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MORALITY BEFORE KNOWLEDGE

The effectiveness of siwak extract as an antibacterial agent against *Staphylococcus aureus, Streptococcus mutans*, and *Escherichia coli*

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ABSTRACT
Background: The growth of bacteria on external wounds can lead to infections, worsening the condition of the wound due to bacterial decay. This is also caused by certain antiseptic substances that possess only minimal antibacterial properties and are less effective in inhibiting bacterial growth. **Methods**: This study employed sterilization tests and resistance or inhibition tests against bacterial growth, such as S. aureus and E. coli. **Findings:** Siwak contains natural minerals that can inhibit bacterial growth, kill bacteria, remove plaque, prevent cavities, and maintain gum health. **Conclusion**: The study on the effectiveness of siwak stem extract as an antimicrobial agent revealed that the extract of siwak stems (Salvadora persica Linn) can effectively inhibit and kill certain microbes. This is due to the antimicrobial substances found in siwak stem extract, which can prevent the growth of specific microbes. **Novelty/Originality of this article:** It is suggested that herbal ingredients can be used to inhibit biofilm formation in certain bacterial species such as S. aureus and E. coli, as the biological activity and antibacterial effects of tannins and flavonoids have been proven. The novelty of this research lies in the testing of the antibacterial effects of siwak as a herbal ingredient to inhibit the formation of bacterial biofilms in species that commonly cause infections in external wounds.

KEYWORDS: antimikrobial; siwak; staphylococcus aureus and escherichia coli.

1. Introduction

Bacterial growth on open wounds can lead to infections, significantly aggravating the condition as harmful pathogens begin to decompose tissue. This process can be further complicated by the use of certain antiseptic agents that possess low antibacterial efficacy, rendering them less effective in curbing bacterial proliferation and preventing the infection from spreading.

An open wound occurs when the skin peels after a fall or being injured by a sharp item. Typically, these wounds induce pain and suffering. Open wounds should be treated immediately because they might not only impact appearance but also cause bacterial infections. There are several approaches of treating open wounds, including the use of antiseptics. Unfortunately, antiseptics containing chemicals might have negative effects. Antisepsis is the prevention of infection through techniques that dramatically reduce germs on the skin or mucous membranes, hence it can also refer to the process of sterilizing items,

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tools, or spaces to prevent sepsis. An antiseptic (germicide) is a chemical that inhibits bacterial development rather than killing bacteria.

Antisepsis was initially used to protect patients from environmental pathogen contamination (disease-causing organisms) by preventing organisms from entering the body through wounds and limiting the development of infectious diseases. In the 1980s, healthcare institutions established "Universal Precautions" for all patients, signaling a shift in thinking about disease transmission via blood and bodily fluids. With this transition, antisepsis and disinfection became critical tools for safeguarding healthcare personnel and patients. To make antiseptics, mix ethanol and alcohol in a 1:1 ratio (160 ml) in a beaker until fully mixed. After homogenization, add 40 ml of distilled water and mix to combine.

Herbal compounds are thought to be capable of inhibiting biofilm formation in numerous bacterial species, including S. aureus and E. coli, due to the biological activity and antibacterial effects found in tannins and flavonoids. Miswak, a herbal ingredient, contains these chemicals. Miswak includes natural minerals that can limit bacterial growth, destroy bacteria, remove plaque, prevent cavities, and keep gums healthy.

1.1 Siwak (Salvadora perdicia)

Chewing sticks are made from a variety of plants that differ by country. In the Middle East, the most common source is the Arak plant (Salvadora persica). Lemon (Citrus aurantifolia) and orange trees (Citrus sinensis) are widely used in West Africa. African Americans use the roots of the Senna plant (Cassia vinea). Sierra Leone uses African Laburnum (Cassia sieberiana), while India uses Neem (Azadirachta indica). The taxonomy of the Siwak plant is as follows:

Kingdom: *Plantae*
Division: *Magnoliphyta*
Class: *Magnoliopsida*
Order: *Brassicales*
Family: *Salvadoraceae*
Genus: *Salvadora*
Species: *Salvadora persica*

The Arak tree (Salvadora persica) is a small, shrub-like plant known for its dense, branched growth. Its branches are sturdy and can reach a diameter of over a foot, while the plant itself generally grows no taller than three meters. Siwak, also referred to as miswak, is a traditional dental stick derived from the roots and fresh branches of the Arak tree. The typical siwak measures between 15 to 20 cm in length and has a diameter ranging from 1 to 1.5 cm. Once the bark is peeled, the inner part reveals a white surface filled with numerous fibrous strands. While the outer root is brown, the inner core is white, emitting a fresh, celery-like aroma and a mildly spicy flavor.

