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Research Report

Sensitivity difference of *Streptococcus viridans* on 35% *Piper betle linn* extract and 10% povidone iodine towards recurrent apthous stomatitis

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ABSTRACT

***Background**: Oral ulceration often becomes the main reason for the patients to see a dentist. Therapy of the oral ulceration is by giving the palliative therapy with topical antiseptic. Nowadays, there are many researches concerning with the traditional medicines as alternative therapy. One of them is Piper betle linn which contains the antiseptic agent. **Purpose**: This research is aimed to observe the sensitivity difference of Streptococcus viridans on 35% Piper betle linn extract and 10%povidone iodine. **Methods**: This laboratory research was conducted by the post test only design with random complete design. The research sampel is Streptococcus viridans culture that was scrapped from the ulcer of the recurrent aphthous stomatitis patient, then it was replicated by using the Federer theory. **Results**: Inhibitory zone of 35% Piper betle linn extract is bigger than 10% povidone iodine. **Conclusion**: Streptococcus viridans are more sensitive to 35% Piper bittle linn extract than 10% povidone iodine. 35% Piper betle linn extract has more antibacterial effect than 10% povidone iodine.*

***Key words**: Bacteriocid test, 35% Piper betle linn extract, 10% povidone iodine, Streptococcus viridans, recurrent aphthous stomatitis*

ABSTRAK

***Latar belakang**: Ulserasi rongga mulut seringkali menjadi alasan utama bagi pasien untuk memeriksakan diri ke dokter gigi. Terapi ulserasi rongga mulut adalah pemberian terapi paliatif kepada penderita, seperti: pemberian obat topikal yang mengandung antiseptik. Saat ini banyak penelitian dalam pengembangan obat tradisional yang dapat dijadikan sebagai obat alternatif. Salah satu diantaranya adalah daun sirih yang mengandung zat antiseptik. **Tujuan**: Penelitian ini bertujuan mengetahui perbedaan sensitivitas Streptococcus viridans terhadap ekstrak daun sirih 35% jika dibandingkan dengan povidone iodine 10%. **Metode**: Penelitian laboratoris yang dilakukan dengan post test only design dengan rancangan acak lengkap. Sampel penelitian adalah kultur Streptococcus viridans yang diambil melalui swab dari hapusan ulser pada pasien yang menderita stomatitis aftosa rekuren, kemudian dilakukan replikasi dengan rumus Federer. **Hasil**: Zona hambat ekstrak daun sirih 35% lebih besar daripada zona hambat povidone iodine 10%. **Kesimpulan**: Streptococcus viridans lebih sensitif terhadap ekstrak daun sirih 35%. Ekstrak daun sirih 35% memiliki efek daya antibakteri yang lebih tinggi jika dibandingkan dengan povidone iodine 10%.*

***Kata kunci**: Uji bakteriosid, ekstrak daun sirih 35%, povidone iodine 10%, Streptococcus viridans, stomatitis aftosa rekuren*

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INTRODUCTION

Ulceration in oral cavity often becomes a major reason for patients to see a dentist. Complaints of oral ulceration can even involve recurrent ulceration, once occurred ulceration or ulceration persistently occured. The etiology of ulcers on the oral mucosa actually involve trauma, recurrent aphthous stomatitis, microbial infections, mucocutaneous disease, drug therapy, and squamous cell carcinoma.[1,2] Recurrent aphthous stomatitis (RAS) is ulcerative oral lesions often found. RAS occurred about 20% of the population, 2% of whom got severe pain.[1,2] The number of women who suffer from RAS is more than men, and more common at age of 20–30 years.[1]

Oral streptococci are actually gram-positive bacteria that can be obtained on all sides of the human oral cavity. Most of *oral streptococci* are classified into viridans group that are opportunistic pathogens. These bacteria can reach the bloodstream because of trauma through oral lesion. *Streptococcus sanguis* and *Streptococcus mitis* are classified into Streptococcus viridans group that can cause secondary infections in RAS, so it can inhibit healing process.[6–8]

Recurrent aphthous stomatitis is actually considered as urcerative lesions often occured on oral cavity. The cases of RAS in patients who arrived at the Department of Oral Medicine, Faculty of Dentistry Airlangga University in 2009 was about 38.99% which was quite high compared to other cases.

