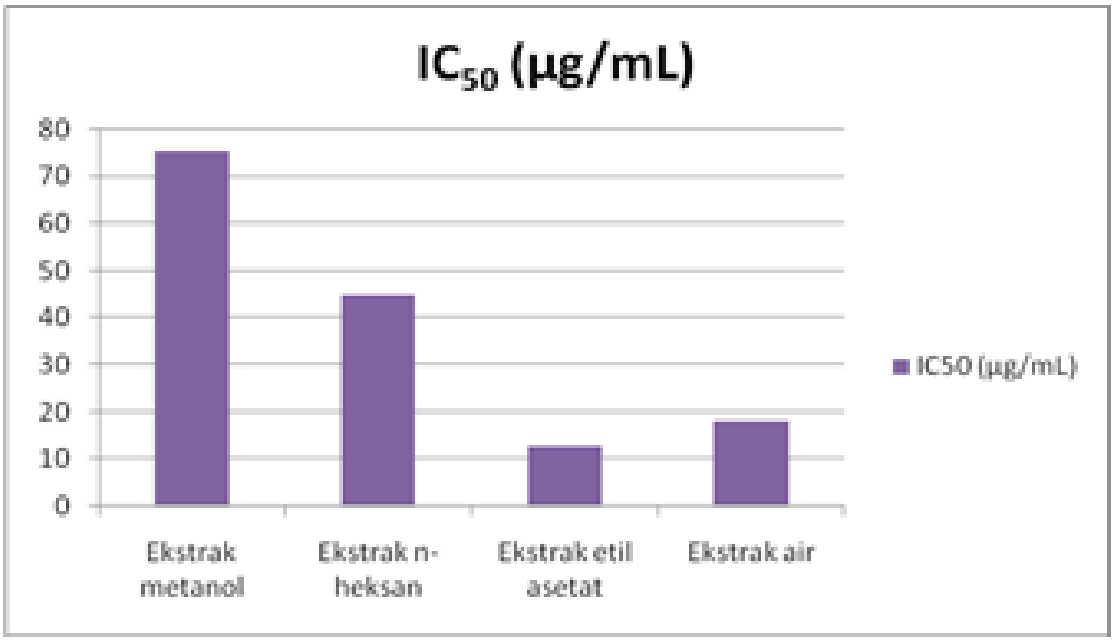

Figure 1. IC_{50} value some extracts *A. excelsa* Nornha

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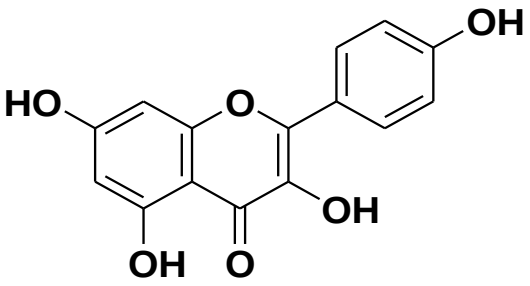
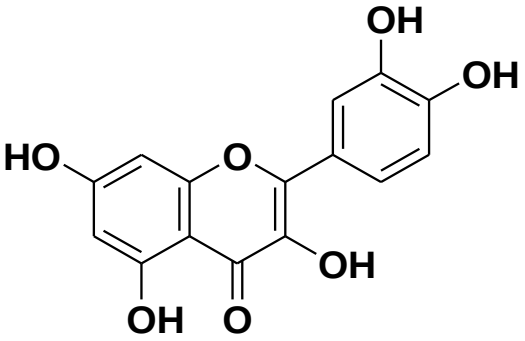
Main compounds of the ethyl acetate fraction are Kaempferol and quercetin. Isolation and purification of the ethyl acetate fraction *A. excelsa* extract to gathered bioactive compounds. The compound exhibited a melting point at 152.7-153˚C and a molecular ion peak at *m/z* 432 in the LC-MS spectrum. According to hydrogen and carbon content, the molecular ion peak and ^{1}H and ^{13}C NMR profiles indicated that the compound has a molecular formula of $C_{15}H_{10}O_7$. Ultraviolet spectrum provides maximal absorbance peaks at λ_{max} 265 and 342 nm, which were characteristic of a flavonoid with flavone skeleton. The addition of NaOH produced bathochromic shift in the absorption bands, indicating the presence of hydroxyl groups in the skeleton, one of which was attached to C-5, as indicated by further bathochromic shift following the addition of H_3BO_3. Absorption bands at 1,675 and 3,197 nm of the IR spectrum indicated where the molecule harbors conjugated carbonyl and hydroxyl groups, respectively.

^{1}H NMR spectrum of the compound shown two hydrogen of aromatic signals with 'meta coupling' at δ 6.35 (1H, *d*, *J* = 2.2 Hertz) and 6.18 (1H, *d*, *J*=2.2 Hertz), which was predicted by hydrogens at C-6 and C-8 of the A ring of the flavone skeleton. Hence, this compound was suggested to have a group of hydroxyl in C-5 and C-7. Furthermore, ^{1}H NMR spectrum revealed two signals with 'ortho coupling' at δ 6.92 (2H, *d*, *J* = 6.7 Hz) and 7.74 (2H, *d*, *J* = 6.7 Hz), the approximation signals from the hydrogens at C-2', C-3', C-5' and C-6' of the B ring. Lack of a specific signal for olefinic hydrogen at C-3 and existence of an anomeric hydrogen signal at δ 5.37 (1H, *d*, *J* = 7.2 Hz) suggested that the compound was a flavonol glycoside. Appearance of anomeric carbon signal at δ 94.9 in the ^{13}C NMR spectrum indicated the availability of a sugar moiety. In consequence to a correlation between the anomeric hydrogen signal (δ 5.37) and the anomeric carbon signal (δ 94.9) that revealed by analysis of the HMBC spectral data, sugar moiety position was established to the C-3 hydroxyl group, signal of methyl.



Chemical structure of Kaempferol



Chemical structure of Quercetin

Quercetin and Kaempferol inhibits SP-C1 cell proliferation in a concentration-dependent manner. Kaempferol and quercetin effects on the viability of SP-C1 cells were evaluated. Treatment of cancer SP-C1 cell lines with kaempferol and quercetin shown that concentration-dependent inhibit of cell growth, as demonstrated by the MTT assay. 24 hours of treatment with kaempferol and quercetin inhibited the proliferation of SP-C1 cells with IC_{50} values 0.72 and 0.70 µg/mL, respectively. Subsequent cell cycle analysis, based investigation applying the IC_{50} concentration of 0.39, 0.78, and 1.56 µg/mL kaempferol and quercetin was applied on SP-C1 cells.

Kaempferol and quercetin inhibited the SP-C1 cell cycle in the G0/G1 phase. To determinate the percentage of SP-C1 cells present in different phases of the cell cycles G0/G1, S, and G2/M, first cells were synchronized with serum deprivation before

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treatment with or without quercetin and kaempferol at several concentrations (C, K 0.39, K 0.78, K 1.56, Q 0.39, Q 0.78, and Q 1.56 µg/mL) for 24 h period, serum-deprived cells were progressively accumulated in the G0/G1-phase, whereas serum-supplied cells were present in the S- and G2/M-phases (Figure 2). Treatment with K (all concentrations) and Q (all concentrations) significantly increased the percentage of cells in the G0/G1-phase and decrease in S and G2/M-phase as compared with the control. Particularly, Cell percentage in G0/G1-phase increased an appropriate increase concentration compared to the control after K treatment. Percentage of cells in the G0/G1 – phase increase compared to the control after Q treatment but the percentage of cell decrease a long increase in concentration.

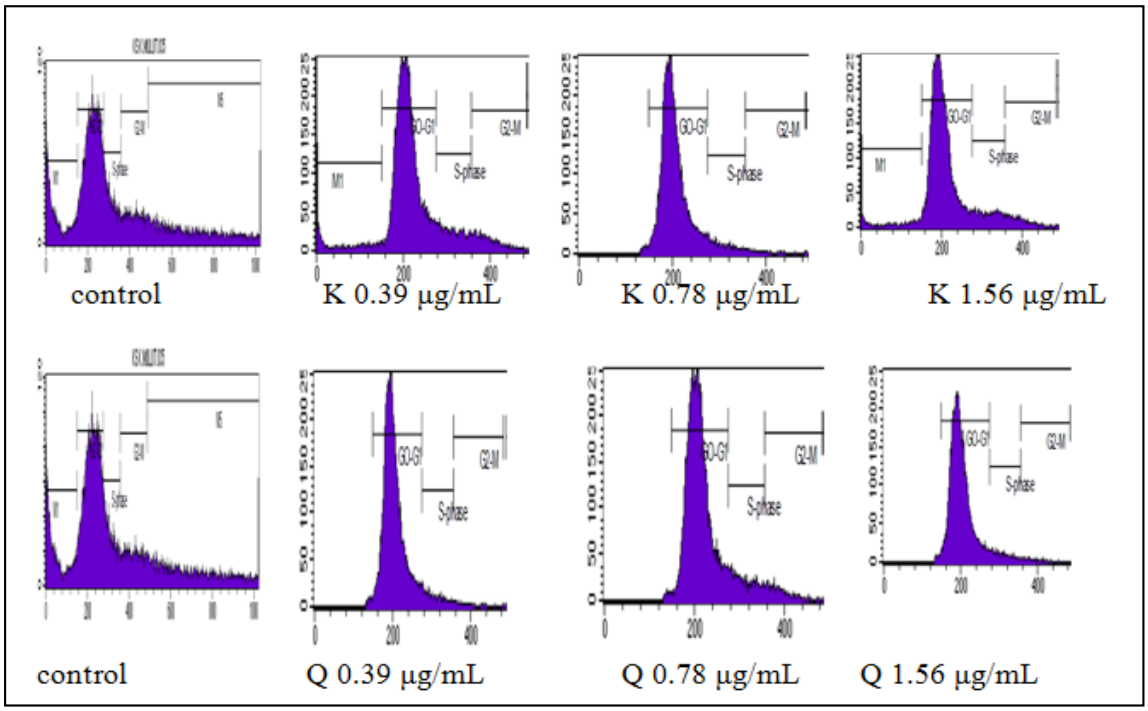

Figure 2. Flowcytometry analysis SP-C1 cancer cell using treated some concentration of kaemperol and quercetin.

DISCUSSION

The ^{1}H- and ^{13}C-NMR spectra of compounds from *A. Excelsa* exhibited resonances due to aromatic systems. The ^{1}H-NMR spectrum of them showed the presence of two doublet signals corresponds to four aromatic protons in ring B, characteristics for the 1′,4′-disubstituted flavone. The ^{13}C-NMR signals of them were assigned with the help of a DEPT experiment. A total of fifteen carbon signals were observed in the ^{13}C-NMR spectrum. The degree of unsaturation was accounted for eight out of the total eleven double bond equivalents. The ^{13}C-NMR spectrum of them showed the presence of 15 aromatic carbon signals. So, from ^{1}H and ^{13}C NMR profiles indicated that the compound had a molecular formula of $C_{15}H_{10}O_7$.

Rural medicinal plants since a long time as sources of potential therapeutic medication, and find out of novel medication or leads compound are usually based on that approach.[12,13] In drug invention, researcher have recently applied a new approach to selecting plants based on the Hammamelidaceae family.[7,8] In previous research, we found that the *A. excelsa* leaves extracts were strongly cytotoxic to the SP-C1 Human tongue cancer cell lines. Therefore, these extracts potential for further investigation. Present study focused on identifying anti-

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proliferative compound from the *A. excelsa* leaves. This study was isolated of two flavonoids, kaempferol and quercetin, which strongly inhibited proliferation of SP-C1 cell lines in a time- and concentration-dependent manner. There is no compound that reported before in relation with its cytotoxicity in these cancer cell lines.

In this study, kaempferol and quercetin with some concentration to arrest G0/G1 phase cell cycle. Cell growth is an additional amount of cells from the cell cycle process. The cell cycle consists of 4 phases, that is G_1, S, G_2, and M. G_1 (Gap 1) is a phase when the cell will synthesis the DNA or go out from cell cycle reversibly or irreversibly to differentiation. The cell that in the G_1 phase will easily controlling cell cycle at a point, that is in the restriction point (R) that will determine the cell to come in back to the cell cycle, go out from cell cycle entering G_0 phase or differentiating.[14]

When the cell cycle phase flow in restriction point dan enter S phase controlled by cyclin-dependent kinase (Cdks) and D, E, and A cyclin. D cyclin function as a growth factor that the expression more depend on the extracellular signal than the cell position on the cycle.[15] When the cell entering the G_0 cycle, on or more of the D cyclin (D1, D2 and D3) were inducted as apart from the first response of a growth factor stimulation, protein synthesis and former complex with catalytic subunit (Cdk4, Cdk6) depend on the mitogenic stimulation.[16] In reverse, if the mitogenic substance were removed, so the D Cyclin synthesis will stop. D cyclin is an unstable protein and the enzymatic activity quickly disappear, so that the cell quickly go out from the cell cycle. A specific inhibitor of Cdk4 and Cdk6 known as Ink4 can directly stop the activity of D cyclin/cdk 4/6 and causing G_1 phase arrest (rest of G_1 phase).[16]

Cell cycle inhibition of tongue cancer (SP-C1 after kaempferol and quercetin intervention as the results of Rasmala leaves isolation. There is a system that controlled the cell cycle. The controlling system known as a checkpoint. There are 4 checkpoints that found on cycle cell, that is G_1, S, G_2, and M. Checkpoint in a cell cycle involving protein cyclin groups and cyclin-dependent kinase (cdk).[17] Protein groups that involved on the cell cycle checkpoint determine cell cycle to stop, DNA repair, or apoptosis if the DNA repair doesn't occur.[18] Kaempferol (4) can inhibit cell cycle on G_0-G_1 phase at 0.39 µg/mL, 0,78 µg/mL and 1,56 µg/mL concentration. This cell cycle inhibition suspected because of kaempferol (4)/ quercetin (5) that can inhibit the work of c-myc. We know that the function from c-myc transcription factor is increasing D cyclin and E cyclin, so that there are increasing activity of G1-CDK (D1-CDK cyclin) and G1/S-CDK (E-CDK6 cyclin). Thus, the quercetin in this research can inhibit tongue cancer SP-C1 cell cycle on G_0-G_1 phase at 0,39 µg/mL, 0,78 µg/mL dan 1,56 µg/mL concentration. This results analog with the research who is conducted by Mocanu *et al,* in 2013 years that shows quercetin can inhibit Epidermoid Cancer Cell line A-431 cell cycle at 5 and 10 µM concentration in 24 hours on G_0/G_1 phase and inhibit Mamary SK-BR-3 cell cycle at 50 and 75 µM concentration in 24 hours on G_0-G_1 phase. Research belonging to Chen *et al*, in 2011 years shows that quercetin can inhibit oral cancer OSCC SCC-25 cell cycle at 50 and 75 µM concentration in 12 hours incubation on the G_1 phase. Cell cycle inhibition which is conducted by quercetin on G_0-G_1 phase possibly because quercetin will synergize with cyclin-dependent kinase inhibitor (CDK1) p21 that that function as inhibitor D-CDK4/6 cyclin activity.[19] With inhibition of D-CDK4/6 cyclin activity so the cell will be obstructed to enter S phase, so that the cell will stop on the G_0/G_1 phase or p21 beside binding cdk and PCNA (proliferating cell nuclear antigen), that is a DNA polymerase α subunit, which needed for replication or DNA reparation. P21 will inhibit cell cycle on the G_1 phase as a result of cell-contact inhibition and because of TGF β.[20]

CONCLUSION

In conclusion, our results suggest that kaempferol and quercetin inhibited the growth of

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SP-C1 cells through inhibited cell cycle in the G0/G1 phase.