Siwak is valued for more than just its use as a toothbrush. Its fibers are naturally elastic, durable, and resistant to breakage, ensuring that even vigorous use does not harm the teeth. The thinner sticks of siwak are especially flexible, making them ideal for reaching every part of the mouth, effectively removing food particles and plaque from teeth surfaces. In addition to its mechanical benefits, siwak contains various natural components beneficial for oral hygiene, further enhancing its utility in maintaining dental health.

1.2 Benefits of Siwak

Siwak has been widely used, particularly in countries where the majority of the population practices Islam, such as the Middle East, Pakistan, Nepal, India, Africa, and Malaysia, as a tooth-cleaning tool that has been scientifically proven to prevent dental damage even when used in the absence of other cleaning and dental care tools or methods.

Siwak goes under numerous names in different nations. In the Middle East, it's known as siwak, miswak, or arak. In Tanzania, it is also known as miswak. In India and Pakistan, it is known as miswak or datan. A recent study on periodontal care undertaken by experts from King Abdul Aziz University in Jeddah, which sampled 480 persons aged 35 to 65 years in the cities of Mecca and Jeddah, indicated that periodontal care in Mecca and Jeddah is lower than in other nations. This indicates that the use of siwak is strongly associated with the lower need for periodontal care among the populations of Mecca and Jeddah.

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Research comparing the effectiveness of miswak powder as an additive in toothpaste with formulations lacking this ingredient shows that toothpaste containing miswak powder provides superior dental cleaning results. The finely ground particles of miswak powder are uniquely capable of reaching narrow, hard-to-clean spaces between teeth, effectively removing stubborn food debris and plaque that conventional toothpaste may miss. This deep-cleaning ability has made miswak powder a key component in promoting oral hygiene, leading many dental care companies worldwide to incorporate it into their products. The World Health Organization (WHO) has also recognized miswak as a valuable natural health resource, highlighting its potential for both current use and future development in oral care practices.

From the above explanation, it can be concluded that miswak is an excellent tool for oral hygiene due to its significant benefits, easy availability, and low cost. Therefore, it is hoped that miswak can contribute to developing countries facing economic barriers and limitations in dental health facilities by improving oral health status in these countries.

1.3 Chemical composition of siwak sticks

Siwak contains natural minerals that inhibit bacterial growth and kill bacteria, remove plaque, prevent cavities, and maintain gum health. The beneficial chemical components of siwak include: (1) Antibacterial acids, such as astringents, abrasives, and detergents, which function to kill bacteria, prevent infections, and stop gum bleeding. The use of fresh siwak sticks may initially feel slightly spicy and somewhat burning due to the mustard-like substances present, which are part of the antibacterial acids. (2) Chloride, potassium, sodium bicarbonate, fluoride, silica, sulfur, vitamin C, trimethylamine, salvadorin, tannins, resins, saponins, flavonoids, sitosterol, and several other minerals, which serve to clean, whiten, and promote the health of teeth and gums. (3) Natural aromatic oils with a fresh taste and smell that can refresh the mouth and eliminate unpleasant odors. (4) Enzymes that prevent plaque formation. (5) Anti-decay agents and antimicrobial systems that act as penicillin to reduce the number of bacteria in the mouth and prevent decay. Siwak also stimulates saliva production, which is an organic component of the oral cavity that protects and cleanses the mouth.

1.4 Staphylococcus aureus

Staphylococcus aureus is a spherical bacterium approximately 1 μm in diameter that stains Gram-positive. Under the microscope, it appears as clusters resembling grapes. This bacterium can withstand temperatures up to 50°C, high salt concentrations, and dryness. Staphylococci colonies are large, measuring 6-8 mm in diameter, and appear transparent. S. aureus is widely distributed in the environment and often exists as normal flora on humans, particularly in the axillary, inguinal, perineal regions, and anterior nasal openings. About 25-30% of people carry S. aureus in their nasal cavities and on their skin.