The diagnosis of oral mucosal ulceration is established based on the history of the disease, and the clinical feature of the ulcer. The biopsy will then be conducted if malignancy or persistent lesions is suspected more than 2 weeks after the causative factors are ommited.[1,4,5]

Therapy for oral ulceration is conducted by giving palliative therapy to patients, such as antiseptic mouthwash (eg. 0.2% chlorhexidine, 1% hydrogen peroxide, 1% povidone iodine) or by giving simple covering agent, such as 10% povidone iodine ointment and topical corticosteroids which can eliminate the symptoms and help healing process.[1,4,5]

Nowadays, a lot of researches concern with the development of traditional medicine that can be used as alternative medicine since the material is easily available with affordable price.[10,11] *Piper betle linn* is a medicinal plant that has many benefits and contains antiseptic substances in all its parts. *Piper betle linn* is widely used to treat bleeding nose, itchy eyes, sore throath, bad breath, bleeding gums, and sore mouth (ulcers).[11,12]

Mouthwash containing 25% *Piper betle linn* can kill dental plaque bacteria, *Streptococcus sanguis* for 30 seconds. Research conducted by Hendratini and Rasyaad shows that mouthwash containing with 25% *Piper betle linn* extract could inhibit plaque better than that containing with 1% povidone iodine, 1.5% hydrogen peroxide, and 0.1% chlorhexidine gluconate.[13]

A research conducted by Siswanto, moreover, shows that *Piper betle linn* extract ointment 35% is the fastest solution of wound healing process in the cheek mucosa of white rats when compared with 15% and 25% ones.[13,14] In vivo research conducted in female rats induced with everfescent tablet containing with Piper betle linn with the highest toxic dose even did not show any organ damage or death in those examined animals.[15] Clinical research was also ever conducted in humans through patch test showed that 35% *Piper betle linn* extract was a non-allergenic material.[16]

Considering to antibacterial effect possessed by Piper betle linn, it has already been able to be used in oral cavity since there are many toothpaste and mouthwash products containing with Piper betle linn extract sold commercially to protect gingival and dental health. Based on the description above, it is necessary to study more about the benefits of *Piper betle linn* extract as a natural antimicrobial in oral cavity therapeutics. Thus, *Piper betle linn* extract later can be used as a therapeutic oral ulceration made in the form of an ointment.

The antibacterial effect of *Piper betle linn* extract also can prevent secondary infections. It is also known that *Piper betle linn* extract in the form of ointment can be used as covering agent to accelerate the ulcer healing process. Before clinical test was conducted, the sensitivity test towards *Streptococcus viridans* was conducted on 35% *Piper betle linn* extract and then compared with the test on 10% povidone iodine commercially available in the form of ointment. Clinical test showed that 10% povidone iodine in the form of ointment is able to cure recurrent aphthous stomatitis ulcers on the fifth day.[9]

Antimicrobial sensitivity test is a test conducted to determine the sensitivity of microbial pathogens towards antimicrobials. Diffusion method of antibacterial test, moreover, is the most frequently used method, agar diffusion method. The greater the inhibitory zone around the medicine is, the more sensitive bacteria to the medicine is.[17] Therefore, the aim of this research is to observe the differences of the sensitivity of *Streptococcus viridans* on 35% *Piper betle linn* extract and 10% povidone iodine.

MATERIALS AND METHODS

Javanese *Piper betle linn* leaves are obtained from Balai Materia Medica Batu. They were then extracted in the phytochemistry laboratory of UPT of Balai Materia Medica Batu by the following steps. First, fresh *Piper betle linn* leaves were separated from their stems, and then washed and dried by being aerated for ±3 days. Then, they were weighed and finely ground to make it become dry powder. After that, they which wanted to be extracted were weighed. The material of *Piper betle linn* powder used was about as much as 75.524 g (put into six reaction tubes). The *Piper betle linn* powder put into those six tubes was given

ethanol solvent 95% about 1020 ml. The extraction unit was programmed into five extraction cycles with drying programs for 60 minutes. The result obtained was 100% *Piper betle linn* extract about 155 ml. The result of the 100% pure extract was finally given sterile aquades, and then diluted up to 35%.

