Isolation and Determination of *Candida albicans* on The Mouth and Siwak Salafi Community

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**Abstract.** Siwak is a Salvadoraceae family that is reported by some researchers to have an antimicrobial effect. The purpose of this study was to determine *Candida albicans* in the mouth and siwak. This research is a type of "true experimental" research with the population of the Salafi community of the Lima Kaum Batusangkar mosque, the sample was taken by purposive sampling as many as three people who showed different ways of using it. The stages of the study were divided into 2 steps, the first step was the isolation of *Candida albicans* in all three types of samples, the second step was by molecular identification, DNA extraction was carried out by following standard procedures from Sambrook using the phenol-chloroform technique. The Polymerase Chain Reaction (PCR) process was carried out using a Thermal Cycler Applied Biosystem Type 2700 with a volume of 25 µl containing 100ng / ml of total DNA, 2 µl 2.5 mM dNTP, 0.62 µl (10 mmol) primary mix forward, and 0.625 µl 10x buffer. The results of the amplification of CO1 gene fragments in electrophoresis using an agarose gel. Readings of the CO1 gene sequences were carried out in Macrogen Co., Korea, using forward primers MI3F (-21) 5'TGTAAAACGACGGCCAGT3' and reverse primer MI3R (-27) 5'CAGGAAACAGCTATGAC3'. The results showed that the isolation of the mouth and wood of washed and unwashed siwak showed positive *Candida albicans* while in samples that varied the use of siwak and toothpaste there was no *Candida albicans* found in the mouth and the wood of the siwak.

1. Introduction
Siwak in the historical sociological review has been used as a tool for cleaning teeth and mouth since 7000 years ago \[^1\]. Siwak is a family plant of Salvadoraceae which is reported by several researchers to have an anti-microbial effect. Maharani, et al reported that Salvadoraceae can inhibit *Candida albicans*. *Candida albicans* is a normal flora found in 80% of healthy people. The results of Farooqi and Srivastava's research in Eva explained that in the siwak plant has been found silica, sulfur, and vitamin C. The chemical content is very beneficial for oral health. Trimethylamine and vitamin C help cure and repair gingival tissue. According to research on siwak, it is proven that the use of siwak ingredients can help cure and repair gingival tissue.

The habit of cleansing has become a culture in Muslim communities, especially those who are members of the Tanah Datar Salafi congregation. Based on interviews on August 10, 2017, the pilgrims obtained information that the use of siwak has been carried out routinely, especially before
the fard prayer time. This custom is based on the sunnah of the Prophet Muhammad who always has used siwak every time before prayer. In the implementation, there are various methods. From the three worshipers interviewed, information was obtained that there was a combination of using siwak with toothpaste, meaning that siwak was used every time they would pray Fardhu but in the morning and evening they still used normal toothpaste to brush their teeth. The other two worshipers only use Siwak wood in maintaining oral and dental hygiene, but there are differences in the treatment of Siwak wood, one of whom always wash Siwak wood every time it is used while the other does not wash Siwak wood. The above facts are interesting to study because in the Tetet Kartilah study reported that there are differences in the level of dental hygiene between siwak wood and modified siwak wood

Mohd and Turner in Hasan Suliman also reported that newly cut siwak wood had a good effect compared to non-fresh ones, and it was suspected that there were dangerous components if used after 24 hours [2]. So based on the facts above, it is necessary to research the determination of Candida albicans as microorganisms that can be found in the mouth to see the effect of using siwak. The purpose of this study is to determine the existence of Candida albicans in the mouth and siwak wood of Salafi Tanah Datar worshipers.

2. Materials and Methods

2.1 Materials
The materials used was a siwak wood (Salvadora persica), Spriger / Drigalsky stem, Volume pipette, bunsen lamp, NA media in Petridishes, Pure bacterial culture, Diluent solution (BPW or 0.9% physiological NaCl).

