RESEARCH ARTICLE

The Effectiveness Of Anchovy Concentration (Stolephorus insularis) as Antimicrobial to Streptococcus mutans (In Vitro)

Almira Fa'Izah,[*] Istien Wardani,[**] Diana Soesilo[***]
[*] Undergraduate Fakultas Kedokteran Gigi Universitas Hang Tuah
[**]IKGA Fakultas Kedokteran Gigi Universitas Hang Tuah
[***]Konservasi Gigi Fakultas Kedokteran Gigi Universitas Hang Tuah

ABSTRACT

Background: *Dental and oral diseases which are often found in children is dental caries. Streptococcus mutans is the main cause of caries. Caries can be prevented by using a topical application of fluoride. The Anchovy (Stolephorus insularis) contains protein, vitamins (A, B_1, C), and minerals (Fe, Ca, K, F). Calcium fluoride (CaF_2) within the anchovy can inhibit the occurrence of dental caries.* ***Purpose:*** *The aim of this study was to determine the antimicrobial ability of anchovy extract (Stolephorus insularis) to Streptococcus mutans.* ***Materials and Methods:*** *This study was a laboratory experimental research with post test only control group design. Diffusion method were applied with 2 controls: negative control used DMSO 1%, positive control used NaF solution, and 3 concentrations of anchovy extract (Stolephorus insularis) 3%, 6%, and 12%, each group were composed of 6 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 antimirobial zone diameter. The average zone of antimicrobial at the concentration of 3% were 7,11 mm, 6% 9,5 mm, 12% 10,78 mm, for the negative control DMSO 1% 6 mm and the positive control NaF solution 8,16 mm. The largest diameter of the clear zone was at concentration of 12% (P < 0,05).* ***Conclusion:*** *The anchovy extract (Stolephorus insularis) had an antimicrobial effect to the growth of Streptococcus mutans.*

Key words: *Anchovy extract (Stolephorus insularis), Streptococcus mutans, caries prevention, antimicrobial effect.*

Correspondence: *Istien Wardani, Bagian IKGA, Fakultas Kedokteran Gigi Universitas Hang Tuah., Arif Rahman Hakim 150, Surabaya, Phone 031-5945864, 5912191, Email: istienwardani@yahoo.com*

BACKGROUND

Dental and oral diseases which are often found in societies is dental caries. This disease not only affects adults, but also often suffered by children.[1] Based on the basic health research (Riskesdas) in 2007 the prevalence of active caries in Indonesia amounted to 46.5%.[2] Meanwhile, according to the data from Health Ministry of Republic Indonesia (Kemenkes) in 2009, as many as 89% of Indonesian children under the age of 12 years suffer from dental caries.[1]

The main problem in the oral health of children is dental caries. Dental caries is an infectious disease that is closely related to the consumption of foods and beverages that contain cariogenic ingredients.[2] In children the necessary precautions through plaque control, dietary habits (consumption of foods and drinks containing sugar), oral hygiene, fluoride usage, sealant, and mouthwash.[3]

Dental caries is an infectious disease that came from the demineralization in hard tissue of the crown and root surface of the tooth that can be prevented.[3] Caries is progressive because of the accumulation of activity that can be detected by tissue damage from the tooth surface (pit, *fissure,* and the interproximal zone) to extend into the pulp tissue. The main factor that caused caries is the host (teeth), the microorganisms (bacteria), the substrate, and the time.[1]

Demineralization of dental caries caused by susceptible (host), the bacteria that caused caries, and the substrate for bacteria. Caries bacteria include *Streptococcus*, *Lactobacilli*, and *Actinomyces.*[4] The microorganisms produce organic acids especially lactic acid by fermenting carbohydrates on the surface of the teeth resulting in decreased salivary pH (below 5,5) which resulted demineralised totth surface and then forming small holes called dental caries.[4] *Streptococcus mutans* is very meaningful recognized as the cause of dental caries.[5]

Emphasizing prevention of caries in the oral environment returns imbalance as a protective mechanism of remineralization.[6] One of caries prevention is by using fluorine as a topical application, mouthwash, and toothpaste.[7]