This research was an experimental clinical research conducted by post test only design with complete randomized block design and has been declared by the Ethical Clearance Commission of Health Research, Faculty of Dentistry, Airlangga University. Samples of this research is *Streptococcus viridans* bacterial culture derived from ulcer swabs on recurrent aphthous stomatitis based on the certain following criteria: namely untreated ulcer diagnosed as major type of recurrent aphthous stomatitis with diameter > 3 mm.

Retrieval specimens then were obtained from lesions of recurrent aphthous stomatitis in adult patients aged between 22–44 years old (Figure 1). Patients were given oral and written explanation about the purpose and methods of how the research will be conducted, and the patients were then asked to fill out and sign informed consent voluntarily. Afterwards, the patients were asked to rinse with water. Their ulcers were then dried with sterile cotton. Then, swab was conducted by using cotton bud that had been sterilized by autoclave. The results were then sent to the microbiology laboratory to be cultured and also to have bacterial identification.

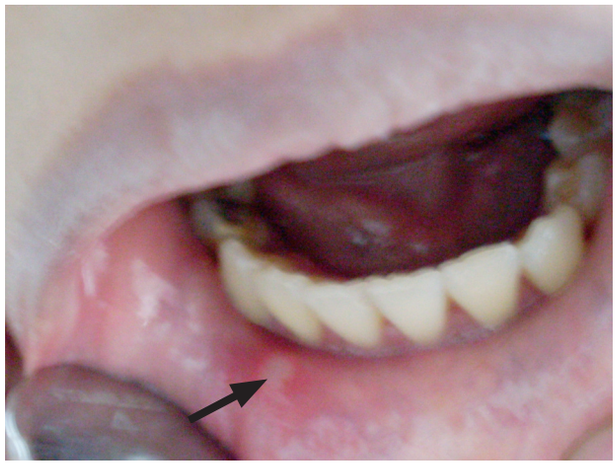

Figure 1. Patient with recurrent aphthous stomatitis on lower labial mucosa (arrow = ulcer).

The bacterial identification of Streptococcus viridans was conducted. First, the swab results were inserted into Brain Heart Infusion (BHI) media. They were incubated for 1 day at 37 centigrade degree. Then, they were planted in *Blood Agar Plate* (BAP) for 1 day at 37 centigrade degrees. The colonies identified as *Streptococcus* were then planted in chocolate agar slant (CAS) for 1 day at 37 centigrade degrees. In other word, the results which were seemed as green strains around the colonies in CAS could be identified as *Streptococcus viridans*, and then were planted in BAP for antimicrobial sensitivity test.

BAP containing with *Streptococcus viridans* was then divided into 3 parts, namely the filter paper with 35% *Piper betle linn* extract (as the treatment group), the filter paper, 10% povidone iodine (as the positive control group), and the untreated group (negative control). The inhibitory zone diameters of those both groups, the treatment group and the positive control group were then compared after one day incubation. Next, those groups were replicated as much as 9 times by using Federer formula. The date was analyzed using independent t test.

RESULTS

Based on the observation and calculation results of the inhibitory zone diameter of oral *Streptococci viridans* on the groups using 35% *Piper betle linn* extract and 10% povidone iodine with nine times of replication for each the mean of the inhibitory zone diameter of *Streptococcus viridans* on the group using 10% povidone iodine is about 10.22 mm lower than that on the group using 35% *Piper betle linn* extract about 13.77 mm (Table 1).

Table 1. The mean and standard deviation of the inhibitory zone diameter of *Streptococcus viridans* on the two research groups

Group	N	Mean (mm)	Standard Deviation
10% Povidone iodine	9	10.2222	0.97183
35% *Piper betle linn* extract	9	13.7778	1.56347

Before conducting the test and analysis on those research groups, normality test was conducted on each of those groups by using Kolmogorov Smirnov test. The result of the test showed that all of those groups had greater values than 0.05 ($p > 0.05$). It indicates that the data of those groups has normal distribution. Next, a different parametric test, Independent T-test, was also conducted to see the significance differences among those research groups.