2.2 Isolation of Candida albicans
Isolation was carried out on all three types of samples with the condition of the mouth before cleansing, after cleansing, wood that has been used. The isolation process is carried out in a way; Make a dilution of 10-1 - 10-6 from a pure bacterial culture with a diluent solution, take a test tube containing a pure bacterial culture, open and burn the tube neck. Transfer 0.1 ml of bacterial culture aseptically to the surface of the NA media in a petri dish, burn the spreader that has been previously dipped in alcohol, allow it to cool, spread / spread the bacterial culture with a spreader evenly and allow the surface to dry, after the surface to dry, then incubate in reverse for 24 hours at room temperature and observe the growth, compare the growth of each dilution and compare the growth with the results of the spread plate technique in experiment 2 (sterilization by filtration).

2.3 Molecular Identification
DNA extraction was carried out by following the standard procedure of [3] using the phenol-chloroform technique. The Polymerase Chain Reaction (PCR) process uses Thermal Cycler Applied Biosystem Type 2700 with a volume of 25 µl containing 100ng / ml of total DNA, 2 µl 2.5 mM dNTP, 0.62 µl (10 mmol) primary mix forward, and 0.625 µl 10x buffer. PCR conditions include predenaturation of 94 ° C for 30 seconds, 50 ° C for 40 seconds, after which a final elongation of 72 ° C is carried out for 10 minutes. The results of the amplification of CO1 gene fragments in electrophoresis using 2% AGE (Agarose Gel Electrophoresis). The CO1 gene sequence is carried out using DNA sequencing services in Macrogen Co., Korea. The CO1 sequence is performed using the primary forward MI3F (-21) 5'TGTAAAACGACGGCCAGT3' and reverse primer MI3R (-27) 5'CAGGAAACAGCTATGAC3'.

3. Results and Discussion
The use of siwak for Muslims in Tanah Datar has become its lifestyle. Although in practice, there are still many who do not fully believe in the efficacy of siwak so they feel the need to combine the use of
siwak and toothpaste. In the results of research on samples that use a combination of siwak and toothpaste together as shown in Table 1, the results showed that almost all isolates were detected negatively to the content of *Candida albicans*.

This is probably caused by the antimicrobial effect produced by toothpaste and siwak wood. The content of thiocyanate oxidase and hydrogen peroxide as an antimicrobial contained in siwak turns out to kill several bacteria such as *Staphylococcus intermedius*, *A. actinomycetemcomitans*, *Veillonella parvula*, *Actinomyces israelii*, *Selenomonigena spuigena*, *Streptococcus salivarius*, *Streptococcus oralis*, and *Actinomyces naeslundii*.[4]

| No | Prayer Times | Treatments | Results |
|----|--------------|------------|---------|
|    |              | Before Siwak | After Siwak | Siwak Wood | Rep I | Rep II |
| 1  | Subuh        | √ (isolate 1) | Negative   | Negative   |       |       |
|    |              | √ (isolate 2) | Positive   | Positive   |       |       |
|    |              | √ (isolate 3) | Positive   | Positive   |       |       |
| 2  | Zhuhur       | √ (isolate 4) | Negative   | Negative   |       |       |
|    |              | √ (isolate 5) | Positive   | Negative   |       |       |
|    |              | √ (isolate 6) | Negative   | Negative   |       |       |
| 3  | Asyar        | √ (isolate 7) | Negative   | Negative   |       |       |
|    |              | √ (isolate 8) | Negative   | Negative   |       |       |
|    |              | √ (isolate 9) | Negative   | Negative   |       |       |
| 4  | Maghrhib     | √ (isolate 10)| Negative   | Negative   |       |       |
|    |              | √ (isolate 11)| Positive   | Positive   |       |       |
|    |              | √ (isolate 12)| Negative   | Negative   |       |       |
| 5  | Isya         | √ (isolate 13)| Negative   | Negative   |       |       |
|    |              | √ (isolate 14)| Negative   | Negative   |       |       |
|    |              | √ (isolate 15)| Negative   | Negative   |       |       |

