



## Effectiveness of *Piper betle* leaf infusion as a palpebral skin antiseptic

Husnun Amalia<sup>\*a</sup>, Ratna Sitompul<sup>\*\*</sup>, Johan Hutauruk<sup>\*\*</sup>,  
Andrianjah<sup>\*\*\*</sup> and Abdul Mun'im<sup>\*\*\*\*</sup>

### ABSTRACT

The goal of an antiseptic is to eliminate or greatly reduce the number of microorganisms in the surgical field at the time of the surgery. The objective of this study was to verify the effectiveness of 20% *Piper betle* leaf infusion as an antiseptic solution in pre-surgery cataract patients. A clinical trial with partner-matching design was conducted on 31 pairs of eyelids. From each pair of eyelids, one eyelid was assigned to the *Piper betle* infusion group and the opposite one to the povidone-iodine group. The microorganisms were collected by swab from the patient's palpebral skin, inoculated on nutrient agar, and incubated at 37°C for 20 hours. The antiseptic effectiveness was measured by counting the microbial colonies before and after administration of the antiseptic solutions. This study demonstrates that the mean colony counts after application of 20% *Piper betle* leaf infusion showed a significant reduction of 27-100% compared with those before administration ( $p=0.001$ ). Mean colony counts after 10% povidone-iodine administration showed a significant reduction of 88-100% compared with the mean counts before the solution was applied ( $p=0.000$ ). The 20% *Piper betle* infusion has an antiseptic potential. Nevertheless, the 10% povidone-iodine solution has more effective antiseptic capability.

**Keywords:** Antiseptic, palpebral skin, *Piper betle* leaf infusion, povidone-iodine

\*Department of Ophthalmology,  
Medical Faculty,  
Trisakti University  
\*\*Department of Ophthalmology,  
\*\*\* Department of Microbiology,  
\*\*\*\*Department of Mathematics  
and Natural Science,  
Faculty of Medicine,  
University of Indonesia

#### Correspondence

\*dr. Husnun Amalia, SpA  
Department of Ophthalmology,  
Medical Faculty,  
Trisakti University  
Jl. Kyai Tapa No.260 Grogol  
Jakarta 11410  
Telp. 021-5672731 ex. 2614

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### INTRODUCTION

The Indonesian Basic Health Survey of 2007 (*Riset Kesehatan Dasar tahun 2007*) indicates that the prevalence of cataracts in Indonesia is up to 1.8%.<sup>(1)</sup> The management of

cataract is by cataract surgery. Good surgical results are dependent on pre- and intra-operative procedures as a whole, including treatment with antiseptic solutions. The use of antiseptics before surgery is a standard procedure. In 1867, Joseph Lister for the first time introduced the

preoperative use of antiseptics.<sup>(2)</sup> Preoperative antisepsis is performed by cleansing the periocular skin with an antiseptic solution in order to prevent contamination by normal palpebral microbial flora at the time of surgery.<sup>(3)</sup>

Povidone-iodine is an antiseptic that has been known for more than four decades and is extensively used to date.<sup>(4)</sup> Povidone-iodine is a broad-spectrum antimicrobial agent that is effective against both gram-positive and gram-negative bacteria, fungi, bacterial spores, viruses, protozoa, mycobacteria and yeasts. This solution contains iodine as the active principle, which has an excellent and rapid antimicrobial action.<sup>(4)</sup> The concentration used for periocular skin cleansing is 5-10%.<sup>(5,6)</sup> The topical application of povidone-iodine in a significantly high concentration can result in irritation and sensitivity reactions in 0.7-20% of the population.<sup>(4,7)</sup> Although the incidence of allergic reactions to povidone-iodine is not high, if these reactions occur preoperatively, they may become an impediment to the course of surgery and the patient will suffer.