The use of fluoride recommended by doctor and dentist so that the teeth become harder and more resistant to caries.[5] The purpose of the use of fluorine is an attempt to protect the teeth from caries. *Fluor* works by inhibiting the metabolism of plaque bacteria that can ferment carbohydrates through changes hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) the enamel becomes fluorapatite ($Ca_{10}(PO_4)_6F_2$).[3] Fluorapatite formation can decrease solubility in acidic email, speed up the remineralization process, and inhibit the action of bacterial enzymes (antimicrobial activity). *Fluor* residing in biofilms will inhibit bacteria work in synthesizing enzyme enolase so that bacteria can not produce acid.[8] The mechanism of caries inhibition by fluoride can be achieved at lower concentrations (< 100 μg/ml). If the fluoride is used too much, can lead to accumulation of fluoride in the matrix, forming a "mottled enamel".[5] The recommended fluoride adequacy rate was 1.5–4 mg/day.[9]

One of the natural ingredients that contain high concentrations of fluoride is anchovy (*Stolephorus insularis*) as many as 15,7-38,3 ppm mainly in the form of CaF_2 compound.[10] Besides containing fluorine, *Stolephorus insularis* also contains energy, protein, fat, carbohydrates, calcium, iron, phosphorus, vitamin A, vitamin B, and vitamin C.[11]

CaF_2 compound can give most fluoride results because of its ability gradually removing fluorine and also acts as a fluorine backup. However, these compounds are not widely used in dentistry because it is difficult to obtain in dosage forms and expensive. [8] So that the fluorine content of *Stolephorus insularis* studied by students of Dentistry University of Indonesia in dosage forms the substrate as a topical application in vivo.

Stolephorus insularis very easily obtained in Indonesia and most people eat.[10] This is because *Stolephorus insularis* is one of the most abundant resources in Indonesia especially nearshore area.[12] Sedati coastal subdistrict, Sidoarjo regency is an area of inshore waters, so *Stolephorus insularis* is one of the local resources.

Antimicrobials are compounds of biological or chemical that is capable of disrupting the activity of microbial growth.[13] *Fluor* in bacteria works bacteriostatic, inhibits cell proliferation by inhibiting the synthesis of nucleic acid which is a very vital part for cell development. The mechanism works by binding to the enzyme-RNA polymerase (the subunits) that inhibit the synthesis of RNA DNA.[14]

Based on the reasons above, the researcher wanted to know the effective concentration of *Stolephorus insularis* extract as antimicrobials against the growth of *Streptococcus mutans* bacteria.

MATERIALS AND METHODS

This research is kind of true experimental, with the study design post test only control group design. The samapling technique using simple random sampling, is to divide the subjects into five groups, each group was given a different treatment. On the negative control group K(-) using a DMSO 1% solution, on the positive control group K(+) using NaF solution, on the treatment group P1 were given *Stolephrous insularis* 3% extract, P2 were given *Stolephrous insularis* 6% extract, P3 were given *Stolephrous insularis* 12% extract.

The tools used are mask, rubber gloves, test tube rack, test tubes, petridish, micropipet, bacti zepper (sterilizer osse), osse, autoclave, incubator, anaerobic jar, gaskit, anaerobic indicator, blender, scales, porcelain bowl, spatula glass, oven, waterbath, rotary evaporator, caliper with precision 0,05 mm, circular disc Ø 6 mm, cotton swab, syringe, densicheck (bacterial turbidity measuring device), cryotube, cryotube rack, tweezer, biological safety cabinet (place to conduct experiments on bacteria). Materials used are *Streptococcus mutans* bacteria in the MH blood agar, *Stolephorus insularis* extracts with various concentrations (3%, 6%, 12%), NaF solution, ethanol 96%, DMSO 1% solution, NaCl sterile liquid, TYC agar.

The process of making *Stolephorus insularis* extracts performed at the Laboratory of

Phytochemistry Faculty of Pharmacy, University of Widya Mandala Surabaya. Purchase *Streptococcus mutans* bacteria and experimental research conducted at the Laboratory of Microbiology of the Center for Health Surabaya.