Besides, the content of Potassium Chloride and sodium chloride which is also found in siwak wood also has antibacterial power and cleaning effect.[5]. Toothpaste also contains several chemical compounds that are similar to siwak. It was found that toothpaste containing SLS detergent turned out to also contain calcium carbonate, water, sorbitol, hydrated silica, flavor, cellulose gum, potassium citrate, DMDM hydantoin, and fluoride.[6] Allegedly the content of chemical compounds that are similar between toothpaste and siwak has a dual effect on antimicrobials so that the average is obtained negatively on the content of *Candida albicans*. However, it was also found that positive isolates containing *Candida albicans* were isolated 2, isolate 3, and isolate 11. Isolate 2 and isolate 11 were samples taken in the mouth with the condition of the mouth after cleansing was detected to contain *Candida albicans* while isolate 3 was samples taken on wood siwak that has been used.

In isolate 2, molecular identification was carried out using BanI PCR RFLP of SADH, data obtained that isolates were identified with 3 strains identified by DNA ribosome sequence analysis and deposed with BankGen. Can be seen in the following table 2.

The strain identified 100 percent is KU058170.1. *Candida orthopsilosis* strain JCABC010, AY520277.1 *Candida orthopsilosis* strain BG02-7-15-020A-F-2, KU058171.1 *Candida orthopsilosis* strain JCABC018. *Candida orthopsilosis*. Candida is known to have diverse types, known as *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metaphsilosis* are genetically different types both psychologically and morphologically.[7]. Candida species can attack the mouth, vagina, skin, and lungs, and some are even found as food contaminants such as *Candida orthopsilosis* NN 14, Other species that are often isolated from clinical specimens are *C. dubliniensis*, *C. tropicalis*, *C. kefyr*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. lusitaniae*. *C.
guillermondii, C. stellatoidea, C. pseudotropicalis, and C. famata. Mixed Candida species can be found in oral candidiasis, but the main cause is C. albicans\cite{8}.

Table 2. BLAST isolate 2 result

| Isolate                                      | % Identiti | E-value | Max score |
|----------------------------------------------|------------|---------|-----------|
| KU058170.1. Candida orthopsilosis strain JCABCO10 | 100,000    | 0.0     | 2998      |
| AY520277.1 Candida orthopsilosis strain BG02-7-15-020A-F-2 | 100,000    | 0.0     | 2998      |
| KU058171.1 Candida orthopsilosis strain JCABCO18 | 100,000    | 0.0     | 2996      |
| HE681725.1 Candida orthopsilosis Co 90-125    | 99,938     | 0.0     | 2992      |
| FN812686.1 Candida orthopsilosis 5S rRNA gene | 99,938     | 0.0     | 2992      |
| KY118177.1 Candida parapsilosis isolatee XS2  | 99,877     | 0.0     | 2987      |

In Table 3, information can be obtained that almost all samples of Siwak wood are identified as containing Candida albicans, while mouth samples both before cleansing and after cleansing are identified as negatively containing Candida albicans.

Table 3. The objects with the condition of siwak washed every use

| No | Prayer Times | Treatments : | The Result |
|----|--------------|--------------|------------|
| 1  | Subuh        | √ (isolate 16) | Positve    |
|    |              | √ isolate 17) | Negative   |
|    |              | √ isolate 18) | Positive   |
| 2  | Zhuhur       | √ isolate 19) | Negative   |
|    |              | √ (isolate 20) | Negative   |
|    |              | √ (isolate 21) | Positive   |
| 3  | Asyar        | √ (isolate 22) | Positive   |
|    |              | √ isolate 23) | Negative   |
|    |              | √ isolate 24) | Positive   |
| 4  | Maghrhib     | √ (isolate 25) | Negative   |
|    |              | √ (isolate 26) | Positive   |
|    |              | √ (isolate 27) | Positive   |
| 5  | Isya         | √ (isolate 28) | Positive   |
|    |              | √ (isolate 29) | Negative   |
|    |              | √ (isolate 30) | Positive   |