REFERENCES

1. Liviu F and Johan L. Oral Squamous Cell Carcinoma: Epidemiology, Clinical Presentation and Treatment. Journal of Cancer Therapy. 2012; 3:263-268.
2. Juliana HG, Scheidt LS, Yurgel KC, Maria AZ, Figueiredo FGS. Characteristics of oral squamous cell carcinoma in users or non users of tobacco and alcohol. *Rev Odonto Cience*. 2012; 27(1): 69-73.
3. Sankalp A G, Munira M, and Chintan B. Oral Squamous Cell Carcinoma: Current Treatment Strategies and Nanotechnology-Based Approaches for Prevention and Therapy. Critical Review in Therapeutic Drug Carrier Systems. 2016; 33(4): 363–400.
4. Tantry M, Aulia I, Andri H, Endang S, Mantra N, Alwin K, and Harmas Y Y. The incidence of oral squamous cell carcinoma (OSCC) and its relationship with orofacial pain in oral cancer patients in West Java Province, Indonesia. Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology. 2017; 29: 29–32
5. Tan W, Lu J, Huang M, Li Y, Chen M, Wu G, Gong J, Zhong Z, Xu Z, Dang Y, Guo J, Chen X, and Wang Y. Anti-cancer natural products isolated from chinese medicinal herbs. *Chinese Medicine.* 2011; 6: 27-37.
6. Anna L and Krzysztof G. Anticancer Activity of Natural Compounds from Plant and Marine Environment. Int. J. Mol. Sci. 2018; 19: 1-38
7. Kim HH, Yi HS, Hwan MO, Hyuk KH, Ra KS, and Lee MW. Anti oxidative and anti-proliferative activity on Human Prostate Cancer Lines of the phenolic compounds from *Corylopsis coreana* Uyeki. *Molecules*. 2013; 18: 4876-4888.
8. Yang YN, Chen JH, Zhou GS., and Tan YP. Pentacyclic triterpenes from the resin of *Liquidambar formosana*. *Fitoterapia*. 2011; 82: 927-931.
9. Ashok A. Structural elucidation of compounds using different types of spectroscopic techniques. JCHPS. 2014; Special Issue 5:22-25.
10. Arlette SS, Roosje RO, Supriatno, Willyanti S, Sidik and Unang S. 8-Hydroxycudraxanthone G Suppresses IL-8 Production in SP-C1 Tongue Cancer Cells. NatProdComm. 2014; 9 (1):75-78.
11. Vanzyl EJ, Rick KRC, Blackmore AB, MacFarlane EM, McKay BC. Flow cytometric analysis identifies changes in S and M phases as novel cell cycle alterations induced by the splicing inhibitor isoginkgetin. PLoS ONE. 2018; 13(1): 171-191.
12. Ritva T and David J N P. Mitogen-Activated Protein Kinases: Functions in Signal Transduction and Human Diseases. Int. J. Mol. Sci. 2019; 20: 2-5
13. Zhang D, Liu J, Mi X, Liang Y, Li J, and Huang C. The N-terminal region of p27 inhibits HIF-1α protein translation in ribosomal protein s6-dependent manner by regulating PHLPP-ras-ERK-p90RSK axis. *Macmillan Publisher Limited: Cell Death and Disease*. 2014; 5:1535-1540.
14. Liu JD, Wang YJ, Yu CF, Chen LC, Lin JK, Liang YC, Lin SY, and Ho YS. . Molecular mechanisms of G_0/G_1 cell-cycle arrest and apoptosis induced by terfenadine in human cancer cells. *Molecular carcinogenesis*. 2013; 8 (3): 26-31
15. Denkert C, Furstenberg A, Daniel PT, Koch I, Kobel M, Weichert W, Siegert A, and Hauptmann S. . Induction of G0/G1 cell cycle arrest in ovarian carcinoma cells by the anti-inflammatory drug NS-398,but not by COX-2-specific RNA interference. *Oncogene* . 2013; 22, 8653–8661.
16. Xiao LS, Yun SZ, Xue FW, Wen JZ, Zheng W, Fei Z, Yi JZ, Jian HL, Jia WM , Yun PH, Lei C, Huai PL, Yuan YY, Ying BL and Jun G.. Casticin induces apoptosis and G0/G1 cell cycle arrest in gallbladder cancer cells. *Cancer Cell Int*. 2017; 17(9):111-116.
17. Marcella. L. B. C, Elaine. P. P, Andréia. H. O, Roger. C, Sônia. N. B, and Lidia. A. G.. Morphological alterations and G0/G1 cell cycle arrest induced by curcumin in human SK-MEL-37 melanoma cells. *Braz. arch. biol. technol*. 2010; 53 (2): 6-11.
18. Cai. X, Hu X, Tan X, Cheng W, Wang Q, and Chen X.. Metformin Induced AMPK Activation, G0/G1 Phase Cell Cycle Arrest and the Inhibition of Growth of Esophageal Squamous Cell Carcinomas In Vitro and In Vivo. *PLoS ONE*. 2015; 10(7):133-139.
19. Xinying L, Hongxia S, Feng K, Yanxia G, Yuan C, Lu Z, Dongfang G, Xiaofei Z and Han Z.. Pemetrexed exerts anticancer effects by inducing G0/G1-phase cell cycle arrest and activating the NOXA/Mcl-1 axis in human

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esophageal squamous cell carcinoma cells. *oncology letters*. 2018; 17 (2): 1851-1858.

20. Che. J. H, George. H, Wei. L. N, Shih. W. W, Chun. P. C, Li. Y. L, Jih. H. G, Tzong. W. L and Chi. L. C. Cephalochromin Induces G0/G1 Cell Cycle Arrest and Apoptosis in A549 Human Non-Small-Cell Lung Cancer Cells by Inflicting Mitochondrial Disruption. *J. Nat. Prod.* 2014; 77 (4): 758-765.

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RESEARCH ARTICLE

The antibacterial effect of Anchovy (Stolephorusinsularis) Extract Against P.aeruginosa

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ABSTRACT

Introduction: *Root canal infection is largely a continuation of the caries process that is not treated and develops so that it involves the root canal. Sterilization is a part of multivisit root canal treatment and important for the success of root canal treatment. The Anchovy (Stolephorus insularis) contains protein, vitamins (A, B1, C), and minerals (Fe, Ca, K, F). Calcium fluoride (CaF2) contained in anchovies can inhibit bacteria that cannot be removed by chemo-mechanical processes such as instrumentation and irrigation* ***Purpose:*** *The aim of this study was to determine the antimicrobial ability of anchovy extract (Stolephorus insularis) to P. aeruginosa.* ***Materials and Methods:*** *This study was a laboratory experimental research which with post test only control group design. Diffusion method were applied with 2 controls: positive control using ChKM solution, negative control using 1% DMSO and 3 concentrations of jengki anchovy extract (Stolephorus insularis) 18%, 24%, and 30% as treatment groups, where each group consisted of 5 samples. Antimicrobial was assessed by measuring the diameter of the clear zone around the discs contained the anchovy extract (Stolephorus insularis). Data were analyzed by Kruskal-Wallis test followed by Mann-Whitney test.* ***Result:*** *The results from this study showed clear zone around the discs of the anchovy extract (Stolephorus insularis). The more concentration of the extract showed the more antimicrobial zone diameter. The average inhibitory zone at 18% concentration was 6.03 mm, 24% 7.59 mm, 30% 8.69 mm, positive control of ChKM solution 31.43 mm for negative control DMSO 1% 6.03 mm. The largest diameter of the clear zone is at a concentration of 30%.* ***Conclusion:*** *The results obtained showed that the inhibition zone of the Stolephorus insularis extract concentration of 30% had the largest average among the other concentrations of 8.69 mm.*

Keywords: *Anchovy extract (Stolephorus insularis), P. aeruginosa, root canal sterilization, antimicrobial effect.*

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INTRODUCTION

Dental and oral health is an important issue and needs to be considered. The most common dental and oral health problems are caries and periodontal disease.[1] Caries can affect both adults and children. Caries process mostly progressed as root canal infection. Untreated caries will continue to grow and become a gateway for microorganisms to enter the pulp and activated inflammatory response than continue to become pulp necrosis.[1,2]In necrosis teeth, pulp tissue will be a media of bacterial products accumulation and microorganism breakdown products[3,4] Bacteria and their products and inflammatory mediators will accumulate in the root canal and exit to the periapical tissue through the apical foramen so that abnormalities occur in periapical tissue such as chronic apical periodontitis [3,5]

Chronic apical periodontitis is a polymicrobial infection, an infection caused by various types of bacteria, namely Pseudomonas aeruginosa (P.aeruginosa), S.aureus, A.faecalis, Actinomyces spp, Streptococcus spp, Porphyromonas spp and Eubacterium[6] One of them is P.aeruginosa is a bacterium found in the root canals that causes endodontic treatment failure. P. aeruginosa is an opportunistic component of root canal microbes that can be found in primary endodontic infections, in tooth necrosis, abscesses, extraction wounds, endodontic infections, sinus fluid, and injury to injury [6]

Root canal treatment can be divided into three main stages, namely root canal preparation or cleaning and forming and smoothing, root canal disinfection or sterilization and root canal filling.[7] Sterilization is a part of multivisit root canal treatment and is important for successful root canal treatment. The use of root canal sterilization agents will kill pathogenic microorganisms in the root canal. Root canal sterilization materials must meet several requirements, including effective in killing microorganisms in the root canal with a long-term effect, not toxic, does not irritate the root canal and can inhibit pain [3]

Root canal treatment is one of the dental treatment, that involves treating diseases or injuries in pulp and periapical tissue. The purpose of this treatment is to restore the condition of a tooth that is biologically acceptable to the surrounding tissue so that the tooth can be maintained as long as possible in the mouth. This means the tooth does not cause complaints and can functioning fine. [8]

Although done irrigation in the root canal, but microorganisms may still be left behind, especially in the dentine tubules. Other researchers mention that cleaning and forming root canals and irrigation of root canals significantly reduce or kill microorganisms from the root canals. But the complete elimination of microorganisms cannot always be achieved clinically, due to the anatomical shape of the root canal and the limitations of instrumentation and irrigation[6] These bacteria are mostly habitats in the mouth area especially saliva, tongue and gingival. This bacterium is quite unique and has not been isolated from other habitats. Just like Enterococcus faecalis, Actinomyces is also a gram-positive bacteria that are anaerobic and do not form spores. Aerobic bacteria such as Diphtheroids, Staphylococci, Streptococci, E.coli, Pneumoniae, P. aeruginosa are also found in root canals of teeth that experience necrosis [9,10]

Provision of root canal sterilization is intended to kill bacteria that cannot eliminated by chemo-mechanical processes such as instrumentation and irrigation. Based on their chemical properties, root canal sterilization materials are divided into two groups, namely specific drug classes and non-specific drugs which can be in the form of one or a combination of several antibiotics. One of the root canal sterilization drugs that are often used is the phenol group, such as ChKM (Chlorophenol Kamfer Menthol) which has a disinfection ability and has a broad spectrum so that it can be used in root canal treatment [11] However, this phenol sterilization drug has

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several weaknesses that are toxic, can kill cells that are still potential, are allergens that can cause immune reactions and have a pungent odor and an unpleasant taste that causes discomfort in patients [3]

In a study conducted by Fa'izah (2016) using anchovy extract (Stolephorus insularis) has antibacterial activity against Streptococcus mutans at concentrations of 3%, 6% and 12%. In this study it can be seen that the fluoride contained in the extract of anchovy (Stolephorus insularis) has antibacterial activity against Streptococcus mutans.[12] Stolephorus insularis contains antibacterial substances such as fluoride. Besides that, Stolephorus insularis also contains energy, protein, fat, carbohydrate, calcium, iron, phosphorus, vitamin A, vitamin B and vitamin C.[13] In Indonesia, Stolephorus insularis is very easy to obtain and most people consume it.[14] This is because Stolephorus insularis is one of the most abundant resources throughtout Indonesia, especially in coastal waters.[12,15]

Antimicrobials are biological or chemical compounds that can interfere with microbial growth activities.[16,17] Fluoride in bacteria works bacteriostatically, which inhibits cell proliferation by preventing the synthesis of nucleic acids which are a vital part of cell development. The mechanism of action is by binding to the polymerase-RNA enzyme (in subunits) so that it inhibits RNA and DNA synthesis.[18]

Based on the above reasons, the researchers wanted to know the concentration of Stolephorus insularis extract which was effective as an antimicrobial against the growth of P. aeruginosa bacteria.

MATERIALS AND METHODS

This research is an experimental laboratory research. The design of this study is the post test only control group design. For the study design, group objects are divided into 5 groups. The study design group 1 (negative positive control group) using 1% DMSO ChKM, group 2 (negative control group) using 1% DMSO and groups 3, 4, and 5 were the treatment groups that were given jengki anchovy extract (Stolephorus insularis) with different concentrations, namely 18%, 24%, and 30%.

The experimental unit or sample used in this study is pure P. aeruginosa bacteria from the Microbiology Laboratory of the Surabaya Health Laboratory Center with the criteria that the number of P. aeruginosa bacterial colonies is synchronized with a Mc Farland solution of 0.5.