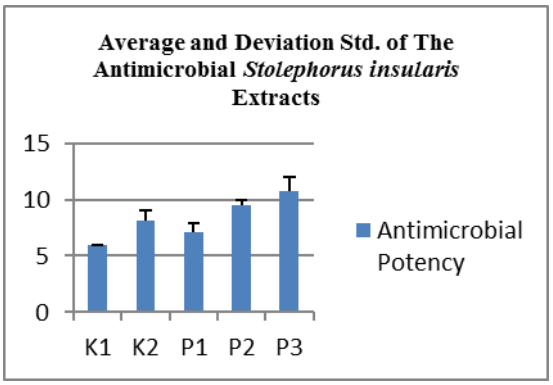
The initial step of this study begins by creating a culture of *Streptococcus mutans* in NaCl liquid sterile which is comparable to 0,5 McFarland standart. Then prepare for sterile TYC media into five research groups, each sample consisting of two control groups and three treatment groups. *Streptococcus mutans* bacteria taken from NaCl liquid that has been synchronized with the turbidity of Mc Farland 0,5 solution and then rubbed on the entire surface of the plate in TYC agar by using a sterile swab. After that, the disc is dipped in each solution was treated for 10 seconds. Then the disc affixed to the TYC agar which have inhaled the *Streptococcus mutans* bacteria in accordance with the zone that has been provided. Incubated for 2x24 hours at 37^{o}C in anaerobic jar in the incubator. After that, measured the diameter of the antimicrobial zone is formed in the form of clear zone around the disc three times using callipers (mm).

RESEARCH RESULT

Data obtained from the research results were tabulates and analyzed descriptively that aims to obtain an illustration of the distribution and summarizing data to clarify the presentation of the results, then test hypotheses using analytic statistical significant level of 95% (p<0,05) using SPSS version 20.

Table 1. Results of average and standart deviation of the antimicrobial potency in each treatment group

Groups	Average ± Deviation Std.
K 1	6 ± 0,00
K 2	8,16 ± 0,93
P1	7,11 ± 0,74
P2	9,50 ±0,50
P3	10,78 ± 1,24



Picture 1. Diagram of average results and the deviation standart of antimicrobial potency in each treatment groups

Before performing hypothesis testing research results, then normality test first, because the normality test is one of the requirements parametric test. Normality test used is *Shapiro-Wilk* test, because the number of research subjects are less than 50 subjects. This test aims to determine whether the data obtained normal distribution or not with a significance level of 0.05 (p=0,05). If the data were normally distributed (p > 0,05) can proceed with parametric test, and if the data are not normally distributed (p < 0,05), then followed by a non parametric test.

The test results for normality using the *Shapiro-Wilk* test in table 2 shows all groups, those are group K2, P1, P2, and P3 normal distributed (p > 0,05).

Homogenity test is also one of the prior conditions to the parametric test. The homogeneity test using *Levene test*. Data can be said to satisfy

the homogenous variance when p>0,05. If p<0,05 then the data does not have a homogenous variances thus qualified to perform parametric tests are not met.

Significancy Test homogenity of variance showed score 0,000 (p < 0,05), It can be concluded that there is a difference of variance between groups of data are compared.

From the results of homogenity test data, It can be concluded that the data have variances those are not homogenous. Thus, the parametric test requirements are not met.

Therefore the terms of parametric test are not met, the data are normal distributed and the data are not homogenous, then tested the non-parametric *Kruskal-Wallis*. *Kruskal-Wallis* test was used to determine the potency antimicrobial differences in *Streptococcus mutans* each treatment groups.

Based on the *Kruskal-Wallis* test results in table 4 of significance value of 0,000 (p < 0,05), then there is a difference in the antimicrobial potency of each groups.

Mann-Whitney test is the continuous non-parametric test from *Kruskal-Wallis* test which is useful to know that the group has significant difference potency antimicrobial by comparing between the two groups with a significance level of p<0,05.