This research was conducted on objects that used to use siwak wood to maintain oral health without being combined with the use of toothpaste. This proves that siwak wood is effective as an antibacterial which is very effective in preventing and killing bacterial and anti-fungal growth\cite{8}. This research also proves that antimicrobial substances possessed by siwak wood can be produced when siwak fibers are rubbed into teeth so that it stimulates an increase in the quantity of saliva at the same time that siwak will emit fluoride ions, silicon, tannic acid, sodium bicarbonate and various other chemical compounds.
that can inhibit bacterial growth \[1\]. However, this antimicrobial compound cannot overcome the growth of microbes, especially \textit{Candida albicans} attached to siwak wood, this is evidenced by the detection of microbes in the wood of siwak. This reinforces the allegation conveyed by M. Ibrahim that there may be harmful microbes after 24 hours on siwak wood if it is not refreshed \[9\]. However, DNA identification in siwak wood was not detected by microbes.

Table 4 below explains the results of tests on objects that normally use Siwak every time they will pray Fardhu. However, in this condition, Siwak wood is always washed every time it is used.

**Table 4.** The objects that are not washed during use

| No | Prayer Times | Treatments : | The Result |
|----|--------------|--------------|------------|
|    |              | Before Siwak | After Siwak | Siwak Wood | Rep I | Rep II |
| 1  | Subuh        | √ ( isolate 31) | √ ( isolate 32) | Negative | Negative |
| 2  | Zhuhur       | √ ( isolate 34) | √ ( isolate 35) | Positive | Positive |
| 3  | Asyar        | √ ( isolate 37) | √ ( isolate 38) | Negative | Positive |
| 4  | Maghrhib     | √ ( isolate 40) | √ ( isolate 41) | Negative | Negative |
| 5  | Isya         | √ ( isolate 43) | √ ( isolate 45) | Positive | Positive |

From the results of the study obtained information that almost all isolates were detected negative Candida, there were only two isolates that were positive Candida namely isolates 34 and 45. Isolate 34 continued with molecular identification while 45 was not identified further. The results of molecular identification show that isolate 34, which is a sample of the mouth before cleansing, identified the \textit{Candida albicans} strain ATCC 18804. It can be seen in Table 5 below:

**Table 5.** BLAST isolate 34 result

| Isolat | % Identiti | E-value | Max score |
|--------|------------|---------|-----------|
| HQ876034.1 \textit{Candida albicans} strain ATCC 18804 | 99.106 | 0.0 | 2211 |
| AF114470.1 \textit{Candida albicans} | 99.024 | 0.0 | 2207 |
| CP025165.1 \textit{Candida albicans} strain SC5314-P0 | 99.024 | 0.0 | 2206 |
| CP025157.1 \textit{Candida albicans} strain SC5314-P0 | 99.024 | 0.0 | 2206 |
| CP025182.1 \textit{Candida albicans} strain SC5314-GTH12 | 99.024 | 0.0 | 2206 |

It was explained earlier that \textit{Candida albicans} is a normal flora found in 80% of healthy people although it can turn into a pathogen when there is a pre-deposition factor \[8\]. The pathogenic nature of siwak causes oral candidiasis. In a study conducted by Setiawati Maharani, it was found that siwak extract was effective in inhibiting the growth of \textit{Candida albicans} by 50%. The same results were also found in this study that the use of siwak although not combined with the use of toothpaste was successful in inhibiting the growth of \textit{Candida Albican}. This is evidenced by the small number of isolates that were detected negatively in studies of samples using siwak wood routinely even though
the presence of *Candida albicans* as normal flora cannot be completely removed from the human body.

4. Conclusions
The conclusion of the research is The results showed that the isolation of the mouth and wood of washed and unwashed siwak showed positive *Candida albicans* while in samples that varied the use of siwak and toothpaste there was no *Candida albicans* found in the mouth and the wood of the siwak.

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