P. aeruginosa bacteria obtained in the form culture of blood agar, then taken as much as 2 ose then incubated on liquid BHI (Brain Heart Infusion) media for 2x24 hours at 37 ° C in an anaerobic atmosphere. The turbidity of the P. aeruginosa bacterial suspension was then synchronized with the Mc Farland standard 0.5 to obtain a bacterial suspension containing 1.5x108 CFU / ml (Colony Forming Units) with the eye carried out by holding the test tube next to each other and looked at him on a white background with black stripes. If the turbidity of the bacterial suspension is still not the same, the bacterial suspension can be diluted or given additional bacteria, then homogenized by centrifuge.[9] The process of equalizing bacteria with the Mc Farland 0.5 standard is carried out at the Oral Biology Laboratory of the Faculty of Dentistry, Hang Tuah University, Surabaya.

Antibacterial power was examined by measuring the diameter of the clear zone that appeared around the filter paper placed on the BHI agar media by the diffusion method. Measurement using a digital caliper (with a diameter of mm). In order to obtain more valid data, measurements were made three times, namely the longest, medium and shortest diameters and then taken an average of the three length measurements.

The anchovy extract (Stolephorus insularis) is a sea fish obtained from Kenjeran Beach fishermen. The anchovy (Stolephorus insularis) used for this research is fresh the anchovy. The anchovy (Stolephorus insularis) is extracted by maceration method by means of

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the anchovy (Stolephorus insularis) is roasted at <100ºC for three hours in five days, blended, then sifted until 100 mesh fineness. The anchovy fish powder (Stolephorus insularis) is taken as much as 500 grams, then soaked using 96% ethanol until submerged for 3x24 hours. Then the thick extract of the anchovy (Stolephorus insularis) with semisolida consistency made three concentrations of 18%, 24%, and 30% using 1% DMSO.

Prepare BHI media so that it is sterile for 5 research samples, each sample consisting of 3 treatment groups and 2 control groups. Taking P. aeruginosa culture from liquid BHI (Brain heart Infusion) media that has been equaled with turbidity with Mc Farland 0.5 solution (bacterial suspense containing 1.5x108 CFU / ml), then rub the bacterial culture on the entire surface of BHI media (Brain Heart Infusion)) so that by using a sterile cotton swab.[9]

In the positive control group, the disc was immersed in 1 ml of ChKM solution for 10 seconds. In the negative control group, the disc was immersed in 1 ml DMSO 1 ml for 10 seconds. In the treatment group, the disc was immersed in Stolephorus insularis extract with a concentration of 18%, 24% and 30% for 10 seconds, then placed the disc in one of the BHI media zones using sterile tweezers and was slightly pressed. Insert petridish into anaerobic jar with gaskit and anaerobic indicator, then incubated in an incubator for 2x24 hours at 37ºC.

Inhibited zones formed were measured and the data obtained were analyzed by statistical tests using SPSS version 23.

RESULTS

The research data were analyzed descriptively to get a picture of the distribution and summation of data in order to clarify the presentation of research results.

Table 1shows that there is an antibacterial power of Stolephorus insularis extract at a concentration of 18% (6.03 mm); 24% (7.59 mm); 30% (8.69 mm); and in the positive control, ChKM (31.43 mm) against P. aeruginosa. While the negative control of 1% DMSO did not show any antibacterial power against P. aeruginosa.

Table 1. Average diameter of inhibition zone and standard deviation of Stolephorus insularis extracts against the growth of P. aeruginosa bacteria.

Group	Replication	Average	Std. Deviation
K+	5	31,43	2,68
K-	5	6,03	0,01
P1	5	6,03 7,59	0,01
P2	5	8,69	0,43
P3	5		0,77
Total	25		

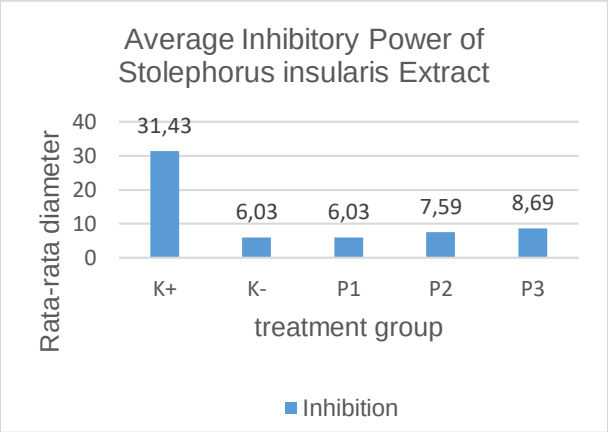

Figure 1. Graph of average diameter of inhibition zone

In Figure 1, the results of the study showed that there were inhibitory zones of Stolephorus insularis extract on the growth of Pseudomonas aeruginosa in the K +, P2 and P3 groups, whereas in the K- and P1 groups there was no inhibitory zone. The treatment was then tested for significance with an error rate of 5% (p = 0.05).

Normality test is done before the research hypothesis test. The normality test is one of the One Way ANOVA test requirements. Furthermore, the results of the study were tested for normalitydistribution using Shapiro-Wilk, because the number of research subjects is less than 50. This test aims to determine whether the data obtained are normally

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distributed or not with a significance level of 0.05 (p = 0.05). If the data is normally distributed (p> 0.05) it can proceed with homogeneity test and if the data is not normally distributed (p <0.05) then the data transformation is carried out.

Table 2.*Shapiro-Wilk test* results

Group	Shapiro-Wilk	Sig.
K+	0,896	0,391
K-	0,833	0,146
P1	0,833	0,146
P2	0,974	0,902
P3	0,967	0,854

Based on the results of the normality test it can be seen that the Stolephorus insularis inhibition zone variable in each treatment group is normally distributed because it has a p value > 0.05. Then it can be continued with homogeneity test and if the data is not normally distributed (p<0.05) then the data transformation is carried out.

Homogeneity test

Table 3.*Levene test* results

Levene Test	Sig.
8,48	0,000

From the results of the homogeneity test (Levene Test) it is known that the significance value of 0,000 has a significance value of less than 0.05 (p <0.05). , namely the Kruskal-Wallis test to determine the difference between the positive control with the treatment group concentrations of 18%, 24%, and 30% of the Stolephorusinsularis extract in each sample.

Hypothesis testing

Table 4.*Kruskal-Wallis Test*

Chi-Square	21,610
Df	4
Asymp. Sig	0,000

Table 4 obtained a significance value of 0,000 (p <0.05). This shows the difference in meaning between positive controls with each treatment group that has different concentrations. Based on this, the Mann-Whitney test was continued.

Mann-Whitney Test

Post Hoc analysis to see a significant difference in the inhibition of Stolephorus insularis on the growth of Pseudomonas aeruginosa bacteria from each group. To do the Post Hoc analysis of the Kruskall-Wallis test is to use the Mann-Whitney test

Table 5.*Mann-Whitney test results(LSD)*

	K+	K-	P1	P2	P3
K+		0,009*	0,009*	0,009*	0,009*
K-			1,000	0,009*	0,009*
P1				0,009*	0,009*
P2					0,047*
P3					

From the results of the Mann-Whitney test it is known that all treatments compared with K + (positive control / ChKM) showed significant differences. In P1 (Stolephorus insularis extract 18%) when compared to K- (negative control), there was no significant difference because the significance value was greater than 0.05 (p> 0.05), in P2 (Stolephorus insularis extract 24%) showed significant differences when compared with K- (negative control / DMSO1% solution), whereas in P3 (Stolephorus insularis extract 30%) showed significant differences when compared with K- (negative control / DMSO1% solution) To determine a significant difference determined by the average antimicrobial extract of Stolephorus insularis.

Based on table 5 the LSD test results there was a significant difference in inhibition of P. aeruginosa bacteria (p <0.05) in the K + group with the K-, P1, P2 and P3 groups, the K- group with the P2 and P3 groups. While K- with group P1 there was no significant difference because the significance value was greater than 0.05 (p> 0.05). While between group P1 with group P2, P3 and group P2 with group P3 there were significant differences.

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DISCUSSION

This study aims to determine the antibacterial power of Stolephorus insularis extract against Pseudomonas aeruginosa. Stolephorus insularis contains between 15.7-38.3 ppm fluoride, mostly in the form of CaF2 compounds which are shortly decomposed in liquid form, so they can provide sufficient quantities of F ions in their environment.[19] CaF2 provides the best fluoridation results because of its ability to release fluoride gradually and also acts as a fluoride reserve.[15]In a previous study using Stolephorusinsularis extract, it had antibacterial activity against Streptococcus mutans at concentrations of 3%, 6%, and 12%.[12] Based on these studies it can be seen that the fluoride content contained in the extract of Stolephorus insularis has antibacterial activity against the bacterium Streptococcus mutans.

In this study, the extract of Stolephorus insularis was tested for its inhibition against the Pseudomonas aeruginosa bacteria which are obligate gram-negative aerobic bacteria. The prevalence of infection in cases of failure after root canal treatment caused by Pseudomonas aeruginosa ranges from 24%-77%. This is due to various virulence and resistance factors of Pseudomonas aeruginosa.[19]

The inhibitory study of Stolephorus insularis extract on the growth of Pseudomonas aeruginosa bacteria was carried out by a diffusion method that used BHI agar media, because it was quite practical to do and was an effective media used to determine the growth of Pseudomonas aeruginosa bacteria which is a gram-negative aerobic obligate. [16] Diffusion method used in this study because researchers can see the level of sensitivity of Pseudomonas aeruginosa against Stolephorus insularis extract. In addition, the diffusion method is a simple and easy procedure and can be used to test aerobic and facultative anaerobic bacteria.[10]

DMSO 1% is used as a dilute extract of Stolephorus insularis as well as a negative control (K-) because the researchers conducted a comparison test of the homogeneity of the extract first using diluent aquades and 1% DMSO. From these results it is known that the extract of Stolephorus insularis is more homogeneous in 1% DMSO solution compared to distilled water. The use of 1% DMSO is also due to DMSO being one of the solvents that can dissolve almost all compounds both polar and non-polar.[20] DMSO does not show any influence on bacteria to 2% DMSO concentration, even there is no visible effect on 3% DMSO concentration. New bacteria showed a decrease in the number when exposed to a DMSO concentration of 4% or more.[17] The statistical test results also showed that 1% DMSO had a significant difference compared to all positive concentrations and controls.

The choice of ChKM as a positive control (K +) because it is one of the root canal sterilizers. The mechanism of action of ChKM in inhibiting bacterial growth is the same as the mechanism of action of fluorine in inhibiting bacterial growth ie inhibiting cell proliferation by inhibiting nucleic acid synthesis which is a vital part for cell development, by binding to the polymerase-RNA enzyme (in sub units) thereby inhibiting RNA synthesis and DNA.[18] In addition, ChKM is included in the derivatives of phenol compounds, the mechanism of action of phenol compounds in inhibiting bacterial growth, namely by denaturing bacterial cell proteins, inhibits cell membrane function (transporting substances from one cell to another) and inhibits nucleic acid synthesis so that bacterial growth can be inhibited.[11]

In this study Stolephorus insularis extract was examined at concentrations of 18%, 24% and 30%. The choice of concentration was based on research conducted by Fa'izah (2016), where Stolephorus insularis extract was shown to have antibacterial activity against Streptococcus mutans at concentrations of 3%, 6% and 12%. Stolephorus insularis extract with a concentration of 12% is the most effective concentration to inhibit the growth of Streptococcus mutans bacteria.[12] In this case

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researchers feel the need to increase the concentration to a concentration of 18%, 24% and 30%, this is due to various factors of virulence resistance and resistance which is found in the bacterium Pseudomonas aeruginosa. In this study it was seen that Stolephorus insularis extract had antimicrobial power against P. aeruginosa bacteria in the treatment group with concentrations of 24%, and 30%. At 18% concentration the antimicrobial power of Stolephorus insularis extract did not show any inhibitory zone with the antimicrobial power of the ChKM solution as a positive control, or with a negative control.

This is caused by anchovy (Stolephorus insularis) containing antibacterial substances, namely fluorine. The mechanism of action of fluorine in inhibiting bacteria, namely inhibiting cell proliferation by inhibiting the synthesis of nucleic acids which is a vital part of cell development. The mechanism of action of fluorine is by binding to the polymerase-RNA enzyme (in subunits) so that it inhibits RNA and DNA synthesis. [18]

In addition, fluorine also limits the supply of reserves for acid production in the synthesis of polysaccharides. The making of this acid is carried out by cariogenic bacteria that are metabolizing carbohydrates at low pH (5.5 or <5.5). However, at low pH conditions, fluoride can easily diffuse into Hydrofluoric Acid thereby inhibiting carbohydrate metabolism carried out by the cariogenic bacteria.[21] Even bacteria can experience death due to the inhibition of the effects of glycolysis in their cells so that the bacteria do not get energy.[22]

In this study it was seen that the greater the concentration of Stolephorus insularis extract, the greater the diameter of the inhibition zone. Stolephorus insularis extract with a concentration of 30% has the largest average inhibition zone of 8.69 mm, compared with an concentration of 18% with an average inhibition zone of 6.03 mm and a concentration of 24% with an average inhibition zone of 7.59 mm. However, ChKM has a greater inhibition zone compared to the whole concentration of Stolephorus extract insularis that is equal to 31.43 mm. This was also seen in statistical tests which showed that ChKM had a significant difference with the entire concentration of Stolephorus insularis extract and DMSO 1% (6.03 mm). This happens because ChKM has a fairly large phenol content consisting of 60% chloro-phenol, 40% camphor and 6% menthol. The camphor content contained in ChKM can extend its antimicrobial effect.[11] So that the inhibition of ChKM can be greater than that of Stolephorus insularis extract. Pseudomonas aeruginosa is a gram-negative bacterium that has the characteristics of a thick cell wall structure in the form of peptidoglycan.[23] Because of the characteristics of the thick bacterial cell wall, it will be very difficult for the active substance extract of Stolephorus insularis to penetrate the cell wall. In addition, gram-negative bacteria also have a defense against acidic conditions in the form of a proton pump mechanism so that it is able to balance the pH in cells and the antimicrobial substrate cannot penetrate into the cytoplasmic membrane.[24]

This study is still qualitative in nature because it only shows differences in the antibacterial power of Stolephorus insularis fish extracts against Pseudomonas aeruginosa at concentrations of 18%, 24%, 30% and research cannot be carried out in the form of quantity to measure the presence of antibacterial compounds. The results obtained showed that the inhibition zone of the Stolephorus insularis extract concentration of 30% had the largest average among the other concentrations of 8.69 mm.