Table 5 The results of *Mann-Whitney* test

Groups	K1	K2	P1	P2	P3
K1		0,000*	0,000*	0,000*	0,000*
K2			6,000	3,000	0,000*
P1				0,000*	0,000*
P2					6,500
P3					

* There are significant differences

The results of *Mann-Whitney* test in table 5 showed there are significant difference of antimicrobial potency (p < 0,05) in group K1 with the entire treatment group, group K2 with group P3, and group P1 with group P2 and group P3. While the K2 group with P1 and P2 group showed no significant difference potency antimicrobial (p > 0,05). So also in the P2 group with P3 group showed no significant difference potency antimicrobial (p > 0,05).

DISCUSSION

This study used *Stolephorus insularis* extract with concentration of 3%, 6%, and 12%. Selection is based on the concentration in vivo studies using substrate *Stolephorus insularis* with 5% concentration which can be used as a topical application of fluoride.[8] MIC fluoride against *Streptococcus mutans* (0,75%)[15] with the highest fluoride content of 38.3 ppm *Stolephorus insularis*,[10] so the researcher tried concentration of 3% as the initial concentration. Then researcher increased the concentration be doubled to 6% and 12%.

Making *Stolephorus insularis* extract using ethanol at 96% concentration because this solvent is universal (can dissolve polar and nonpolar compound) so expected by using ethanol 96%, active substance required may be fully extracted.[17] Then *Stolephorus insularis* powder which already dissolved in 96% ethanol, evaporated by using a rotary evaporator until It becomes thick. Rotary evaporator is used as a solvent vaporizer because It can vaporize until below its boiling point with the aid of a pressure drop so that the chemical

compound contained in the solvent is not damaged or decomposed.[16]

The method used in this research is the diffusion method because the method can be used to test aerobic and facultative anaerobic bacteria.[18] *Streptococcus mutans* bacteria is facultative anaerobic bacteria.[19]

The used of *Streptococcus mutans* bacteria due to these bacteria are the most dominant agent of human dental caries.[20] Caries can be triggered by consumption of foods and beverages that contain cariogenic ingredients [2] which is often favored by children.

Streptococcus mutans incubation using anaerobic jar and gaskit because It can create an anaerobic atmosphere perfectly,[18] allowed to stand at a temperature of 37^{o} for 48 hours.[20]

TYC media used as a medium to determine their inhibition in this study. TYC media contains sucrose which is as high as 50 gr/L, so that *Streptococcus mutans* can ferment to multiply .[22]

The negative control used was DMSO 1% because It doesn't have antibacterial properties that will affect inhibition of bacteria and the extracts tested are natural materials.[23] In addition, the solution serves as a solvent DMSO is rapidly absorbed into the epithelial extract without damaging the cells and is often used in medicine and health.[24] On the positive control using NaF solution because this solution is the topical application material most often used to prevent caries, that is inhibit the growth and development the oral flora that play a role in the caries process.[25] The use of fluoride as a topical application has been done a long time and has been shown to inhibit acid formation and growth of microorganisms.[26]

The results of this study showed that *Stolephorus insularis* extract had antimicrobial power against the *Streptococcus mutans* bacteria in all treatment groups with a concentration of 3%, 6%, and 12%. At a concentration 3% and 6% antimicrobial power of *Stolephorus insularis* extract not much different than antimicrobial power of NaF solution as a positive control. At a concentration of 12%, the antimicrobial power of *Stolephorus insularis* extract is greater than NaF solution. It's because *Stolephorus insularis* contains antimicrobial substances such as fluoride. Fluoride can inhibit the growth and development of microorganisms,[25] inhibits many *Streptococcus* oral bacterial enzyme system,[19] thus inhibiting the activity of cariogenic bacteria in the metabolism of carbohydrates to form acids and polysaccharides adhesive.[26]

Based on the results obtained *Kruskal-Wallis* test, p = 0,000 ($p<0,05$) which showed a significant difference in all groups, then followed by *Mann-Whitney* test to see the significance of the two data groups. Based on the research, It seemed that the greater concentration of *Stolephorus insularis* extract also has a greater diameter of antimicrobial zone. It's because the higher concentration of *Stolephorus insularis* extract, the concentration of active ingredient contained therein are also getting bigger, so the antimicrobial zone is also greater. The averages of antimicrobial at a concentration 3% (7,11 mm), 6% (9,5 mm), 12% (10,78 mm), negative control DMSO 1% (6

mm), and positive control NaF (8,16 mm).