The betel vine (*Piper betle* Linn., *sirih*), the leaves of which are extensively consumed as betel quid in the Indian subcontinent, has been used as a medicinal plant by Southeast Asian peoples for more than 3000 years. Betel leaf has broad antiseptic properties and to date there have been no reports in the literature on the occurrence of cases of allergy to the plant. The use of betel leaf as an antiseptic is increasing.<sup>(9)</sup> The active substances in betel leaf are phenol and its derivatives. The phenolic derivatives contained in betel leaf have a five-fold greater antibacterial potency than phenol itself.<sup>(10)</sup> Hydroxychavicol, a major phenolic compound present in the aqueous extract of the *Piper betle* leaf, is known for its antioxidant and anticancer properties.<sup>(11,12)</sup> The betel leaf has been demonstrated to be effective against *Streptococcus* sp., *Staphylococcus* sp., *Escherichia coli*, *Vibrio*

*elitor*, *Salmonella typhosa*, *Shigella shigae*, *Micrococcus luteus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and others.<sup>(13,14)</sup>

Cases of allergy to povidone-iodine encountered in the population need an alternative antiseptic solution. An infusion of betel leaves have the potential for becoming an alternative antiseptic because of its bactericidal action that has been demonstrated in a number of studies. This is the reason for the present study, which aims to compare the effectiveness of the antiseptic action of a betel leaf infusion to that of povidone-iodine as a control in the antisepsis of the palpebral skin in pre-surgery cataract patients.

## METHODS

### Study design

The present study was an experimental trial conducted in the operating room of the Department of Ophthalmology of a government hospital in Central Jakarta in November 2007 with matching approach. The laboratory part of the investigations were performed at the University of Indonesia, respectively in the laboratory of the Department of Microbiology, Faculty of Medicine, and the Phytochemical Laboratory of the Department of Pharmacy, Faculty of Mathematics and Natural Sciences, both of which are located in Depok.

### Study subjects

The sampling in this study was done consecutively. The prospective samples for this study were patients who were planned to undergo cataract surgery in the operating room of the Department of Ophthalmology. The eyelids of selected patients were assigned by matching into the S group comprised eyelids of non-surgical patients, which underwent antisepsis with betel leaf infusion and the B group comprising eyelids of surgical patients, which underwent antisepsis with povidone-iodine solution. Each matched

pair in the S and B groups came from the same patient, namely the left and right eyelids, that were subjected to different treatments.

The inclusion criteria were patients presenting to the self-supported Ophthalmology Operating Room of a government hospital in Central Jakarta who were planned to undergo cataract surgery and consenting to participate in the study; all eyes and eyelids that belonged to surgery and non surgery candidates and were healthy, without previous surgery, and free of tumors, infections, or wounds; and the selected patients had to be willing to fill out an informed consent form for this study.

#### **Extraction method for *P. betle* leaves**

All stages of the study were performed in conditions of antiseptis. The test substances in this study were betel leaf infusion and povidone-iodine solution. The betel leaves were from *Piper betle* Linn., as identified by the Biological Research Center of the Indonesian Scientific Institute (*Lembaga Ilmu Pengetahuan Indonesia - LIPI*) in Cibinong. The betel leaves were washed, dried and comminuted by rubbing them between the hands. Of the reduced *simplicia*, 10- and 20-twenty gram portions were weighed on an analytical balance (Shimadzu, type AW120) and subsequently made into infusions in sterile glass beakers. Each of the beakers was filled with 100 mL distilled water, which was supplemented with 20 and 40 mL of distilled water (twice the dry weight of the *simplicia*). The contents of the beakers were subsequently heated for 15 minutes at 90°C, with occasional stirring. The betel leaf preparations were left to cool and strained through sterile filter paper. Hot distilled water was poured through the strained leaves and this was added to the infusions up to a volume of 100 mL. The resulting infusions had a concentration of 20% (w/v). The infusions were filtered by means of bacterial filters of 0.22 mm pore size (Millipore filters) and kept in sterile

bottles at room temperature in a dry place and protected from direct sunlight. The povidone-iodine solution used as test substance was a commercially available solution under the brand name of Betadine.

The clinical specimens were collected by using cotton swabs before and after antiseptic treatment of the palpebral skin on both eyes of the patients. A preliminary study was performed to determine the concentrations of the betel leaf infusions (10% atau 20%), the type of transfer medium (normal saline [NaCl 0.9%] or nutrient broth), the transfer volume (1 mL, 2 mL, or 3mL), and the appropriate dilution.

The collected clinical specimens were then serially diluted in normal saline and nutrient broth (NB), to obtain inoculum concentrations of 1, 1:10, 1:100, 1:1000, and 1:10.000. Volumes of 100 mL of the clinical specimen suspensions were taken with sterile micropipettes for inoculation on nutrient agar (NA) plates. The inoculation was performed in duplicate for both treatment groups by spreading sterile glass beads onto the plates, following which the inoculated plates were incubated for 20 hours at 37°C. The effectiveness was assessed by the reduction in colony counts and the percentage of reduction. The number of colony-forming units (CFUs) was determined manual the agar culture.