Staphylococcus aureus (S. aureus) is a catalase-positive bacterium that has the ability to ferment mannitol, distinguishing it from other species of Staphylococcus. When cultured on Mannitol Salt Agar (MSA), S. aureus metabolizes mannitol, producing acidic by-products. These acidic by-products lower the pH of the medium, leading to a color change in the pH indicator, phenol red, which shifts from red to yellow, indicating fermentation. This characteristic is a key differentiator between S. aureus and other Staphylococcus species, such as Staphylococcus epidermidis (S. epidermidis), which does not ferment mannitol. When S. aureus is cultured on Columbia agar supplemented with 5% defibrinated sheep blood and incubated at 37°C, it exhibits beta-hemolysis. This is observed as a clear zone surrounding the bacterial colonies, due to the lysis of red blood cells, further aiding in its identification and differentiation from other species.

1.5 Eschericia coli

Escherichia coli, commonly known as E. coli, was first identified in 1885 by Theodore Escherich, a German pediatrician. During his observations, Escherich noted the widespread presence of this bacterium in the intestinal tracts of healthy individuals, especially within the fecal flora of newborns and infants. It was discovered that E. coli is a natural inhabitant of the human gut and is also found in the intestines of many other warm-blooded animals. Remarkably, this bacterium coexists with humans throughout their lifetime, forming part of the normal microbiota in the digestive system. Although E. coli is generally harmless, it is considered an opportunistic pathogen. It has the potential to cause primary infections in the intestines, contributing to approximately 5-10% of such cases. Furthermore, it has been closely linked to nosocomial infections in healthcare settings and is a major causative agent of urinary tract infections, particularly in women.

Escherichia coli is classified as a Gram-negative, rod-shaped bacterium that does not form spores, is not acid-fast, and measures 2-3 x 0.6 µm. Its characteristics include: (1) Being part of the Gram-negative bacterial group. (2) Having a rod (bacillus) shape ranging from short to coccoid forms. (3) Existing either as single cells or in pairs (diplobacilli), with some forming short chains. (4) Not forming spores or capsules. (5) Possessing peritrichous flagella. (6) Pathogenic E. coli has a cell wall with pili. (7) Featuring a thin peptidoglycan layer. (8) Its outer membrane comprises lipoproteins, lipopolysaccharides, and phospholipids. (9) Being an aerobic and facultative anaerobic bacterium.

Based on the diseases it causes, E. coli can be categorized into two groups: (1) Opportunistic E. coli, which can cause disease under certain conditions such as nutritional deficiencies or other underlying illnesses. (2) Enteropathogenic or enterotoxigenic E. coli, which has adhesive antigens and produces enterotoxins, leading to disease.

Pathogenic types include: (1) ETEC (Enterotoxigenic Escherichia coli). (2) EPEC (Enteropathogenic Escherichia coli). (3) EIEC (Enteroinvasive Escherichia coli). (4) EHEC (Enterohemorrhagic Escherichia coli). (5) EAEC (Enteroaggregative Escherichia coli). (6) DAEC (Diffuse-Adherence Escherichia coli).

2. Methods

2.1 Siwak stick sterilization test

The sterilization of the siwak stem extract obtained by filtering via a germ filter was tested by introducing 5 ml of the solution to two culture tubes containing heart infusion broth. The tubes were then incubated at 37 °C for 24 hours. If the tubes show no turbidity, the extract is regarded sterile (Lilis S, Yoni A).

2.1.1 Preparation of Staphylococcus aureus bacterial samples

The bacteria employed are Staphylococcus aureus strains. The bacteria are subcultured on blood agar plates for 24 hours at 37 degrees Celsius. 4-5 colonies are picked

from the growing colonies using a sterile loop and incubated at 37°C for 2-5 hours, or until bacterial growth is observable. To make a bacterial suspension, dilute it with sterile physiological saline until it matches the turbidity of the Brown III standard suspension. This corresponds to a bacterial concentration of 10^8 CFU/ml. The solution is then diluted with BHI broth to reach a bacterial concentration of 10^6 CFU/ml (Lilis S, Yoni A. 2016).

2.1.2 Preparation of Streptococcus mutans and Escherichia coli bacterial samples

Streptococcus mutans (S. mutans) bacteria were cultured on Brucella agar at 37°C in an anaerobic environment for 24 hours. Escherichia coli was first grown on MHA agar for 24 hours at an optimum temperature of 37°C. Subsequently, colonies of E. coli were collected and transferred into test tubes containing sterile aquades, NaCl, or Water Broth as the liquid medium. The mixture was then shaken until turbidity appeared.