This study was a qualitative difference that shows the power of antimicrobial *Stolephorus insularis* extract against the growth of *Streptococcus mutans* bacteria at a concentration of 3%, 6%, dan 12%, and an initial study. The results showed that the highest concentration of the antimicrobial power which is 12% greater than antimicrobial power of NaF as a positive control. Henceforth, quantitative research needs to be done to determine the decrease in the number of bacterial colonies.

CONCLUSION

Based on the results of the study, the anchovy extract (*Stolephorus insularis*) has effective antimicrobial power against the *Streptococcus mutans* bacteria at a concentration of 12%.

BIBLIOGRAPHY

1. Wala HC, Wicaksono DA, Tambunan E. 2014. Gambaran Status Karies Gigi Anak Usia 11-12 Tahun pada Keluarga Pemegang Jamkesmas di Kelurahan Tumatangtang I Kecamatan Tomohon Selatan. Kandidat Skripsi, Fakultas Kedokteran Gigi Universitas Sam Ratulangi Manado. H. 2
2. Worotitjan I, Mintjelungan CN, Gunawan P. 2013. Pengalaman Karies Gigi serta Pola Makan dan Minum pada Anak Sekolah Dasar di Desa Kiawa Kecamatan Kawangkoan Utara. Jurnal e-GIGI (eG), 1(1): 60.
3. Angela A. 2005. Pencegahan Primer pada Anak yang Berisiko Karies Tinggi. Majalah Kedokteran Gigi (Dent. J.), 38(3): 134-130.
4. Aini BN. 2013. Pengaruh Cara Pengolahan dan Jumlah Daun Sirih Merah (*Pipercrocatum*) terhadap Pertumbuhan *Lactobacillus acidophilus* (Kajian *in Vitro*). Skripsi Fakultas Kedokteran Gigi Universitas Gajah Mada. H. 1.
5. Houwink B, *et al.* 1993. Ilmu Kedokteran Gigi Pencegahan, Alih Bahasa Suryo S & Abyono R. Yogyakarta: Gajah Mada University Press. H. 230-229.
6. Sasmita IS, Pertiwi ASP. 2013. Identifikasi, Pencegahan, dan Restorasi sebagai Penatalaksanaan Karies Gigi pada Anak. Fakultas Kedokteran Gigi Universitas Padjadjaran Bandung. H. 4
7. Herdiyanti Y, Sasmita IS. 2010. Penggunaan *Fluor* dalam Kedokteran Gigi. Program Profesi Fakultas Kedokteran Gigi Universitas Padjadjaran. H. 7
8. Zabrina S. 2012. Pengaruh Aplikasi Substrat Ikan Teri Jengki (*Stolephorus insularis*) terhadap Tingkat Retensi *Fluor* pada Email Gigi Tikus *Sprague Dawley* (*in Vivo*). Skripsi, Fakultas Kedokteran Gigi Universitas Indonesia. H. 22, 2-1.
9. Oktanovia SY. 2013. Ikan Teri Dapat Meningkatkan Ketahanan Email Gigi. Makalah Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Negeri Padang. H. 9
10. Gunawan HA. 2006. Pengaruh Tingkat pH Larutan Teri terhadap Perubahan Dimensi dan Kelarutan Kristal Apatit. Jurnal Anatomi Indonesia, 1(1): 29-25.
11. Daradjatun AN. 2014. Pemanfaatan Tepung Kepala Ikan Teri Jengki (*Stolephorus insularis*) sebagai Bahan Substitusi Tepung Ikan dalam Pakan Buatan Ikan Lele Masamo (*Clarias sp.*). Skripsi, Fakultas Pertanian Universitas Lampung. H.7.
12. Mardjudo A, Rahman ARA. 2014. Usaha Perikanan Ikan Teri (*Stolephorus sp.*) dengan Alat Tangkap Bagan Tancap di Desa Bukit Aru Indah Kecamatan Sebatik Timur Kabupaten Nunukan Provinsi Kalimantan Utara. Jurnal Ilmiah AgrIBA (2): 198.
13. Andayani T, Hendrawan Y, Yulianingsih R. 2014. Minyak Atsiri Daun Sirih Merah (*Piper crocatum*) sebagai Pengawet Alami pada Ikan Teri (*Stolephorus indicus*). Jurnal Bioproses Komoditas Tropis, 2(2): 123.
14. Karina, VN. 2012. Perbedaan Daya Antibakteri Bahan Perekat Braket Ortodonsi antara Semen Ionomer Kaca dengan Resin Komposit Berfluor terhadap *Lactobacillus acidophilus*. Skripsi, Fakultas Kedokteran Gigi Universitas Jember. H. 18-17.