#### **Data analysis**

The primary data were statistically analyzed using SPSS version 12, by means of the t-test. The primary data were transformed into their log<sub>10</sub> to obtain a normal distribution of the data. The transformed data were tested for normality, if  $p \geq 0.05$  indicated that the obtained data were distributed normally. Paired t-test was used for analyzing within-group data, whereas unpaired t-test was for between-group data. The data in the preliminary study was tested by ANOVA. A p value of  $< 0.05$  (2-tailed) was considered to be significant.

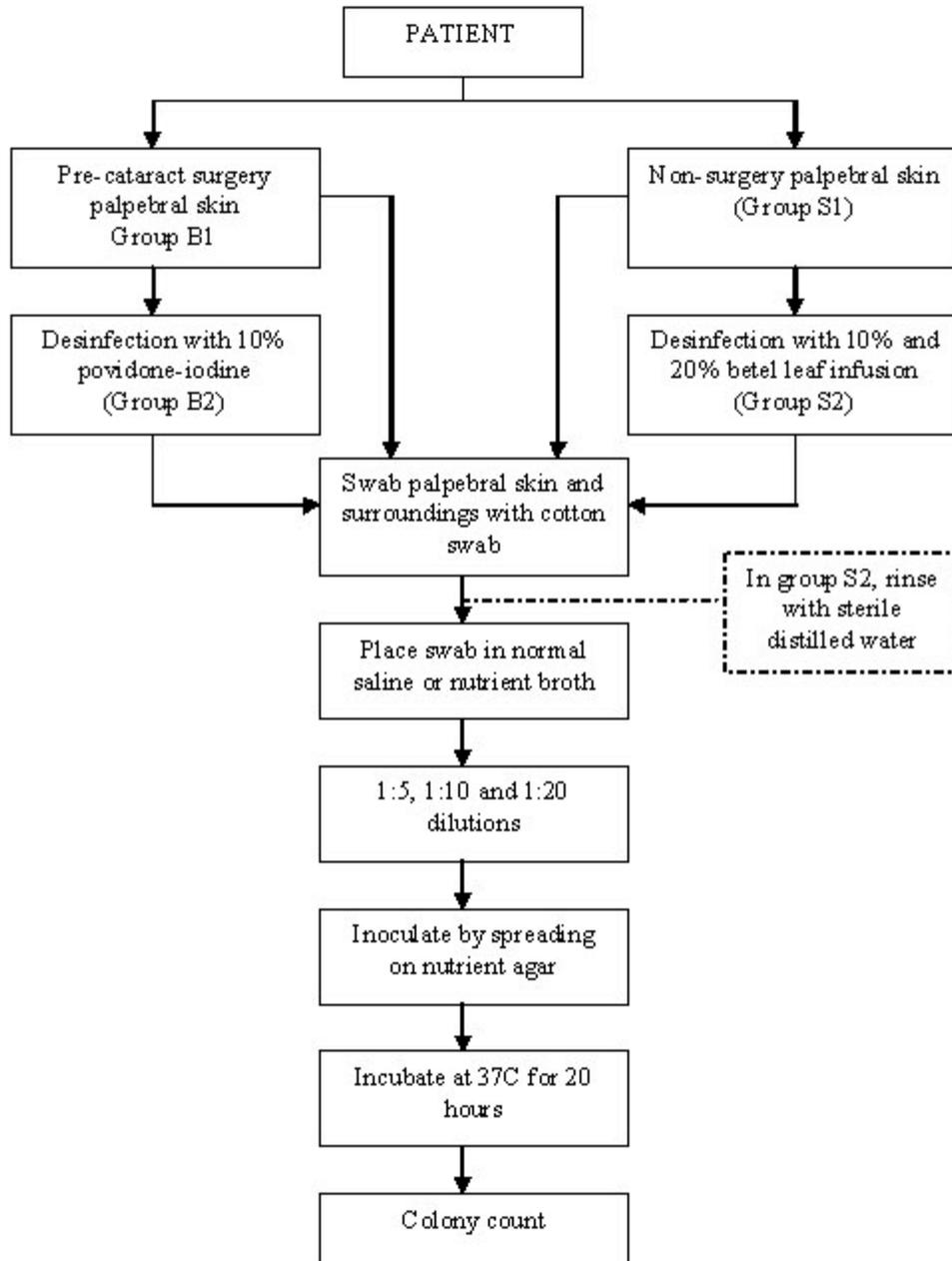


Figure 1. Flow of study

## RESULTS

The infusions made in this study had concentrations of 10% and 20% (w/v), were brownish in color, had the characteristic betel