2.2 Resistance Test (Inhibition)

2.2.1 Staphylococcus aureus Resistance Test

Determination of the minimum inhibitory concentration (MIC) of siwak stem extract using the serial dilution method in broth: Ninety test tubes were prepared for three series of dilutions with three repetitions, where each series of dilution in one repetition involved ten tubes. In tubes 2 through 9, 1 ml of sterile distilled water was added. Next, 1 ml of siwak extract was added to tubes 1 and 2, resulting in a concentration of 50% in tube 1 and 25% in tube 2. Serial dilutions were then performed from tubes 2 through 9 by transferring 1 ml of the solution from tube 2 to tube 3. Tube 3 was mixed until homogeneous, then 1 ml was transferred to tube 4, and so on, until tube 9 was transferred to tube 10. Tube 10 contained the remaining dilution of the siwak extract and served as a negative control.

2.2.2 Streptococcus mutans resistance (inhibition) test

Prepare Brain Heart Infusion (BHI) with added yeast, and then prepare 8 test tubes filled with BHI and yeast. Two of these tubes should be prepared in duplicate. The 100% extract (1 g/ml) is diluted twice. Fill the first seven tubes with the diluted extract, while the eighth tube serves as the control (without miswak extract). Bacteria grown on Brucella agar media under anaerobic conditions are used as the standard inoculum. Add 1 ml of bacterial suspension to 9 ml of BHI and yeast, then homogenize. Transfer 1 ml of the bacterial suspension, which has been added to BHI and yeast, into the 7 prepared tubes. Incubate the tubes at 37°C under anaerobic conditions for 48 hours. Observe the turbidity to determine the Minimum Inhibitory Concentration (MIC).

2.2.3 Escherichia coli Resistance (Inhibition) Test

The parameter being tested is the Total Escherichia coli, using the ISO 9308-1:2014 method for Enumeration of Escherichia coli and Coliform Bacteria in water samples that have been treated with extracts and residues of miswak. The variations in dosage for the miswak extract are 1, 5, 10, and 15 mL, while for the miswak residue, the variations are 1, 5, 10, and 15 mg. These dosage variations for the extract and residue are independent variables, similar to the optimum contact times which are 1, 5, 10, 15, 30, and 60 minutes. All samples are agitated at a speed of 200 rpm according to the specified contact times. For each test, a control without any addition of extract or residue is also established to determine the initial condition of Total E. coli.

3. Results and Discussion

3.1 Minimal Inhibitory Concentration (MBC) Test

3.1.1 Staphylococcus aureus

The table below illustrates the average minimum inhibitory concentration (MIC) of Salvadora persica stem extract against Staphylococcus aureus, based on the results obtained from a comprehensive study conducted by Lilis S. and Yoni A. in 2007. This research aimed to evaluate the antimicrobial properties of the extract, particularly its effectiveness in inhibiting the growth of Staphylococcus aureus.

Table 1. Minimum Inhibitory Concentration (MIC) of Salvadora persica Stem Extract Against Staphylococcus aureus from the Study by Lilis S. and Yoni A. (2007)

No	Microbial Species	MIC (gr%)
1	*Staphylococcus aureus ATCC 25923*	6,25
2	*Staphylococcus aureus (wild strain)*	6,25

Table 1 demonstrates that the extract of siwak stems is capable of inhibiting and killing both pure and local strains of S. aureus. This indicates that siwak stem extract possesses antimicrobial properties against S. aureus that are bactericidal in nature. To inhibit the growth and kill S. aureus, siwak stem extract requires only a concentration of 6.25%.

3.1.2 Streptococcus mutans

In the study conducted by Zaenab, Mardiastuti, Anny, and B Logawa in 2004, the minimum inhibitory concentration (MIC) of siwak stick extract against S. mutans was found to be 6.25% w/v, equivalent to 62.5 mg/ml. Additionally, S. mutans growth was observed at a concentration of 3.125% w/v, or 31.25 mg/ml.