CONCLUSION

Based on the results of this study, it is known that the extract of Stolephorus insularis at a concentration of 24% and 30% has an antibacterial power against Pseudomonas aeruginosa. The 30% concentration has the most effective antibacterial power to inhibit the growth of Pseudomonas aeruginosa bacteria

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REFERENCES

1. Kurniawati SW. Aktivitas Antibakteri dar iEkstrak Etanol Daun Asam Jawa (TamarindusIndica Lin) Terhadap Kultur Aktif Staphylococcus aureus dan Escherichia colli [Skripsi]. Jakarta: Universitas Negeri Syarif Hidayatullah; 2008. p. 50.
2. Yustina AR, Suardita K, W Dian A. Peningkatan jumlah osteoklas pada keradangan periapikalakibatinduksilipopolisakarida Porphyromonas Gingivalis (suatu penelitian laboratories menggunakan tikus) JBP. 2012;14(3): 140-4.
3. Walton RE, Torabinejad M. Prinsip & praktek ilmu endodonsia. Ed. 3. Jakarta: Penerbit buku kedokteran EGC; 2008.p. 36, 205 230, 324-6.
4. Hojo K, Nagaoka S, Oshima T, Maeda N. Bacterial Interactios in Dental Biofilm Development. J Dent Res. 2009;88(11): 982-990.
5. Mulyawati E. Peran Bahan Disinfeksi Pada Perawatan Saluran Akar. Maj Ked. 2011;18(2): 205-9.
6. Preethee T, Kndaswamy D, Hannah R. Molecular identification of an Enterococcus faecalis endocarditis antigen efaA in root canals of therapy-resistant endodontic infections. J Conserv Dent. 2012;15(4): 319-22.
7. Wintarsih, O., Partosoedarmo, M., Santoso, P., Kebocoran Apikal pada Irigas idengan EDTA Lebih Kecil Dibandingkan yang Tanpa EDTA (A Comparative Study of Apical Leakage on Irrigation Using and Without EDTA), Jurnal PDGI. 2009;58(2):14-19.
8. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus Faecalis: Its role in root canal treatment failure and current concpetsbin retreatment. Journal of endodontics, 2006;32(2):98-93.
9. Hermawan A. Pengaruh Ekstrak Daun Sirih (Piper betle L) Terhadap Pertumbuhan Staphylococcus aureus dan Escherichia coli Dengan Metode Difusi disk. Fakultas Kedokteran Gigi Universitas Airlangga. Jakarta: Salemba Medika; 2007.p. 3-4.
10. Bauman RW. Microbiology International Edition. San Fransisco: Pearson Education Inc and Person Benjamin Cummings; 2004.p. 299-297.
11. Osswald R. The Problem of Endodontis and Managing it Trough Conservative Dentistry. 2005.p. 134-44.
12. Fa'izah A. Konsentrasi Efektif EkstrakIkan Teri Jengki (Stolephorus insularis) Sebagai Antimikroba Terhadap Bakteri Enterococcus faecalis [Skripsi]. Surabaya: Fakultas Kedokteran Gigi Universitas Hang Tuah; 2016. p.31-41.
13. Daradjatun AN. Pemanfaatan Tepung Kepala Ikan Teri Jengki (Stolephorus insularis) sebagai Bahan Subtitusi Tepung Ikan dalam Pakan Buatan Ikan Lele Masamo (Clarias sp.) [Skripsi]. Lampung: Fakultas Pertanian Universitas Lampung; 2014. p. 7.
14. Mardjudo A, Rahman ARA. Usaha PerikananIkan Tri (Stolephorus sp.) dengan Alat Tangkap Bagan Tancap di Desa Bukit Aru Indah di Kecamatan Sebatik Timur Kabupaten Nunukan Provinsi Kalimantan Utara. Jurnal Ilmiah Agr IBA. 2014;(2): 198.
15. Zabrina S. Pengaruh Aplikasi SubstratIkan Teri Jengki (Stolephorus insularis) terhadap Tingkat Retensi Fluor pada Email Gigi Tikus Sprague Dawley (in Vivo) [Skripsi]. Jakarta: Fakultas Kedokteran Gigi Universitas Indonesia; 2012. p.1-2, 22.
16. Uttley AHC, George RC, Naidoo J, Woodford N, Johnson AP, Collins CH et al,. High-level Vancomycin-resistant Enterococci Causing Hospital Infections. Epidem. Inf, Great Britain. 2009; 103:173-82.
17. Wadhwani T, K Desai, D Patel, D Lawani, P Bahaley, P Joshi, V Kothari. Effect of various solvents on bacterial growth in context of determining MIC of antimicrobials. The Internet Journal of Microbiology. 2008; 7(1); 1-6.
18. Karina VN. Perbedaan Daya Antibakteri Bahan Perekat Braket Ortodonsi antara Semen Ionomer Kacadengan Resin Komposit Berfluor terhadap Lactobacillus acidophilus [Skripsi]. Jember: Fakultas Kedokteran Gigi Universitas Jember; 2012. p. 17-18.
19. Gunawan HA. Pengaruh Tingkat pH Larutan Teri terhadap Perubahan Dimensi dan Kelarutan Kristal Apatit. Jurnal Anatomi Indonesia. 2006;01(1): 25-29.
20. Handayani D, Maipa D, Marlina, Meilan. Skrining Aktivitas Antibakteri Beberapa Biota Lautdari Perairan Pantai Painan, Sumatera Barat. Jurnal, FakultasFarmasiUniversitasAndalas. 2009.p. 5.
21. Handini AD. PerbedaanTopikalAplikasiBahan Casein Phosphopeptide-Amorphous Calcium Phospate (CPP-ACP) danBahan Sodium

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Fluoride terhadap Jumlah Koloni Streptococcus mutans pada Saliva AnakUsia 6-12 Tahun [Skripsi]. Makassar: Fakultas Kedokteran Gigi Universitas Hasanuddin; 2013.p. 13-16.
22. Oktanovia SY. Ikan Teri Dapat Meningkatkan Ketahanan Email Gigi. Makalah Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Negeri Padang; 2013.p. 9.
23. Cooper GM, Hausman RE. The Cell: A Molecular Aproach 3rd ed. Washinton D.C: ASM Press; 2004. p. 518-19.
24. Poeloengan M. Penguian Yoghurt Probiotik pada Pertumbuhan Bakteri. Balai Besar Penelitian Veteriner. 2012. p. 303, 307.

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RESEARCH ARTICLE

The Effect of Silica Dioxide (SiO_2) Nanoparticle Coating and Duration of Coffee Immersion on Discoloration of Thermoplastic Nylon Denture Base

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ABSTRACT

Introduction: *Silica dioxide (SiO_2) nanoparticle have long been used as a denture base coating. Thermoplastic nylon denture base material is prone to discoloration due to its amide bonds absorb water easily. Meanwhile, coffee contains chlorogenic and tanic acid, which can change the color of denture bases.* ***Purpose:*** *This study was to examine the effect SiO_2 coating and duration of coffee immersion on discoloration of thermoplastic nylon denture base.* ***Method:*** *Samples consisted of 24 thermoplastic nylon in square-shaped (30 x 30 x 2 mm), divided into 4 groups (n = 6) which were control (without SiO_2 coating) and treatment (with SiO_2 coating) groups, which then were immersed in coffee solution for 15 and 30 days. Discoloration test was conducted using spectrophotometer by measuring the delta absorbance of light before and after coffee immersion.* ***Result:*** *The lowest delta absorbance was in the 15-day treatment group (0.019 ± 0.006) and the highest was in the 30-day control group (0.085 ± 0.028). Two-way ANOVA test showed that SiO_2 coating and coffee immersion had an effect on discoloration of thermoplastic nylon ($p < 0.05$). Post hoc LSD test showed that there were significant differences between the control and treatment group at 15 and 30 days of coffee immersion ($p < 0.05$).* ***Conclusion:*** *SiO_2 as a thermoplastic nylon denture base coating can reduce discoloration by coffee immersion for 15 and 30 days.There were no differences between 15 and 30 days of coffee immersion on thermoplastic nylon's discoloration in the control and treatment groups.*

Keywords: *Coffee immersion; discoloration; spectrophotometer;silica dioxide (SiO_2) coating;thermoplastic nylon.*

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INTRODUCTION

The denture base is a part of a denture that has a contact with oral mucosa, supports the artificial teeth, distributes occlusal pressure to the supporting tissue, and gives retention and stability to the denture.[1] An ideal denture base is determined based on several criteria, such as biocompatibility in the oral cavity, aesthetics, bonding with artificial teeth, radiopaque, ease of repair, and good physical and mechanical properties.[2]

Thermoplastic nylon material had been introduced as a denture base material since the 1950s for hard or soft tissue undercut cases, repeated fracture of acrylic denture, patient aesthetic issue, and acrylic allergies.[3]Thermoplastic nylon is produced by condensation polymerization reaction between hexamethyl diamine NH_2-$(CH_2)_6$-NH_2 and dibasic acid COOH-$(CH_2)_4$-COOH, which produces linear polymer chain NH_2-$(CH_2)_6$-NH-CO-$(CH_2)_4$-COOH and residual products in the form of water condensation.[4] The weakness of thermoplastic nylon is amide (-NH_2) and chromophores (C = O) groups, which easily absorb water and stain, thus susceptible to discoloration and the surface becomes rough.[5,6] The higher the concentration of amide groups, the greater the absorption of water and discoloration.[7]

Discoloration of denture base material occurs due to intrinsic and extrinsic factors.[8] Intrinsic factors are caused by changes in physics and chemical conditions of aging polymer matrix. Extrinsic factors occur due to absorption and adsorption of water around the material through water diffusion mechanism. Other factors influencing discoloration are staining, dehydration, surface roughness, oxidation, and water absorption.[8]

Several drinks such as coffee, tea, soft drinks, yoghurt, and red wine change the color of denture base.[9,10] Coffee changes the color of denture base due to its chlorogenic acid, which releases H^+ ions that disrupt the bonding chain of thermoplastic nylon.[11] Coffee also contains tanic acid, which gives yellowish brown stain.[12] The duration of coffee immersion also affects the amount of water diffusion so that the longer the thermoplastic nylon is immersed, the more water will be absorbed. Moreover, the more water that is absorbed, the greater the discoloration that occurs.[13]

Several methods have been applied to prevent discoloration and water absorption such as denture base coatings with nanoparticles.[14] Silica dioxide (SiO_2) nanoparticle has biocompatible properties to cell[15] and widely used as antimicrobial.[16] Coating the acrylic resin deture base material with 0.5% silica dioxide nanoparticles can reduce water absorption[17] and prevent *Candida albicans* adhesionto denture base.[16] Silane (3-methacryloxypropyl trimethoxy silane) is used as a coupling agent in the coating process to bridge the bonding of organic (thermoplastic nylon) and inorganic (silica dioxide) material.[18]

To evaluate the discoloration of thermoplastic nylonafter coffee immersion, a spectrophotometer is used to measure the light absorbed by thermoplastic nylon before and after the immersion, which is based on the light and dark measurement. This study aims to investigate the effect of silica dioxide (SiO_2) nanoparticles coating and the duration of coffee immersion on the color changes of thermoplastic nylon denture base.

MATERIALS AND METHODS

This study was an experimental laboratory using 24 subjects in the form of square-shaped thermoplastic nylon samples (30 x 30 x 2 mm). Twenty four samples were divided into 4 groups (n = 6): the control (without SiO_2 coating) and treatment (with SiO_2 coating) group, which were immersed in robusta coffee for 15 and 30 days. Materials that were used in this study were thermoplastic nylon (Valplast, USA), SiO_2 nanoparticles (Sigma-Aldrich, Germany), silane (Monobond, Ivoclar, Netherland), robusta coffee (Singa Brand, Indonesia), and ethanol as SiO_2 solvents. The tools used in this study were digital scales and measuring

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cups, nirbaken, microwave oven, staining jar as coffee immersion containers, and UV Vis spectrophotometer (Pharmaspec 1700, Shimadzu, Japan) to measure the discoloration based on the absorbance of light.

The coating method that was used was dip-coating. The treatment group was given a pre-treatment with silane (Monobond, Ivoclar) using a micro brush on the entire surface, then it was allowed to dry. Subsequently, it was immersed in coating solution that contained 0,5% of SiO_2 nanoparticles (0,5 grams SiO_2 in 100 ml ethanol) for 5 seconds and dried in an oven (Panasonic, Japan - 220V / 50Hz) at 70°C for 10 minutes. After control and treatment groups were ready (Figure 1), the initial color measurement was performed using spectrophotometer before coffee immersion.

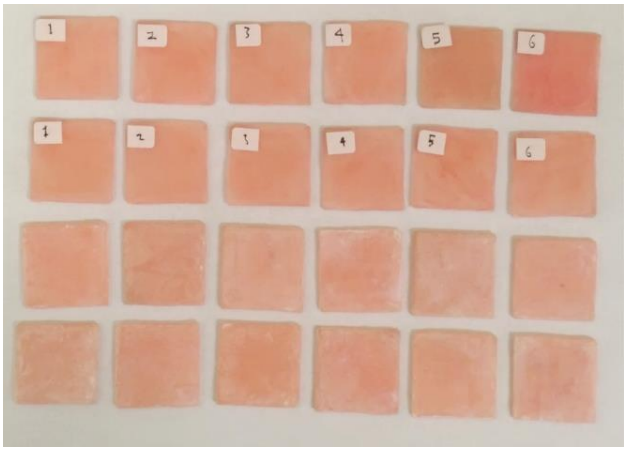

Figure 1. The color of all thermoplastic nylonsample before coffee immersion

All samples were immersed in robusta coffee (6 grams of coffee powder in 180 ml of water) using a staining jar so that the entire surface of the sample was exposed to the coffee. Immersion was carried out for 15 and 30 days. The coffee solution then was changed every day (Figure 2).