leaf odor, with pH = 6 for the 10% and pH = 5.2 for the 20% infusions. The control solution used in this study was a 10% povidone-iodine solution under the brand name of Betadine. The latter was an opaque reddish-brown solution with pH = 4.

Tests using the 20% betel leaf infusion showed a higher percentage reduction in microbial colony count in comparison with the 10% betel leaf infusion. Therefore, in this study the 20% infusion was subsequently used. The preliminary study with nutrient broth yielded substantial numbers of microbial colonies. In contrast, normal saline yielded smaller colony counts, which were however adequate for quantitative analysis. Thus in this study normal saline was used as the transfer medium. The tested transfer volumes in the preliminary study were 3 mL, 2 mL, and 1 mL, respectively. The colony counts in the preliminary study were inversely related to the transfer volumes, where the smallest transfer volumes yielded the highest counts; thus the best transfer volume was 1 mL. The serial dilution tests in the preliminary study indicated that the microbial counts could be easily determined from undiluted samples. Therefore the samples contained in the transfer medium could be directly inoculated on nutrient agar plates.

#### Effectiveness of the antiseptics

The present study involved 31 pre-surgery cataract patients meeting the inclusion criteria. Table 1 shows that the majority of patients were elderly persons with a mean age of  $\geq 60$  years (64.5%), and that there were 54.8% female and 45.2% male elderly. Both age and gender were not correlated with the initial colony count (before antiseptic treatment, respectively with betel leaf infusion and povidone-iodine solution).

Table 1. Distribution of demographic characteristics (n=31)

Characteristics	N (%)
Gender	
Male	14 (45.2)
Female	17 (54.8)
Age (yr)	
< 60	11 (35.5)
> 60	20 (64.5)
Transportation	
Bicycle	7 (22.5)
Public	6 (19.4)
Taxi	6 (19.4)
Private	12 (38.7)

There was no significant difference for colony counts in both groups before antiseptic treatment with betel leaf infusion and povidone-iodine solution ( $p > 0.05$ ), and thus the antiseptic power of both types of antiseptic could be compared between groups (Table 2).

Mean colony count after antiseptics with 20% betel leaf infusion was slightly lower in comparison with mean colony count before antiseptics ( $p=0.001$ ) (Figure 2). This indicates that the 20% betel leaf infusion had a potential antiseptic capability. Mean colony count after antiseptics with povidone-iodine solution was always within the range of 0-7, irrespective of the colony count before antiseptics ( $p=0.000$ ) (Figure 3), indicating that antiseptic effectiveness of povidone-iodine solution was extremely high for all types of microorganisms present on palpebral skin.

Table 2. Mean colony counts before and after treatment, by treatment group

	Before antiseptics	After antiseptics	p
Betel leaf infusion	577	134	0.001
Povidon-iodine	510	1	0.000
p	0.452	0.000	