15. Pratiwi R. 2005. Perbedaan Daya Hambat terhadap Streptococcus mutans dari Beberapa Pasta Gigi yang Mengandung Herbal. Fakultas Kedokteran Gigi Universitas Hasanuddin Makassar. H. 67.
16. Tamara R. 2014. Daya Hambat Ekstrak Teripang Emas (*Stichopus hermanii*) terhadap Bakteri *Enterococcus faecalis*. Skripsi, Fakultas Kedokteran Gigi Universitas Hang Tuah Surabaya. H. 60.
17. Amalia S. 2012. Efek Antibakteri Ekstrak Etanol Pegangan (Centella asiatica (L.) Urban) sebagai Alternatif Medikamen Saluran Akar terhadap Porphyromonas fingivalis (secara In Vitro). Skripsi, Fakultas Kedokteran Gigi Universitas Sumatera Utara. H. 3.
18. Bauman RW. 2004. Microbiology International Edition. San Francisco: Pearson Education Inc and Pearson Benjamin Cummings. H. 299-297.
19. Handini AD. 2013. Perbedaan Topikal Aplikasi Bahan Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) dan Bahan Sodium Fluoride terhadap Jumlah Koloni *Streptococcus mutans* pada Saliva Anak Usia 6-12 Tahun. Skripsi, Fakultas kedokteran Gigi Universitas Hasanuddin. H. 16-15, 13.
20. Adrianto WDA. 2012. Uji Daya Antibakteri Ekstrak Daun Salam (*Eugenia polyantha Wight*) dalam Pasta Gigi terhadap Pertumbuhan *Streptococcus mutans*. Skripsi, Fakultas Kedokteran Gigi Universitas Jember. H. vii.
21. Wilson DE dan Chosewood LC. 2009. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. U.S. Department of Health and Human Services. H. 291
22. Wan AKL, Seow WK, Walsh LJ, Bird PS. 2002. Comparison of Five Selective Media for The Growth and Enumeration of *Streptococcus mutans*. Australian Dental Journal, 47(1): 26-21.
23. Patel JD, Anshu KS, Vipin K. 2009. Evaluation of Some Medicinal Plants Used in Traditional Wound Healing Preparations for Antibacterial Property Against Some Pathogenic Bacteria. Journal of Clinical Immunology and Immunopathology Research, 1(1): 012-007.
24. Alfath CR, Yulina V, Sunnati. 2013. Efek Antibakteri Ekstrak Kulit Buah Delima (*Granati fructus cortex*) pada *Streptococcus mutans In Vitro*. Journal of Dentistry Indonesia, 20 (1): 8-5.
25. Setyo D. 2009. Topikal Fluor. Available from https://www.scribd.com/doc/30062773/TOPIKAL-FLUOR. Accessed. December 25, 2015
26. Amnur AND. 2014. Pengaruh Pasta Gigi Mengandung *Xylitol* dan *Fluoride* Dibandingkan Pasta Gigi Mengandung *Fluoride* terhadap Plak Gigi. Skripsi Fakultas Kedokteran Umum Universitas Diponegoro. H. 20.