Figure 2. The coffee immersion of control and treatment group in the staining jar for 15 and 30 days

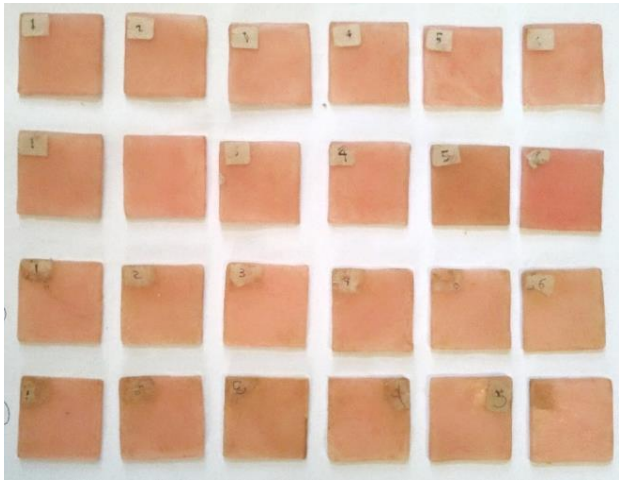

Figure 3. The color of all thermoplastic nylonsample after coffee immersion

After the coffee immersion was complete, (Figure 3) the final color measurement was performed using a spectrophotometer. Statistical analysis was performed using two-way ANOVA test with 95% significance level followed by LSD post hoc test.

RESULTS

The means and standard deviation of delta light absorbance of thermoplastic nylon before and after coffee immersion were shown in Table 1, the data were also presented in the form of bar diagrams in Figure 4. As presented at Table 1 and Figure 4, the treatment group with SiO_2 coating showed a smaller delta light absorbance than the control group. It implies that the treatment group had fewer discoloration than control group.

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Table1. The means and standard deviation (sd) of delta light absorbance of control and treatment groups before and after coffee immersion for 15 and 30 days

Groups	Duration of Coffee Immersion (*mean ± sd*)	
	15 Days	**30 Days**
Control (without SiO_2 coating)	0.065 ± 0.022	0.085 ± 0.028
Treatment (with SiO_2 coating)	0.019 ± 0.006	0.032 ± 0.011

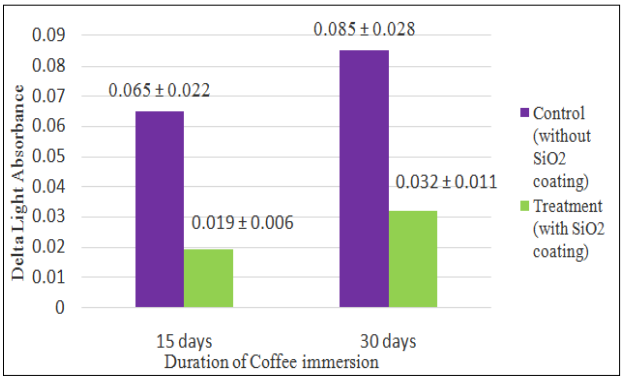

Figure 4. The diagram of means and standard deviation of delta light absorbance of control and treatment groups before and after coffee immersion for 15 and 30 days.

Shapiro-Wilk normality test results showed that the data in each group had a normal distribution (p> 0.05). The homogeneity test results using Levene's test also exhibited the value that all population variances were the same (p> 0.05). Two-way ANOVA test was performed to compare the mean difference between group of SiO_2 coating nanoparticles and the duration of coffee immersion on the color change of thermoplastic nylon denture base (Table 2).

Table 2. The two-way ANOVA results of the effect of SiO_2 coating and duration of coffee immersion on the discoloration of thermoplastic nylon

Variabel	F	p value
SiO_2*coating*	45.204	0.000
Duration of Coffee Immersion	4.854	0.039
SiO_2*coating* * Duration of Coffee Immersion	0.264	0.613

*: significant difference if p <0.05

The results of the two-way ANOVA test showed a significant effect of SiO_2 coating on thermoplastic nylon discoloration (p <0.05). Moreover, it exhibited a significant effect of the duration of coffee immersion on thermoplastic nylon discoloration (p <0.05). There was no interaction between SiO_2 coating and duration of coffee immersion to thermoplastic nylon discoloration (p> 0.05).

The results of the post hoc LSD test of the treatment group immersed in 15 days of coffee (treatment, 15 days) exhibited a significant difference compared to the whole control group (p <0.05)(Table 3). The control group without SiO_2 coating which was immersed in coffee for 30 days showed the highest delta light absorbance compared to all groups with a value of 0.077 ± 0.027. Post hoc LSD test in Table 3 also exhibited significant differences compared to all treatment groups (p <0.05). However, there were no significant differences between treatment groups immersed in coffee for 15 and 30 days (p> 0.05). There were no significant differences between the control group immersed in coffee for 15 and 30 days (p> 0.05).

Table 3. Post hoc LSD test for each group

Groups	Control, 15 Days	Control, 30 Days	Treatment,15 Days	Treatment,30 Days
Control,15 Days	-	-.0198333	.0453333*	.0330000*
Control, 30 Days	.0198333	-	.0651667*	.0528333*
Treatment ,15 Days	-.0453333*	-.0651667*	-	-.0123333
Treatment, 30 Days	-.0330000*	-.0528333*	.0123333	-

*: The mean difference is significant at the 0.05 level

Test investigation of thermoplastic nylonsurface using a light microscope (Leica, DM 500) with 100 times magnification showed that the SiO_2 coating group had a smoother surface than the control group because of the covering of SiO_2 nanoparticles on the surface of porous microstructure (Figure 5). The color surface examination using a digital microscope (Dino-Lite, 20 times magnification) also showed

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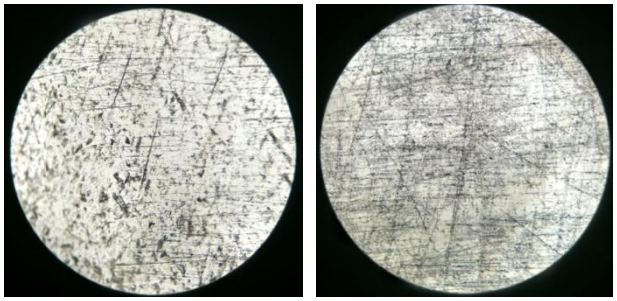
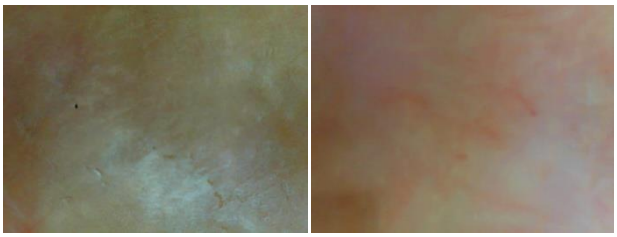
the effect of coffee immersion in the control group whose surface became darker than that of the treatment group (Figure 6).



(A) **(B)**

Figure 5. Description of thermoplastic nylon surface using a light microscope (Leica, DM 500) 100 times magnification, (A) Control group without SiO_2 coating, (B) Treatment group with SiO_2 coating.



(A) **(B)**

Figure 6. The color of thermoplastic nylon surface after coffee immersion (digital microscope, Dino-Lite, 20 times magnifying) (A) Control group, (B) Treatment group.

DISCUSSION

The result of this study indicated that SiO_2 coating on the thermoplastic nylon is effective to reduce discoloration due to coffee immersion for 15 days. The time taken for someone to consume coffee is about 30 menit, so that coffee immersion for 15 days is equivalent to exposure of coffee consumption for 2 years. These results imply that the SiO_2 coating is effective to prevent discoloration for 2 years due to coffee exposure.

SiO_2 nanoparticles coating will make a thin layer that covers the porous microstructure, which inhibits water and stain absorption. Zuo et al.[17] stated that coatings with organic and inorganic materials reduce absorption and solubility of water. Feng's study showed that the coating process close the porous microstructure formed after polymerization.[19] Closure of porous microstructure prevents absorption of water into the matrix of thermoplastic nylon to maintain color stability.Grumezescu stated that the layer formed on the thermoplastic nylon surface is due to the very small size of nanoparticles (<100 nm) that could enter through a micro gap.[20] The layer formed is very thin with the thicknes only about 1 - 1000 nm, which is stable against dimensional change.[21]

The high difference in light absorbance showed that the control group became darker, thus it absorbed lighter than the treatment group. It occurred because the amide -NH group absorbed more water and coffee stain without any protection layer.The ability of coffee to change the color of the thermoplastic nylon is due to its chlorogenic acid, which disrupts the thermoplastic nylon bond chain, thus making it easier for the diffusion process of water molecules. Amaliyah et al.[11] stated that chlorogenic acid disrupts the thermoplastic nylon bond chain by releasing H^+ ions that break the linear bond of thermoplastic nylon so that the deformation occurs. Coffee has tanic acid, which gives brownish yellow stain on the material attached. Guler et al.[12] stated that tannic acid in coffee provides yellowish stain on composite resin polymers. The two-active substances in coffee which are chlorogenic acid and tanic acid contribute to alter the color of thermoplastic nylon by damaging chemical bonds and giving brownish yellow stain.

The results showed no significant difference between the control groups immersed in coffee 15 and 30 days. It can be explained that the absorption of coffee solution by the thermoplastic nylon reaches its maximum point at 15 days as a result the absorption of coffee solution is no longer signficant after 15 days. This result is in accordance with Navarro et al.[22] study, which stated that there were no significant differences between acrylic and thermoplastic nylon denture base immersed in coffee for 15 and 30 days.

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CONCLUSION

Silica dioxide (SiO_2) nanoparticles as a coating material on thermoplastic nylon denture base prevent discoloration due to coffee immersion for 15 and 30 days.There were no significant differences between 15 and 30 days of coffee immersion on thermoplastic nylon discoloration in the control and treatment groups.

REFERENCES

1. Gunadi HA, Margo A, Burhan LK, Suryatenggara F, Setiabudi I. Buku ajar ilmugigitiruansebagianlepasan, Jilid I Jakarta: Hipokrates; 2013: 11-12.
2. Anusavice KJ, Ralph WP, Chiayi S, H Ralph R. Phillips' science of dental materials, St. Louis: Mosby Elsevier; 2013: 143 – 170.
3. Vojdani M, Rashin G. Polyamide as denture base material: literature review. J Dent Shiraz Univ Med Sci. 2015; 16(1): 1-9.
4. McCabe JF, Walls WG. Applied dental material. 9th Edition. UK: Blackwell Munksgard; 2009: 100 – 110.
5. Shah J, Bulbule N, Kulkarni S, Shah R, Kakade D. Comparative evaluation of sorption, solubility, and microhardness of heat cure polymethylmethacrylate and flexible denture base resin. J ClinDiagn Res. 2014; 8(8):1- 4
6. Yunus N, Rashid AA, Azmil LL, Abu_hasan MM. Some flexural properties of a nylon denture base polymer. J. Prosthet. Dent. 2005; 32(1): 65 – 71.
7. Takabayashi Y. Characteristic of denture thermoplastic resins for non-metal clasp dentures. Dent Mater J. 2010; 29(4): 353-361.
8. Goiato MC. Santtos DM, Haddad MF, Pesquiera AA. Effect of accelerated aging on the microhardness and color stability of flexible resins for dentures. J Braz Oral Res. 2010; 24 (1): 114 - 119.
9. Keyf F, Ilker E. Evaluation of gloss changes of two denture acrylic resin materials in four different beverages. J Dental Materials. 2004; 20: 244 - 251.
10. Lai YL, Lui HF, Lee SY. In vitro color stability, stain resistance, and water sorption of four removable gingival flane material. J Prosthet Dent. 2003; 90: 293 - 300.
11. Amaliyah R, Agus S, Lusi H. Plastic deformation of thermoplastic nylon after immersed in robusta coffee bean extract. JurnalPustakaKesehatan, 2015; 3(1): 117 - 121.
12. Guler AU, Yulmaz F, Kulunk T, Guler E, Kurt S. Effects of different drinks on stainability of resin composite provisional restorative materials. J Prosthet Dent. 2005; 94: 118 - 124.
13. Garcia LFR, Lourenco MRR, Fabrício MM, Fernanda CP, Simonides C. Influence of artificial accelerated aging on dimensional stability of acrylic resins submitted to different storage protocols. J Prosthodont. 2010; 19(6): 425 - 431.
14. Mori K. Color and glass evaluation of titanium dioxide coating for acrylic resin denture base. J Prosthodont Res. 2015; 59(4): 249 - 253.
15. Kumar R, Roy I, Ohulchanskky TY, Vathy LA, Bergey EJ, Sajjad M.. In Vivo Biodistribution and Clearance Studies Using Multimodal Organically Modified Silica Nanoparticles. ACS Nano J.2010.(4): 699-708.
16. Azuma A, Akiba N, Minakuchi S. Hydrophilic surface modification of acrylic denture base material coating and its influence on candida albicans adherence. J Med Dent Sci. 2012: (59): 1-7.
17. Zuo W, Feng D, Song A, Gong H, Zhu S. Effects of organic-inorganic hybrid coating on the color stability of denture base resins. J Prosth Dent. 2016; 115(1): 103 - 8.
18. Lung CYK, Matinlinna JP. Aspects of silanes coupling agets and surface conditioning in dentistry: an overview, J.Dental Materials. 2012. 28: 416-677.
19. Feng DF, Gong H, Zhang J, Guo X. Effects of antibacterial coating on monomer exudation and the mechanical properties of denture base resins. J Prosth Dent. 2016; 117(1): 171 - 177.
20. Grumezescu A. Nanobiomaterials in Dentistry: Applications of Nanobiomaterials, Elsevier , Amsterdam. 2016. 49 – 77.
21. Brinker CJ, Scherer GW. Sol-gel Science: The physics and chemistry of sol–gel processing. Academic, San Diego. 1990. 786 - 837.
22. Navarro WFS, Beatriz EAC, Cristiane PFB, Janaina HJ, Vanessa MU, Nara HC. Color stability of resins and nylon as denture base material in beverages. J. Prosthodontic. 2011; 20: 632 - 638.