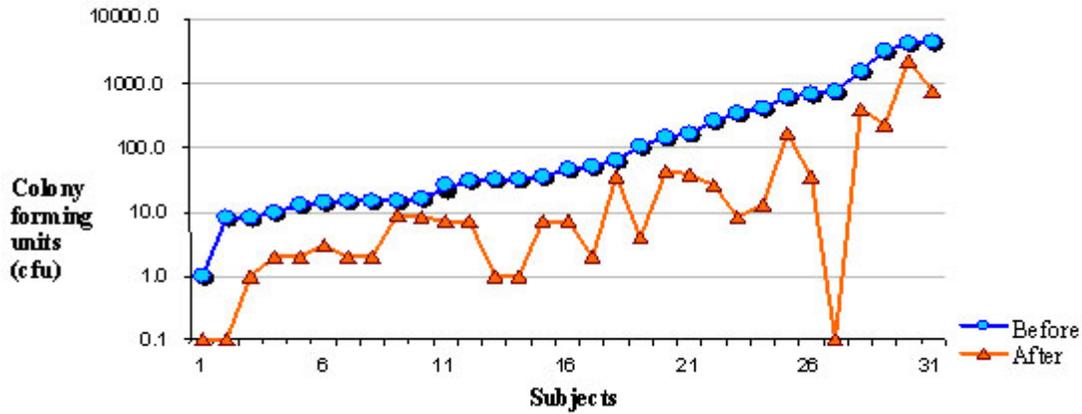


Figure 2. Mean colony counts with 20% betel leaf infusion

Figure 4 shows that the effectiveness of povidone-iodine solution was in most cases around 100%, implicating that this solution has a maximal antiseptic capability that is superior to that of 20% betel leaf infusion.

**DISCUSSION**

Antiseptic solutions play an important role in preventing sepsis of the skin and mucous

membranes by significantly inhibiting and killing the microorganisms present on these tissues.<sup>(15)</sup> The human skin has a normal microbial flora that is extremely varied in species and numbers, therefore an antiseptic substance needs to have considerable antiseptic capability for use in pre-operative procedures of antiseptis.<sup>(16)</sup>

The present study demonstrates that there was a significant reduction in colony count after antiseptic treatment with povidone-iodine as well