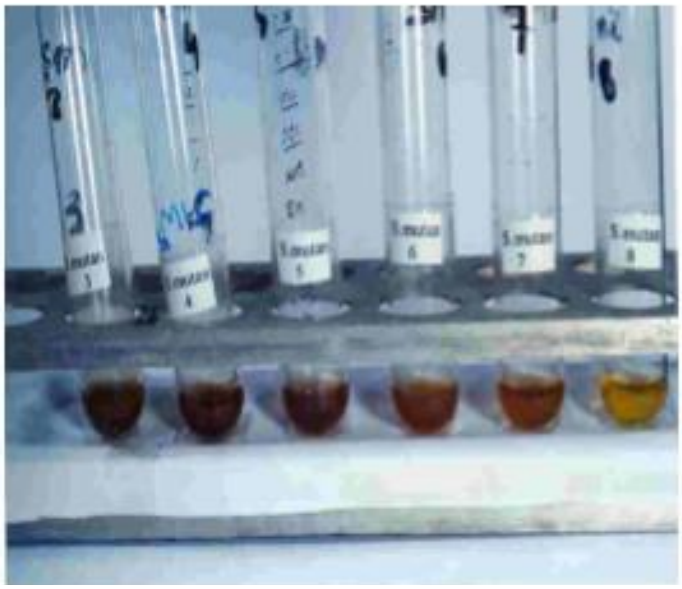

Fig. 1 The results of the MIC test on S. Mutans bacteria obtained MIC results at a concentration of 6.25gr% in the 4th tube and the growth of Ss. Mutans in the 5th tube with a concentration of 3.125gr%

Table 4.2 Minimal Inhibitory Concentration of Siwak Stem Extract Against S.mutan

No	Concentration (%)	MIC of Siwak Stem Extract
1	50	-
2	25	-
3	12,5	-
4	6,25	-
5	3,125	+

No	Concentration (%)	MIC of Siwak Stem Extract
6	1,563	+
7	0,753	+
8	0	+

3.1.3 Escherichia coli

At contact times of 1, 5, and 10 minutes, a sample dilution of 10^-2 was used, whereas for contact times of 15, 30, and 60 minutes, a sample dilution of 10^-1 was applied for observation data. This is because, at shorter contact times of 1, 5, and 10 minutes, the contact time between the extract and E. coli colonies was still too brief, making any changes in colony count less visible when using a lower dilution factor. In contrast, at longer contact times of 15, 30, and 60 minutes, the changes in colony count were more apparent with a dilution of 10^-1.

The overall effectiveness of bacterial eradication of E. coli in response to various quantities of extract used indicates that at an extract volume of 15 ml and a contact duration of 5 minutes, E. coli colonies were eliminated at a rate of 99.980%, corresponding to a decrease of 3.7 log. In contrast, a 10 ml extract volume resulted in a 99.995% reduction, or 4.3 log, in colony count after 10 minutes of contact. Overall, the eradication of E. coli colonies increased with the addition of extract quantities starting from 1 ml across all contact durations, except for the 5, 10, and 15-minute durations, where a preliminary decrease was observed. This decrease was followed by changes in subsequent volume increases and decreases; however, these fluctuations were minor except for the 5-minute contact time. In some variations of miswak extract volume addition, E. coli colonies existed in an ideal environment, resulting in no decline in colony count; instead, the colonies underwent regeneration, yielding negative findings.

Subsequent testing was conducted on the residue (raffinate) of miswak to analyze the extent of E. coli colony inhibition. Dilutions used for each sample were 10^-1 and 10^-2. The choice of dilutions was based on data from previous tests, particularly the observed range of E. coli colonies. Since the results served as comparative data, the dilutions had to be identical. The results indicated a high antibacterial activity, with up to 88.80% or 0.951-log reduction in E. coli colony count after 10 minutes of contact with a residue mass of 15 mg at a 10^-2 dilution. The pattern observed in the inhibition caused by the residue, whether at a 10^-1 or 10^-2 dilution, showed that the greater the mass added, the higher the E. coli colony inhibition. It can also be concluded that the residue contains antibacterial compounds with greater efficacy compared to the miswak extract, as evidenced by the absence of negative results across all mass variations.

4. Conclusions

In the study on the effectiveness of siwak stem extract as an antimicrobial agent, it was found that siwak stem extract (Salvadora Persica Linn) can significantly inhibit and kill certain microbes. This is due to the antimicrobial compounds present in the siwak stem extract that prevent the growth of specific microbes. Other studies have also demonstrated that siwak stem extract can prevent plaque formation on teeth and various dental issues such as tooth decay. Therefore, it is highly recommended for the public to use siwak stem extract as a teeth-cleaning medium. Besides its ability to prevent disease-causing bacteria, using siwak stems also follows the Sunnah of Prophet Muhammad SAW, providing additional benefits to its users. This study also identified aspects that require further research on siwak stem extract, such as the tannins contained in the extract that function as astringents, among other compounds. Furthermore, special attention must be paid during bacterial cultivation to avoid contamination with other microbes, such as fungi and airborne bacteria, which could affect the results. Preventive measures include managing the incubator temperature and ensuring the sterilization of the equipment used.

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Author Contribution
H. F. R & A. N conceived and designed the study, performed the experiments, analyzed and interpreted the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper and approved the final draft.

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