After conducting Independent t-tests to see the comparison of the inhibitory zone diameters of *Streptococcus viridans* between the group using 35% *Piper betle linn* extract and the group using 10% povidone iodine, it is finally known that there were significant differences in inhibitory zone diameter of *Streptococcus viridans* among those groups since the significance value was smaller than 0.05 ($p = 0.001$ or $p < 0.05$).

DISCUSSION

Based on the results of bacteriocid test on *Piper betle linn* extract against *Streptococcus viridans* in recurrent aphthous stomatitis patients, it is known that there was significant difference in inhibitory zone diameter on the sample group using 35% *Piper betle linn* extract ($p < 0.05$).

Compared with 10% povidone iodine group, 35% *Piper betle linn* extract even had greater inhibitory zone diameter. In this research, there were actually three given treatments, 35% *Piper betle linn* extract, 10% povidone iodine, and control. Those three treatments then were replicated by using the Federer formula since this research is considered as a purely experimental research using homogeneous and randomized samples derived from the cultured colonies of *Streptococcus viridans*.

Streptococcus viridans was taken from a sample through a swab on the patients with recurrent aphthous stomatitis ulcer to maintain the homogeneity of the research sample. Then the bacteria are cultured and incubated at 37 degrees for 1 day. Sensitivity test of *Streptococcus viridans* was then conducted by diffusion method. It aims to know the diameter size of inhibitory zone of 35% *Piper betle linn* extract compared with 10% povidone iodine. The larger the diameter of the zone is the higher the inhibitory properties of its bacteriocid *Streptococcus viridans* is actually more sensitive to 35% *Piper betle linn* extract than 10% povidone iodine because of high enough antibacterial properties of phenolic component in the essential oil of *Piper betle linn* extract. Toothpaste with essential oil of *Piper betle linn* extract has high antiseptic power against *Streptococcus* colonies α.[18] Essential oil actually consists of phenol component (propenyl phenol) as much as 60% and non-phenol component. Phenol is antiseptic component consisting of eugenol, estragol, chavibetol (betle phenol),[9] and chavikol which can kill some bacteria, such as gram-positive and gram-negative bacteria.[12]

Propenyl phenol is a toxic compound that can disturb and open three-dimensional structure of *Streptococcus viridans*, which then becomes a random structure without causing damage to the structure of the covalent skeleton, but causing denatured proteins of *Streptococcus viridans*. After the denaturation process, amino acid sequence of protein remains intact, but the biological activities of protein are broken, so it cannot implement its function.[12] The content of propenyl phenol on *Piper betle linn* extract is very strong and able to kill bacteria since it has bacteriocid, five times greater than phenol. There is great phenolic components in 35% *Piper betle linn* extract, such as eugenol, estragol, chavibetol, and chavikol. This condition makes the diameter of inhibitory zone of 35% *Piper betle linn* extract greater than that of 10% povidone iodine. As standard antiseptic, povidone iodine has high antiseptic power by interacting in the cell walls of bacteria causing the formation of permanent pores, so it then causes the loss of cytoplasmic material and the reducing of enzyme activity, and later the bacteria become lysis.[19]

To obtain high antibacterial power, the selection of *Piper betle linn* must be considered. The use of *Piper betle linn* that is still young is better than the old one since the content of volatile oil in the young is higher than the old one.[18] Another thing that must be considered to get the essential oil of *Piper betle linn* is selecting the fresh one with bright color, perfect shape, free of disease (fungus or pests), and without color changing.[10]

In this research, 95% ethanol solvent was used. In the extraction process of *Piper betle linn*, organic solvents, such as ether, alcohol, and chloroform, should be used. It is because the essential oil is not soluble in the water solvent.[20] Twenty five percent *Piper betle linn* extract and methanol solvent has better antibacterial power that that with bacitracin 10 U, chloramphenicol 30 μg, streptomycin 10 μg, sulfonamides 300 g, and vancomycin 30 μg.[21]

It can be concluded that *Streptococcus viridans* is more sensitive to 35% *Piper betle linn* extract since it has higher antibacterial inhibitory effect than 10% povidone iodine. Thus, clinical test research is needed on 35% *Piper betle linn* extract as the active ulcer therapeutic agent to be applied in patients with oral ulcers. Furthermore, bio molecular research is then also needed to know the content mechanism of 35% *Piper betle linn* extract during the healing process of oral cavity ulcer.

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