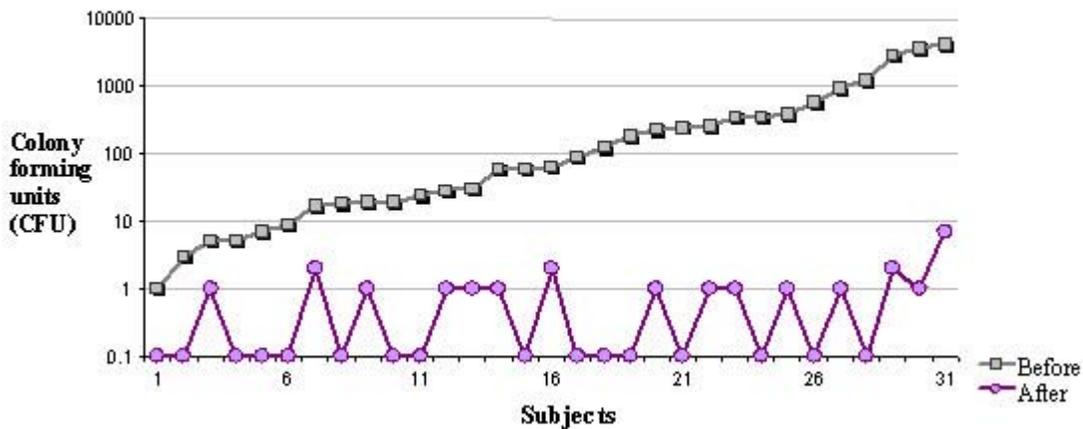


Figure 3. Mean colony counts with 10% povidone-iodine solution

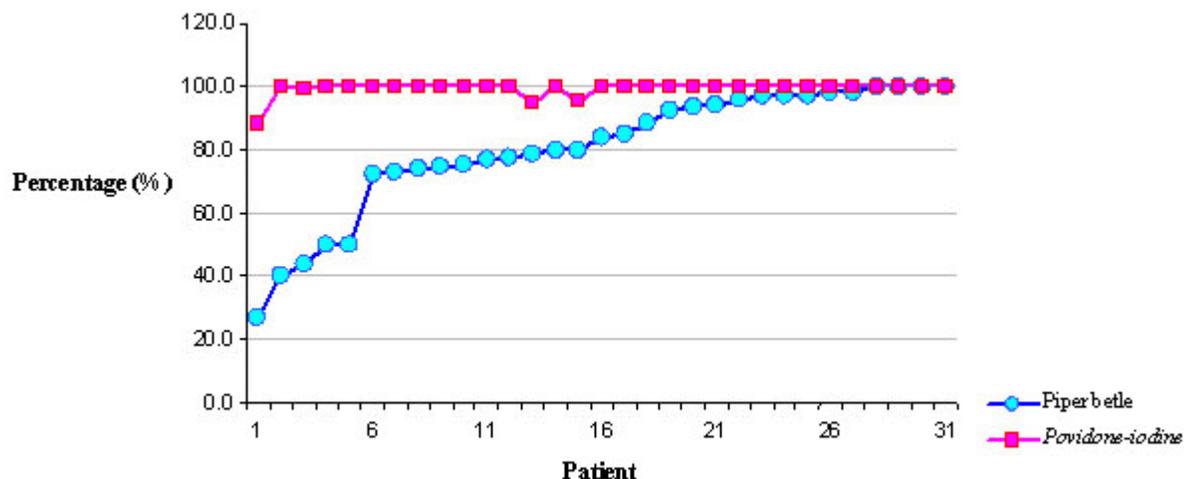


Figure 4. Percentages in reduction of colony counts with 20% betel leaf infusion and 10% povidone-iodine

as with 20% betel leaf infusion. This indicates that both test substances are effective antiseptics and possess antimicrobial properties against the normal palpebral flora.

Twenty-percent betel leaf infusion has a potentially strong antibacterial capability but is less effective. The difference in effectiveness of betel leaf infusion between the *in vitro* study by Murnikusumawatie<sup>(17)</sup> and the results of the preliminary investigation for the present study may possibly be the result of differences in species of microorganisms and in the antiseptic power of the infusions against certain microbial species. Betel leaf infusions are capable of inhibiting the growth of two microbial species in minimal inhibitory concentrations (MIC) of 5% and 20%, but these concentrations are inadequate for inhibiting other microorganisms.<sup>(13)</sup>

Povidone-iodine as a 10% solution exhibits an extremely effective antibacterial capability against all species in the normal human microbial flora, irrespective of their numbers on the palpebral skin. This solution kills all normal flora

within 2-3 minutes, whether present in large or small numbers. Ferguson et al.<sup>(4)</sup> reported that 5% povidone-iodine solution has an effective antiseptic capability as pre-operative antiseptic solution on the palpebrae and adnexa. Irrigation of the fornices with 5% povidone-iodine was associated with significantly fewer positive conjunctival cultures at the time of surgery.<sup>(18)</sup> The present study demonstrates that 20% betel leaf infusion and 10% povidone-iodine had effective antibacterial capability in reducing the colony count. However, 10% povidone-iodine was capable of killing greater numbers of bacteria after antiseptic treatment ( $p=0.001$ ), indicating that the antibacterial capability of 10% povidone-iodine was higher than that of 20% betel leaf infusion.

Povidone-iodine has been shown to be bactericidal against a wide range of bacteria, and is also effective against fungi, protozoa, and viruses.<sup>(19)</sup> Povidone-iodine is an antiseptic solution with a concentration of 10%, which is equivalent to 1% iodine. Houang et al.<sup>(20)</sup>

reported the minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 1% povidone-iodine solution against four species of test bacteria to be in the range of 1:256 – 1:8192. The content of active substance in povidone-iodine is accurately known and has been determined in numerous studies. The betel leaf infusion used in the present study had a concentration of 20% (w/v), but its content of active substances is as yet undetermined.

Suriatmini reported that the total concentration of phenolic derivatives present in a 5-10% betel leaf infusion was 0.72-2.28%, while the concentration of phenolic derivatives used as antibacterial solution was 0.5-2%.<sup>(21)</sup> Betel leaf infusions contain phenolic derivatives with a six-fold greater antibacterial power than phenol itself. However, their concentrations are not known with certainty and there have been no studies on the minimum bactericidal concentration (MBC) of these phenolic derivatives.

The reduction in microbial colony count after antiseptic treatment with 20% betel leaf infusion varied between 27 and 100%, but a value of  $\leq 50\%$  was found in only four patients. This may be due to variation in the normal flora of individual patients and is evidenced by the variation in colonial morphology in each sample, even though no identification of the colonies was performed.

In this study, assessment of allergic reactions was done at 10 minutes, 60 minutes and 24 hours after application of the antiseptic. The signs and symptoms used for assessment were redness of the skin, feelings of warmth, and itching. None of the participants showed any of the above signs and symptoms after application of the antiseptic to the palpebral skin, indicating that 10%- and 20%- betel leaf infusions did not induce allergic reactions on palpebral skin.

## CONCLUSION

Although 20% *Piper betle* leaf infusion is potentially antiseptic, 10% povidone-iodine solution has more effective antiseptic capability. Thus 20% betel leaf infusion has less effective antiseptic capability compared with 10% povidone-iodine solution. 

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