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RESEARCH ARTICLE

The inhibition of leaf extract Moringaoleifera on the formation biofilm bacteria Enterococcus faecalis

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ABSTRACT

Background: *Enterococcus faecalisis the most common bacteria that cause failure to root canal treatment, which can become very resistant under biofilms. Moringaoleifera has antibacterial properties and may affect the multidrug-resistant bacteria.* ***Purpose:*** *This study aimed to observe the inhibition of Moringaoleifera leaf extract on the biofilm formation of bacteria Enterococcus faecalis.* ***Method:*** *This study was true experimental laboratory research with post-test only control group design and tested using biofilm method, divided into six groups, each group consisted of eight samples. The control groups were: K- (CMC 0,1%), K+ (ChKM), and four treatment groups were: P1 (Moringaoleifera 20%), P2 (Moringaoleifera 40%), P3 (Moringaoleifera 60%), P4 (Moringaoleifera 80%). Antibacterial inhibition was determined by the value of Optical Density in the ELISA Reader. Data analysis using Kruskal-wallis followed by Mann-Whitney test.* ***Results:*** *There were significant differences (p <0.05) seen from the percentage value of biofilm inhibition, on the K - (0 %) group compared with K+ (47,69%), P1(7,68%), P2 (21,13%), P3 (42,33%) and P4 (55,78%), as well on K + group (ChKM) compared with P4 group (Moringaoleifera 80%).* ***Conclusion:*** *Moringaoleiferaleaf extract has inhibition effect for the formation of bacteria Enterococcus faecalis biofilm and the effect is 80% greater than ChKM.*

Keywords: *Biofilm, Enterococcus faecalis, Moringaoleifera*

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INTRODUCTION

Root canal treatment is a treatment in dentistry that aims to maintain teeth in oral cavity so that they can be accepted biologically by the surrounding tissues[1]. Root canal treatment also aims to eliminate the bacteria that cause infection in the pulp tissue and periapex, prevent any damage to hard or soft tissue in teeth and prevent reinfection due to bacterial contamination[2,3,4].

The success of root canal treatment can be evaluated based on clinical, radiographic, and histological examination. Research states that the success rate of root canal treatment reaches 90-95%, and the failure rate of root canal treatment which causes the need for re-treatment reaches 5-10%[5]. Microorganisms remaining after root canal treatment are the main factors causing reinfection[6].*Enterococcus faecalis* is a Gram-positive facultative anaerobic bacteria, known as the most resistant species, are often found in cases with abnormalities after endodontic treatment in a high percentage of up to 77% [7,8,9].*Enterococcus faecalis* bacteria are facultative gram-positive anaerobic bacteria that can live with or without oxygen supply[10].*Enterococcus faecalis* is able to survive in an environment that does not support, survive at low temperatures, in a nutrient-poor environment, invasion of the dentinal tubules, suppress the work of lymphocytes, form biofilms[6,11].

Bacteria *Enterococcus faecalis* has virulent factors that are high resistance to root canal medicamentbecause this bacterium has the ability to maintain a pH balance[12].*Enterococcus faecalis* is also able to penetrate the dentinal tubules to allow the bacteria to avoid instrumentation of the preparations and irrigation materials used during biomechanical treatment[6,13]. Biofilms are collection of bacterial cells either of a kind or several types attached to the substrate, tissue, or cells covered by a polysaccharide binding layer resulting from the excretion of bacterial cells, causing damage to the surface of the cells, mucosa and the tissue that it attaches[14]. Biofilms are one of the causes *Enterococcus faecalis* resistant to antimicrobial materials that can allow this bacterium to be 1000 times more resistant to antibodies, antimicrobials, and phagocytosis[10].

Root canal preparation measures are accompanied by insufficient irrigation to be able to free the root canals from bacteria properly so that it is necessary to administer root canal drugs[15] .ChKM also has better antibacterial, antiseptic, and disinfectant properties than other ingredients such as povidone iodine, chlorhexidine digluconate, and polyhexanide and other phenol groups (para-monochlorophenol, thymol, and cresol). ChKM also has shortcomings such as having toxic effects on periapex tissue, can cause irritation and soft tissue necrosis, it feels bad, has a pungent odor, and can cause allergic reactions[15,16]. The lack of root canal drugs encourages the development of research on natural ingredients that contain antibacterial as an alternative ingredient as a drug that can be used to reduce bacterial activity, because the use of natural ingredients has lower levels of danger, risk, and side effects when compared to the use chemical drugs[17].

Plants that can be used as food and medicine are *Moringaoleifera* plants[18]. *Moringaoleifera* is known as The Miracle Tree because it is proven to be a natural source of food nutrition and is efficacious as a drug whose contents are larger than in other plants[19]. All parts of *Moringaoleifera* plants include: leaf, roots, seeds, bark, fruit, flowers, and adult pods, can be used as a heart and blood circulation stimulant, anti-tumor, anti-pyretic, anti-epileptic, anti-inflammatory, anti-ulcer , anti-spasmodic, diuretic, anti-hypertensive, cholesterol-lowering, antioxidant, anti-diabetic, hepatoprotective, anti-bacterial and anti-fungal[20]. The content of secondary metabolite compounds from *Moringaoleifera* leaf is flavonoids, saponins, tannins, and alkaloids[21].

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The results of the antibacterial activity test of *Moringaoleifera* leaf extract against *Escherichia coli* (gram negative bacteria) and *Staphylococcus aureus* (gram positive bacteria) showed strong antibacterial power in all treatment groups, and the treatment of Escherichia coli with *Moringaoleifera* leaf extract , the concentration of 80% has the strongest antibacterial power[22]. The purpose of this study was to determine the ability of *Moringaoleifera* leaf extracts to inhibit the formation of *Enterococcus faecalis* biofilms.

MATERIAL AND METHOD

This study is a true experimental laboratory study, with the Randomized Post Test Only Control Group Design research design, there are 6 groups, namely negative control (CMC solvent 0.1%); positive control (ChKM); P1 (*Moringaoleifera* extract 20%); P2 (Moringaoleifera extract 40%); P3 (*Moringaoleifera* extract 60%); P4 (80% *Moringaoleifera* extract). and each group consisted of 8 samples. In this research, a method has been conducted to qualitatively test the formation of *Enterococcus faecalis*biofilms by using Congo red to incubate for 48 hours and form a black colony that shows a strain of bacteria capable of producing biofilms.

The making of *Moringaoliefera* leaf extract solution was done by maceration technique. A total of 100 g of *Moringaoliefera* leaf simplex powder was put into erlernmeyer, then soaked with 96 mL of 96% ethanol solution, covered with aluminum foil and left for 5 days while occasionally stirring. After 5 days, the samples soaked were filtered using filter paper to produce filtrate 1 and residue 1. The residue was then added with a 96% ethanol 96% solution, covered with aluminum foil and left for 2 days while occasionally stirring. After 2 days, the samples were filtered using filter paper to produce filtrate 2 and residue 2. Filtrate 1 and 2 were mixed into one, then evaporated using a rotary evaporator, to obtain a thick extract of *Moringaoliefera* leaves. The resulting viscous extract was put into a water bath and evaporated until all the ethanol solvents evaporated. The extract was weighed and stored in a closed glass container before being used for testing. 20% test solution is made; 40%; 60% and 80% by weighing 0.2 gram; 0.4 gram; 0.6 gram and 0.8 gram of Moringa leaf thick extract were then dissolved in 1 mL of 0.1% CMC Na solution, respectively. 22% CMC Na solution was used as a negative control and (ChKM) used as a positive control.

The biofilm inhibition test was carried out by mixed bacteria on BHIB and equalized to 0.5 McFarland I standard. Suspension of *Enterococcus faecalis*biofilm bacteria that had been equalized was then diluted to 1: 100. Bacterial suspension was inserted into a 96-well round- bottomed plastic tissue culture plate (microtiter plate) with a total volume of 0.1 mL (100 µl) in each well using a micropipette. The study was conducted on 1 test plate and 1 blank plate. The test plate was filled with bacterial suspension, then the microtiter plate was put in a special container and then incubated at 37°C for 24 hours. After 24 hours the microtiter plate was removed from the incubator then the test extract solution was put into the microtiter plate filled with bacterial suspension, and on the blank plate the test extract solution was filled without bacteria. The microtiter plate is put back into the incubator for 24 hours. After that the microtiter plate is removed from the incubator, then the test solution is removed and washed 3 times with 0.2 mL phosphate buffer saline. The microtiter plate was dried, then each well was added with 1% crystal violet of 0.2 mL (200 µl) and allowed to stand for 15 minutes. Then rinsed using distilled water and dried for 15 minutes in an incubator of 37 ° C. Add Tween 80 2% 0.2 mL to each microtiterplate.The test results in the form of optical density (OD) values are read using an ELISA reader at a wavelength of 570 nm. The inhibitory power of biofilm formation from the test solution is calculated by the following formula[23].

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RESULT

The research data were analyzed descriptively to obtain an overview of the distribution and summary of the data in order to clarify the presentation of results.

Table 1. The mean value of *Enterococcusfaecalis* biofilms

Group	Replication	Mean	SD
K(-)	8	0	0
K(+)	8	47,69	6,70
P1	8	7,68	0,92
P2	8	21,13	4,45
P3	8	42,33	3,31
P4	8	55,78	3,68

Note: K- (CMC 0.1%); K (+) (ChKM); P1 (*Moringaoleifera* extract 20%); P2 (*Moringaoleifera* extract 40%); P3 (*Moringaoleifera* extract 60%); P4 (80% *Moringaoleifera* extract).

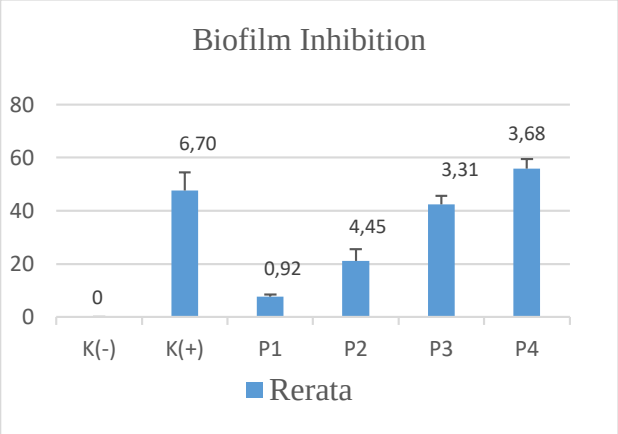

Figure 1. Average bar chart of Enterococcus faecalis biofilm inhibition

The data above shows the largest inhibitory biofilm of *Enterococcus faecalis* is the largest group P4 (*Moringaoleifera* extract 80%), the lowest in the P1 group. Increasing the inhibitory value in the treatment group was seen by the increasing concentration of *Moringaoleifera* leaf extract combination. In K (-) (CMC 0.1%) there was no inhibition, while the K (+) group (ChKM) had inhibitory effect that inhibited the formation of *Enterococcus faecalis* biofilm.

Table 2. Results of normality test with Shapiro-Wilk

Group	Sig
K (+)	0,829*
P1	0,826*
P2	0,238*
P3	0,883*
P4	0,101*

Note : * (p>0,05)

The results of the normality test with Shapiro-Wilk can be seen that each treatment group has a significance value of p> 0.05. This shows that the inhibitory value of biofilms is normally distributed.

Table 3. Test results for the variance homogeneity with Levene

Levene Test	Sig.
5,394	0,001

Note : (p>0,05)

Based on the results of the variance homogeneity test with Levene it is known that the significance value is 0.001 or can be interpreted as p <0.05, so it can be concluded that the biofilm inhibition value is not homogeneous (p <0.05).

Tabel 4. The result of *Kruskal-Wallis* Test

Kruskal-Wallis	**Sig.**
AntarKelompok	0,000*

Note : * (p<0,05)

Kruskal-Wallis test results showed that the value of siginification was 0,000 (p <0.05), it can be concluded that there was a significant biofilm inhibition between the treatment group with the negative control group and the positive control group. To see To see the significant difference in antibacterial power of each group, the Mann-Whitney test was performed

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Table 5. Mann-Whitney Test

Group	K(-)	K(+)	P1	P2	P3	P4
K(-)		0,000*	0,000*	0,000*	0,000*	0,000*
K(+)			0,001*	0,001*	0,059	0,012*
P1				0,001*	0,001*	0,001*
P2					0,001*	0,001*
P3						0,001*
P4						

Note: Group (average inhibition of biofilms); * (p <0.05)

The Mann-Whitney test results showed that there were significant differences in the value of biofilm inhibition (p <0.05), between K (-) with K (+), P1, P2, P3 and P4; between K (+) with P1, P2 and P4; between P1 and P2, P3 and P4; between P2 and P3 and P4; and between P3 and P4.

RESULT

This study aims to determine the inhibitory power of *Moringaoleifera* leaf extracts against the formation of the bacterial biofilm *Enterococcus faecalis.* Biofilms are a community of bacteria that are often well organized, attached to the surface and embedded in the extracellular slime layer which is a sticky material and as a protection produced by bacteria[24]. The formation of biofilms is one of the common mechanisms of a collection of bacteria as a form of defense for survival. Biofilms can protect bacteria from the host's body defenses and can protect from adverse environmental influences such as extreme pH (pH <4.4 and> 9.1), low oxygen content and extreme pressure and temperature (> 100 ° C). Bacteria can become very resistant under biofilm[25].

The method used to identify biofilm formation qualitatively is congo red agar. The results of identification on the media in order to show that the bacteria *Enterococcus faecalis* has the ability to form biofilms shown by the formation of black colonies. While the method used to test the formation of bacterial biofilms quantitatively is a microtiter plate assay. This method is used to observe microbial biofilm attachment[26]. This method was chosen because it is fast, easy, simple, accurate and has a sensitivity of 97.1%, specificity of 97.5% and accuracy of 97.2% in detecting biofilm attachment[27,28].

Statistical test found a significant difference between groups K (-) to K (+), this shows that ChKM is a broad-spectrum antibacterial active against gram-positive and gram-negative bacteria. ChKM began to be known in 1905, ChKM is known to have good antiseptic and disinfectant properties for root canals. This drug is often used in the case of root canal treatment even though ChKM has a toxic content in its use.16 In group K (-) there was no biofilm inhibition, this shows that the 0.1% CMC solution is only a neutral solvent, and is completely has no inhibition and does not have any effect on *Moringaoleifera* leaf extract in inhibiting the formation of *Enterococcus faecalis* biofilms. In all treatment groups P1, P2, P3 and P4 all have significant biofilm inhibitory values this means *Moringaoleifera* leaf extracts at concentrations of 20%, 40%, 60% and 80% contain antibacterial compounds so that they can inhibit the formation of bacterial biofilms *Enterococcus faecalis* .

Moringaoleifera leaves have potential as an antibacterial because the extract contains secondary metabolite compounds including flavonoids, saponins, tannins, and alkaloids. Flavonoids work by interrupting signaling between bacterial cells, so that it will result in the formation of colonies and permanent attachment of bacteria inhibited[21].Saponins work by releasing bonds between bacteria on biofilms into free planktonic bacteria, so that the content of other active substances that are anti microbial can work better[29,30]. Tannins work by coagulating microbial protoplasms, so that

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stable bonds with bacterial proteins are formed which cause inactivation of bacterial proteins[29]. Alkaloids work by reacting with bacterial DNA and amino acid compounds so that the cell nucleus becomes lysis[25]. The magnitude of the active ingredient *Moringaoleifera* leaf extract is directly proportional to the large concentration of the extract, this can be seen from the results of the average inhibition of bacterial biofilms, the greater the concentration of *Moringaoleifera* leaf extracts, the inhibition of *Enterococcus faecalis*biofilms is also greater. There was a significant difference in inhibition of the formation of *Enterococcus faecalis* biofilms (p <0.05) between all treatment groups and in group P4 (*Moringaoleifera* 80% leaf extract) had the greatest inhibitory power. The P4 group and the positive control group (ChKM) also had significant differences (p <0.05) where P4 had a greater inhibition of the formation of *Enterococcus faecalis* biofilms than K (+).

CONCLUSION

Moringaoleifera leaf extract can inhibit the formation of biofilm bacteria *Enterterococcusfaecalis*. *Moringaoleifera* leaf extract concentration of 80% had the greatest inhibitory effect on the formation of *Enterococcus faecalis*bacterial biofilms compared with ChKM as K (+) and *Moringaoleifera* leaf extract concentrations of 20%, 40%, 60%.

REFERENCES

1. Cogulu, D., Uzel, A., Oncag, O., Aksoy, SC., Eronat, C. Detection of *Enterococcus faecalis* in necrotic teeth root canals by culture and polymerase chain reaction methods. European Journal of Dentistry. 2007; (1) :216-21
2. Walton RE dan Torabineiad M. Prinsip dan Praktik Ilmu Endodonsia. Edisi 3. EGC. Jakarta: 2008.
3. Garg N dan Garg A. Text Book of Endodontics. Bangladesh, India. Edisi 3. Penerbit: Jaypee Brothers Medical Publishers; 2014. p.51-55.
4. Sari AN dan Untara TE. Root Canal Retreatment Menggunakan Kombinasi Kalsium Hidroksida dan Chlorhexidine Sebagai Medikamen Intra Kanal Insisivus Sentral Kiri Maksila. *Maj Ked Gi*. 2014; 21(2): 165-170.
5. Michael M. Hoen, DDS, and Frank E. Pink, DDS, MS. Contemporary Endodontic Retreatments: An Analysis based on Clinical Treatment Findings. Journal of Endodontics. 2002; 28(12) : 834-836.
6. Mulyawati E. Peran Bahan Disinfeksi Pada Perawatan Saluran Akar. *Maj KejGi*; 2011; 18(2): p.205-209.
7. Tamara, R., Rochyani, L., Teguh, P. Daya Hambat Ekstrak Teripang Emas (*Stichopushermanii*) terhadap Bakteri *Enterococcus faecalis*. Denta Jurnal Kedokteran Gigi. 2015; 9 (1): 36-46.
8. Karale, R., Thakore, A., Shetty, V.K. "Evaluation of Antibacterial Efficacy of 3% Sodium Hypochlorite, High-Frequency Alternating Current and 2% Chorhexidine on Enterococcus faecalis: An In Vitro Study".J Conserv Dent. 2011; 14(1): 2-5.
9. Jaju, Sushma dan Jaju, dan Prashant P. Newer Root Canal Irrigants in Horizon : A Review. *International Journal of Dentistry*. 2011. Available at http://dx.doi.org/10.1155/2011/851359
10. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB, Enterococcus faecalis: Its role in root canal treatment Failure and current concepts in retreatment. Journal of endodontics. 2006; 32(2): 93-98.
11. Athanassidis, Abbott PV, dan Walsh LJ. The Use of Calsium Hydroxyde, Antibiotics, and Biocides as Antimicrobual Medikamen in Endodontics. *Australian Dental Journal*. 2007; 52(2): 52-57. Available from http://onlinelibrary.wiley.com/doi/10.1111/j.1834-7819.2007.tb00527.x/pdf
12. Kuntari LM, Wignyo H, danMulyawati E. Perbedaan Daya Antibakteri Klorheksidin 2% dan Berbagai Konsentrasi Sodium Hipoklorit Kombinasi Omeprazole 8,5% Terhadap *Enterococcus faecalis*. *J Ked Gi*. 2014;5(2): p.139-149.
13. Siquera JF, Rocas IN, Favieri A, dan Lima KC. Mechanical Reduction of the Bacterial Population in the Root Canal by Three Instrumentation Techniques. *JOE*. 2009; 25(5):

Denta Jurnal Kedokteran Gigi, Februari 2020; Vol.14 No.1; Hal 44-50
Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
DOI: 10.30649/denta.v14i1

p.332-335. Available at http://www.endoexperience.com/documents/5.pdf
14. Wahyudi, D. UjiEfektivitas Ekstrak Seledri (*Apiumgraveolens*) Sebagai Penghambat Produksi Biofilm Pada Salmonella typhi. Jurnal Biomedika. 2014; 7(2) : 1-10
15. Charyadie, F., Adi, S., Sari, R. Daya Hambat Ekstrak Daun Alpukat (*Persea americana, MIII.*) Terhadap Pertumbuhan *Enterococcus faecalis*. Denta Jurnal Kedokteran Gigi. 2014; 8(1): 1-10.
16. Dammaschke T, Jung N, Harks I, dan Schafer E. The Effect of Different Root Canal Medicament on The Elimination of *Enterococcus faecalis ex vivo*. *European Journal of Dentistry*. 2013; 7(4): p. 442-448. Available from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4053669/
17. Adewole, S. & Caxton-Martins, E. Morphological Changes and Hypoglycemic Effects of *Annona Muricata* Linn. (Annonaceae) Leaf Aqueous Extract on Pancreatic B-Cells of Streptozotocin-Treated Diabetic Rats.African Journal of Biomedical Research. 2006; 9: 173-87.
18. Putra IWDP, Dharmayudha AAG, Sudimartini LM. Identifikasi Senyawa Kimia Ekstrak Etanol Daun Kelor (Moringa oleifera L) di Bali. Indonesia Medicus Veterinus. 2016; 5(5): 464–473.
19. Toripah SS, Abidjulu J, Wehantouw F. Aktivitas Antioksidan dan Kandungan Total Fenolik Ekstrak Daun Kelor (Moringa oleifera Lam). Pharmacon. 2014; 3(4): 37-43.
20. Krisnadi AD. Kelor Super Nutrisi. Moringa oleifera. Blora : Pusat Informasi dan Pengembangan Tanaman Kelor. 2015.
21. Rochyani IS, Aryanti E, Suripto. Kandungan fitokimia beberapa jenis tumbuhan lokal yang sering dimanfaatkan sebagai bahan baku obat. Pros Sem Nas Masy Biodiv Indon 1. 2015; (2): 388-391.
22. Dima LLRH, Lolo WA, Fatimawali. Uji Aktivitas Antibakteri EKkstrak Daun Kelor (Moringa oleifera L .) Terhadap Bakteri Escherichia coli dan Staphylococcus aureus. Pharmacon Jurnal Ilmiah Farmasi. 2016; 5(2), 282–289.
23. Dewi ZY, Nur A, Hertriani T. Efek Antibakteri dan Penghambatan Biofilm Ekstrak Sereh (Cymbopogon nardus L.)Terhadap Bakteri Streptococcus mutans. Maj Ked Gi Ind. Desember. 2015; 1(2): 136 – 141.
24. Newman MG, Takei HH, Klokkevold PR, Carranza FA. Carranza's Clinical Periodontology. Elsevier, Singapore. 2012; 11: 239-51, 483-91.
25. Kining E, Falah S, Nurhidayat N. The In Vitro Antibiofilm Activity of Water Leaf Extract of Papaya (Carica papaya L .) against Pseudomonas aeruginosa. 2016; *2*(3): 150–163
26. Merritt JH, Kadouri DE, O'Toole GA. Growing and Analyzing Static Biofilms. Current Protocols in Microbiology1B.1.1-1B.1.18. 2011.
27. Saising J, Singdam S, Ongsakul M, Voravuthikunchai SP. Lipase, protease, and biofilm as the major virulence factors in staphylococci isolated from acne lesions. BioScience Trends. 2012; 6(4):160-164.
28. Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of biofilm formation among the clinical isolates of staphylococci: An evaluation of three different screening methods. Indian Journal of Medical Microbiology. 2006; 24(1) :25-29.
29. Mayantika A. Perbedaan Daya Antibiofilm Antara Ekstrak Kulit Manggis (Garcinia Mangostana L.) 25% Dan Naocl 2,5% Terhadap Biofilm PorphyromonasGingivalis. Skripsi, UniversitasAirlangga, Surabaya. 2013.
30. Donlan RM. Biofilm Elimination on Intravascular Catheters: Important Considerations for the Infectious Disease Practicioner. J. Oxford. Atlanta: Division of Healthcare Quality Promotion, Centers for Disease Control and Pervention. 2011. p. 1043.

Denta Jurnal Kedokteran Gigi, Februari 2020; Vol.14 No.1; Hal 44-50
Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
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RESEARCH ARTICLE

The Risk Analysis of Malnutrition by Tooth Loosing Among Elderly

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ABSTRACT

Introduction: *Tooth loss occurs mostly in the elderly, especially loss of occlusal support can cause disruption of the mastication process and the swallowing of food, so the chewing function is reduced and causes the elderly to prefer soft foods and foods that are easy to chew. The lack of fulfillment of all nutrients needed by the body as the risk of malnutrition in the elderly increases.**Purpose:*** *To evaluate the analysis of tooth loss in the elderly with mal-nutrition based on Mini Nutritional Assessment in Social House Tresna Werdha Kasih Sayang Ibu, Batusangkar.* ***Materials and Methods:*** *Cross-Sectional approach. The total number of the sample was 46 elderly. The Eichner index measured the tooth loss, besides the MNA questionnaire estimated the risk of malnutrition. Data analysis was done using Chi-square* ***Results:*** *76.1% of the elderly have tooth loss (all of the occlusal support) and 69.7% of the elderly at risk of malnutrition. 85.7% of the elderly have tooth loss (all of the occlusal support) with risk malnutrition. The statistical result analyzed by using Chi-square obtained p-value <0.005.* ***Conclusion:*** *There is a risk of malnutrition in the elderly who experience tooth loss*

Keywords:*Tooth loss, malnutrition risk, mini nutritional assessment*

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INTRODUCTION

The elderly age group is growing faster than other age groups. World Population Aging (WPA) projected between 2015 and 2030 expected the elderly population would increase by 56% from 901 million to 1.4 billion. By 2050 it is projected that the number of elderly will double, reaching 2.1 billions.[1]In Indonesia, it is estimated that by 2020 will enter the period of elderly, where 10% of the population will be aged 60 years.[2]The aging process is the process of the disappearance of tissue functions to improve themselves and maintain their standard structure and function, which decreased body endurancein the elderly characterized with partial or complete loss of teeth.[3]

The results of Baseline Health Research stated that the number of teeth lost at the age of ≥65 years was 17.05, at the age of 45-55 years at 5.65 and at the age of 56-64 at 10.13. Tooth loss can lead to disruption of the chewing process and ingestion of food, especially loss of posterior teeth because the performance of digestion based on occlusal contact of the teeth, so that the mastication function becomes reduced. Some authors suggested that individuals with no teeth tend to eat unhealthy foods.[4] Individuals who lose teeth cause decreased efficiency of mastication so they prefer foods that are soft-textured and foods that are easily chewed. Foods consumed are foods that are high in carbohydrates, cholesterol, and calories, but low in fiber, protein, iron, calcium, and essential vitamins. Individuals who lose teeth cause decreased efficiency of mastication so they prefer foods that are soft-textured and foods that are easily chewed. Foods consumed are foods that are high in carbohydrates, cholesterol, and calories, but low in fiber, protein, iron, calcium, and essential vitamins. This causes the lack of fulfillment of all nutrients needed by the body so the risk of malnutrition in the elderly increases.[5]

Malnutrition was defined as "a state of nutrition in which a deficiency, excess or imbalance of energy, protein, or other nutrients, including minerals and vitamins, causes a measurable adverse effect on body function and clinical outcome".[6] Nutritional problems faced by the elderly are closely related to decrease the physiological activity of the body. The lack of balance in food consumption will cause the condition of the elderly to decline naturally.[5] Based on research on the nutritional status of the elderly in Public Health Center according to MNA (Mini Nutritional Assessment), as many as 59.2% have a risk of nutrition, as many as 37.6% are not at risk of malnutrition and as much as 3.2% are experiencing malnutrition.[7]Based on data from the Health Service of West Sumatra Province in 2018 that many elderly people fall into the category of malnutrition in Tanah Datar District, which is 1,228 elderly from the total number of elderly, namely 3,064 elderly.

Based on those above, authors were interested to know The risk analysis of malnutrition by tooth loss among the elderly.

MATERIALS AND METHODS

This was an observational, analytical study with the Cross-Sectional Study approach that was approved by the ethical commission (150/KEP/FK/2018). The samples in this research were selected using a simple random sampling method. The number of samples in this study is 46 elderly in Social House Tresna Werdha Kasih Ibu Batusangkar were contributed to this study and selected by a simple random sampling method. *Simple random* sampling is such a sampling that each basic unit (individual) has the same opportunity to sample it. Characteristics of respondents were elderly people aged ≥ 60 years, without using artificial teeth and able to communicate well were eligible for this study.

Tooth loss can be classified by several methods, one of which is the eichner index. This eichner index classifies teeth according to occlusal support zones. The occlusal support zone is the area that contacts the posterior

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teeth. The occlusal support zone consists of natural teeth which are present in premolars and molars in the maxilla and mandible. In this classification there are as many as four support zones in the right molar, right premolar, left molar and left premolar.[8,9]Tooth loss examination was grouped according to Eichner index (Distribution of tooth loss in the elderly were divided by Eichner index into a group with occlusal supported by group A (having all occlusal support) and B (loss of 1-3 occlusal support), and group with no occlusal support is group C. Malnutrition risk data collection using the Mini Nutritional Assessment questionnaire, including six items cognitive impairment test (6 CIT) with the perceived scale-4 questionnaire. Score ≥12 refers to the risk of malnutrition, score ≤11 no risk.[10] The MNA®-SF was developed by Nestlé and leading international geriatricians and remains one of the few validated screening tools for the elderly. It has been well validated and reliable in international studies in a variety of settings5-7 and correlates with morbidity and mortality.The Mini Nutritional Assessment Short Form (MNA®-SF) is an effective tool to help identify patients who are malnourished or at risk of malnutrition.[11]

The six item impairement test has been validated.The 6 CIT questionnaire consisted of three types of tests namely temporal orientation, attention test, and short-term memory test. The temporal orientation test consists of three questions regarding the year, month and time. The attention test consists of two questions, counting backwards from 20 to one and reading the name of the month of the year from the last month, December to the first month, January. Memory test in the form of an order to remember the names of five items of street names and then respondents were asked to name the street The results of the 6 CIT interview are that if you answer the question correctly you will get a zero score and if the answer is wrong you will get a score of two to four. The total range of scores obtained from this examination is from zero to 28. If the assessment results are zero to seven indicates the respondent's cognitive condition is normal. Mild dementia is marked when getting a score between eight to nine and severe dementia if it scores ten to 28.[12] The data were analyzed by Chi-Square test.

RESULTS

The majority of the study population belonged to the 60-69 years age group (43.5%). More than 65.2% of the respondents is the man. The majority of the elderly live in the social house for less than ten years (58.7%). According to the 6 CIT score,the neuropsychological condition of the respondents was categorized into three groups: normal, mild, and severe. 47.8% of the elderly were severe dementia.

Table 1. Sociodemographcharacteristic

No	Category	Distribution frequency	
		n	%
1.	Age (years)		
	a. 60-69	20	43.5
	b. 70-79	19	41.3
	c. ≥80	7	15.2
2.	Gender		
	a. Man	30	65.2
	b. woman	16	34.8
3.	An extended stay at the social house (years)	27	58.7
	a. ≤ 10	19	41.3
	b. > 10		
4.	Neuropsychological conditions (dementia)	22	47.8
	a. severe	7	15.2
	b. mild	17	37
	c. normal		

Table 2. Classification of tooth loss based on index Eichner

Classification of IndexEichner	Frequency (n)	Percentage (%)
A1	0	0
A2	0	0
A3	0	0
B1	2	4.3
B2	1	2.2
B3	8	17.4

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C1	6	13.0
C2	16	34.8
C3	13	28.3
Total	46	100.0

The distribution A1, A2, A3, B1, B2, B3, C1, C2, and C3 subtype as shown in Table 2 The frequency distribution subgroup tooth loss based on the Eichner index could be seen in Table 1 Respondents in group A were not found in this study. In group B, most were in the B3 subgroup of 8 people (17.4%). In group C the respondents were the most in the C2 subgroup of 16 people (34.8%).

Based on table 3 it can be seen the majority of the respondents without occlusal support were 35 respondents (76.1%). According to the MNA score, more than 69.6% of those who were 'at risk of malnutrition.The psychological stress condition of the respondents can be seen in Table 3, where 25 respondents experienced psychological stress conditions (54.4%). Table 4 showed that the female experienced more loss of all occlusal support (Group C) (81.2%). Table 5 showed that respondents who experienced malnutrition risk most experienced at the age of 75-90 years (73.3%).

Table 3. Occlusal Support

Category	Yes		No	
	n	%	n	%
Available of Occlusal support	11	23.9	35	76.1
The Risk of malnutrition	32	69.6	14	3.4
a condition of psychological stress	25	54.4	21	45.6

Table 4. Frequency distribution of tooth loss based on gender

Gender	have occlusal support group A & B		No occlusal support Group C	
	f	%	F	%
Man	8	26.7	22	73.3
Woman	3	18.8	13	81.2

Table 5. Frequency distribution of Malnutrition risk based on age

Age (years)	No risk		Risk	
	f	%	f	%
60-74	10	32.3	21	67.7
75-90	4	26.7	11	73.3

The bivariate analysis evaluates, there is a a risk of malnutrition in the elderly who experience tooth loss. Table 6 shows 85,7 % who no occlusal support (tooth loss) are at risk of malnutrition in the elderly based on Mini Nutritional Assessment in Social House Tresna Werdha Kasih Sayang Ibu Batusangkar

Table 6. Risk of malnutrition in the elderly who experience tooth loss

Tooth loss	Risk of malnutrition			
	No risk		risk	
	n	%	n	%
Have occlusal support	9	81.8	2	18.2
No occlusal support	5	14.3	30	85.7
Total	14	30.4	32	69.6

DISCUSSION

The World Health Organization (WHO) in Fatmah (2010) classified elderly people into four groups, namely middle age aged 45-59 years; elderly aged 60-74 years; old aged 75-90 years and ancient age above 90 years.[6]The majority of the study population belonged to the 60-69 years age group (43.5%).

The results showed that most of the elderly (76.1%) in social institutions had lost all occlusal support, while those who lost one to three occlusal supports were 23.9%. In a study conducted by Ikebe et al (2012), there were 81% of respondents who entered the C and B groups losing occlusal support according to Eichner.[13]

The results showed that there were (4.3%) respondents who lost one occlusal support, (2.2%) two occlusal support, and (17.4%) three occlusal support. Loss of occlusal support especially on the posterior side affecting the mastication of each individual. According to Ikebe et al. that the less the number of posterior teeth the mastication function will be worse.[13]

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The results showed that the female respondent experienced more loss of all occlusal support (81.2%). This is due to post-menopausal women will undergo hormonal and musculoskeletal metabolism change. Decreased levels of estrogen also occur during menopause; this is associated with increased resorption of alveolar bone, loss of periodontal tissue adhesion and tooth loss. The length of menopause also affects the decrease in bone density resulting in loss of teeth resulting in a reduction of mastication ability.[14]

Malnutrition risk assessment is based on research conducted at Tresna Werdha Social Home, Ibu Batusangkar's compassion using a Mini Nutritional Assessment (MNA). The use of MNA aims to determine the risk condition for malnutrition in the elderly so that the risk of malnutrition can be prevented early on. MNA consists of full MNA and short form MNA in this study researchers used a Mini Nuritional Assessment Short Form (MNA-SF).This study using MNA-SF showed that more than 50% of the elderly were in high-risk of malnutrition (69.6%)and non-malnourished respondents as many as (30.4%). This is in line with research conducted by Nurfantriat Social House Tresna Werdha Minaula Kendari, that there are (58.9%) elderly who were at risk of malnutrition.[15]

This study showed that elderly people aged 60-74 years had lower malnutrition risk (67.7%) than the elderly aged 75-90 years (73.3%). This is because the chewing efficiency will be reduced due to tooth loss as age increased. As age increased, the need for carbohydrate and fat nutrients will also be decreased, while the demand for protein, vitamins, and minerals increase, while the foods consumed by the elderly are foods high in carbohydrates, cholesterol, and calories but low in fiber, protein, iron, and essential vitamins. This causes the non-fulfillment of all the nutrients the body needs so that the risk of malnutrition in the elderly increases.[5] Factors that can affect a person experiencing the possibility of malnutrition in the elderly are found in the MNA-SF questionnaire, namely decreased food intake, weight loss, mobilization, neuropsychological problems or acute illness, neuropsychological problems and the results of BMI calculations.[15]

This study showed that (54.4%) elderly experienced psychological stress. This is in line with the research conducted by Saha et al. in nursing homes of south Suburban Kolkata, India and Indriana et al. in Pustang Gading Parang Ward that there was a high incidence of stress experienced by the elderly. According to Saha et al. a high proportion of at risk of malnutrition and malnutrition associated with the presence of psychological stress[16]. According to Indriana et al. the stress experienced by the elderly in the orphanage was due to changes in daily activities, changes in family associations, partner and family member deaths and changes in the choice of recreation and work.[17]

Table 7. Frequency distribution of condition of psychological stress

condition of psychological stress	Frequency (f)	Percentage (%)
No	21	45.6
Yes	25	54.4
Total	46	100.0

Based on the results of the study (47.8%) respondents experienced severe dementia and (37%) did not have dementia. The presence of dementia disorders in a person can also affect his nutritional intake because dementia can cause the person to forget to eat and they are also not concerned with the nutrients the body needs.[3]Yildiz's research results also showed that dementia especially advanced dementia stage was associated with malnutrition experienced by a person. Malnutrition also seemed to be associated with sleep turbances, psychological problems, immobility, falls and increased hospitalization risk in these patients.[18] So that nutritional intake in the elderly is not fulfilled and if this continues it will cause malnutrition.

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Table 8. Frequency distribution of neuropsychological conditions

Neuropsychological conditions	Frequency (f)	Persentage (%)
Severe dementia	22	47.8
Mild dementia	7	15.2
Normal	17	37
Total	46	100.0

The results showed that respondents who experienced a loss of one to three occlusal support, on average had no risk of malnutrition while respondents who experienced a loss of all occlusal support, on average had a risk of malnutrition. Loss of occlusal support will affect the chewing of the respondent which results in insufficient nutrition needed by the body, so the risk of malnutrition increases. This is in line with research conducted by Zelig et al in 2016, that there is a significant relationship between tooth loss and the risk of malnutrition. Respondents who have less occlusal support will have a greater risk of experiencing malnutrition compared to respondents who have more occlusal support.[19]

Changes in physiological function in the elderly can cause a decrease in food intake which results in a decrease in nutritional status. Decreased physiological function in the elderly has a close relationship with a decrease in nutritional status is decreased ability to chew food and reduced digestive enzyme secretions and result in a lack of food intake in the elderly[5]. The result of the statistical test showed that p-value <0.05, it can be concluded that there was a significant relationship between tooth loss and the risk of malnutrition. The results showed that respondents who experienced one to three occlusal losses, on average did not have malnutrition risk while respondents who lost all occlusal support averaged had the risk of malnutrition. Loss of occlusal support will affect the mastication process and lead to nutrient insufficiency of the body needs, so the risk of malnutrition is increasing

CONCLUSION

There is a significant relationship between tooth loss and the risk of elderly malnutrition based on the Mini Nutritional Assessment at the Tresna Werdha Kasih Sayang Ibu Social House Batusangkar.

Almost of elderly have a high risk of loss occlusalsupport.The more teeth are lost, the risk of malnutrition will increase, so it is recommended to meet nutritional needs.

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REFERENCES

1. World Population Aging. Economic and Social Affairs. 2015.p.1-2.[dikutip 20 Januari 2018]. Available from: https://www.un.org/en/development/desa/population/publications/pdf/ageing/WPA2015_Report.pdf
2. Kementrian Kesehatan RI. Situasi Lanjut Usia di Indonesia.2016 [cited 16 Januari 2018]. Available from: https://www.kemkes.go.id/resources/download/pusdatin/infodatin/infodatin%20lansia%202016.pdf
3. Martono H, Pranaka K. Geriatri. Jakarta: Balai penerbit FKUI; 2009
4. Hanin, I. Hubungan Kemampuan Mastikasi (Analisis Menggunakan Alat Ukur Kemampuan Mastikasi) dengan Kualitas Hidup Wanita Pralansia dan Lansia di Wilayah Bekasi [Thesis]. Jakarta: Universitas Indonesia. 2012. p.1-33.
5. Fatmah. Giziusialanjut. Jakarta: Erlangga.2010. p. 54-103.
6. Puntis,J.W.L. Malnutrition and Growth. JPGN. 2010.
7. Boy E.Prevalensi Malnutrisi pada Lansia dengan Pengukuran Mini Nutritional Assesment (MNA) di Puskesmas.Herb-MedicineJournal. 2019; 2(1): 5-9.

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Available at http://journal-denta.hangtuah.ac.id/index.php/denta/issue/view/14
DOI: 10.30649/denta.v14i1

8. Dhingra, S,Rajesh G, Rao A, Pai UY, Shenoy R,Pai M. 2017. Impact of Occlusal Support and Perceived Chewing Ability on Oral Health-Related Quality of Life Among Patients Attending a Private Dental Institution in India. J Indian Prosthodonti Soc.2017; 17(1): 15-21.
9. Yoshino, K. Relationship Between Eichener Index and Number Of Present Teeth. Bull Tokyo Dent Coll.2012; 53(1): 37-40.
10. Kementrian Kesehatan RI. Pedoman Pelayanan Gizi Usia Lanjut [internet]. Jakarta: Kemenkes RI; 2012 [cited16Januari 2018]. Available from: http://digilib.poltekkesdepkes-sby.ac.id/public/POLTEKKESSBY-Books-399-Pedomanpelayanangizilanjutusia.pdfNestle Nutrition Institute.Nutriion Screening as easy as mna (A guide to completing the Mini Nutritional Assessment-Short Form) [internet]. 2011 [cited Januari 2018]. Available from: https://41e5fc1d-e404-4830-8c07-64690e79acce.filesusr.com/ugd/2a1cfa_ecf925af4e8b443794eebf39ccf05e54.pdf
11. O'SullivanD,O'regan NA, Timmons S.Validity and Reliability of The 6-item Cognitive Impairment: A Review. Dement Geriatrogn Disord.2016;42: 42-49.
12. Ikebe, K. Masticatory Permanence in Older Subjects with Varying Degrees of Tooth Loss. J Dent.2012.
13. Riadiani, B, Dewi RS, Ariani N, Gita F. Tooth Loss and Perceived Masticatory Ability in Post-Menopausal Women. Journal of Dentistry Indonesia. 2014; 21(1): 11-15.
14. Nurfantri, D, Yuniar. Identifikasi Status Nutrisi dan Resiko Malnutrisi pada Lanjut Usia di Panti Sosial Tresna Werdha Minaula Kota Kendari. Dunia Keperawatan. 2016;4(2): 93-99.
15. Saha, S., A.Basu.,S.Ghosh., A.K.Saha., U.Baneriee. Assessment of Nutritional Risk and Its Associated Factors among Elderly Women of Old Age Homes of South Suburban Kolkata, West Bengal, India. J ClinDiagn Res.2014.
16. Indriana, Y. I.Febrian Kristiana, Andrewinata, Sonda, A. Intanirian. Tingkat Stress Lansia di Panti Wredha "Pucang Gading" Semarang. JurnalPsikologi Undip.2010. 8(2): 87-96.
17. Yildiz, D., N.B.Pekel., A.K.Kilic., E.N.Tolgay., F. Tufan. Malnutrition is Associated with Dementia Severity and Geriatric Syndromes in Patients with Alzheimer Disease. Turkish Journal of Medical Sciences. 2015.
18. Zelig. Associations Between Tooth Loss, with or without Dental Prostheses, and Malnutrition Risk in Older Adults: A Systematic Review. Clinical Nutrition.2016;31(3):232-47.

Denta Jurnal Kedokteran Gigi, Februari 2020; Vol.14 No.1; Hal 51-57
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