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The Antimicrobial and Chemical Properties of Salvadora persica; Miswak

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Abstract: Oral microbiota colonization begins at birth and is normally established within one year. Oral infections due to bacterial dysbiosis are most commonly treated with antibiotics. However, the increasing use of antibiotics contributes to global antimicrobial resistance, calling for the exploration of alternative treatments. A potential practice for dental care that has been around for ages is using herbal products such as miswak (Salvadora persica), a chewing twig that serves as a toothbrush alternative and antimicrobial agent. Methanol and aqueous extracts of miswak have been shown to exhibit anti-inflammatory and anti-infectious activity. Common extracted compounds include alkaloids, flavonoids, glycosides, vitamins, essential oils, minerals, sterols, and terpenes. It is crucial to further investigate its potential use as a widely known product and to comprehend any associated side effects or risks. In this study, we selected 17 species of bacteria and one yeast species and conducted susceptibility testing using standard Kirby-Bauer disk diffusion techniques. Additionally, we extracted miswak in methanol and performed Thin Layer Chromatography and Column Chromatography. We found the highest antimicrobial activity in vacuumed miswak, particularly in the 4 species that inhabit the oral cavity. The extraction of that miswak revealed 3 flavonoid compounds with distinct Rf Values. The findings demonstrated that miswak possesses antimicrobial properties and contains compounds essential for antioxidant activity.

Introduction

Oral hygienic practices may heavily influence an individual's quality of life. Poor oral hygiene is associated with an increased risk of a myriad of health complications including insulin resistance, cardiovascular disease, and neurodegenerative disorders [1]. Bacterial colonization of the oral cavity begins at birth; microbiota reside around the tooth, smooth surfaces, and fissures. The human oral microbiome is composed of approximately 700 species of bacteria, most of which play a vital role in maintaining oral homeostasis. Oral-associated bacteria become pathogenic when the bacterium resides beyond the tooth and gums [2]. The number of oral infections, reported by the National Institute of Dental and Craniofacial

Research, reveals that 42.2% of U.S. adults aged 30 or older suffer from periodontitis [3]. Oral infections are primarily treated with antibiotics, with an estimated 142,155 antibiotics prescribed per 1,000 dentists per year [4]. Antibiotic use for oral infection has been associated with tooth loss, and microbial resistance [5]. Beyond the oral cavity, long-term use of antibiotics alters the gut microbial floral diversity, which can further impact composition and metabolic function with increased resistance gene expression [6].

The increasing use of antibiotics calls for the exploration of substitutes for oral infection prevention. An alternative practice for dental care is the use of herbal products such as cloves as an analgesic, rosemary oil as an antitoxin, and miswak as an antimicrobial agent [7]. In ancient times, Egyptians chewed Salvadora persica, also known as miswak, to enhance oral hygiene; miswak is currently used worldwide by Muslims [8]. Miswak refers to the Arabic word for chewing stick; evidence of chewing stick use dates back 7,000 years and use of the stick is currently recommended by the World Health Organization. Chewing upon the stick frays the end to form a brush. The mechanical action of brushing, along with the antimicrobial properties are believed to contribute to the betterment of oral hygiene of users [9]. A comparison of the effectiveness of Salvadora persica chewing sticks and plastic toothbrushes with fluoride toothpaste in plaque and gingival prevention revealed that chewing sticks were most effective in the reduction of dental plaque [10]. Extracts of miswak have been shown to reduce bacterial count by 80% after 45 seconds of washing the mouth with miswak [11]. Using methanol extracts, Salvadora persica was found to inhibit bacterial growth of S. aureus and Streptococcus species [8] and had variable antibacterial activity for species of E. coli, K. pneumoniae, P. aeruginosa, S. marcescens, and S. pyogenes [12]. Common compounds that can be extracted from Salvadora persica include alkaloids, flavonoids, glycosides, vitamins, essential oils, minerals, sterols, and terpenes [13]. Nevertheless, the antimicrobial properties of Salvadora persica in comparison to popular toothpaste and mouthwashes and the chemical properties of miswak remain understudied. This knowledge could be utilized as a preventative measure against oral infections, such as Streptococcus infection, avoiding the need for long-term use of antibiotics. It is crucial to understand the chemical properties of miswak to allow further investigation of its potential use and of any side effects associated with it. The purpose of this study is to elucidate the antibacterial efficacy of Salvadora persica in comparison with commercially available oral hygiene toothpaste and mouthwashes and to comprehend the importance of the chemical composition of miswak.

Materials and Methods

Materials

Salvadora persica products were purchased from Amazon in various forms: miswak sticks in vacuum bags, fresh-dried sticks, and toothpaste. Commercially used toothpaste and mouthwash products purchased from Amazon included Colgate, Crest, Oral-B, and O2 Rinse. Streptococcus mutans, a bacterial species commonly associated with the oral cavity, was purchased from American Type Culture Collection (ATTC). Control antibiotics Bacitracin and Trimethoprim, species of

bacteria and yeast, and all other chemicals were provided by the University of Minnesota-Rochester.

Susceptibility Test

Susceptibility testing of microorganisms was performed using standard Kirby-Bauer disk diffusion techniques. Mueller-Hinton agar is a complete, non-selective, non-differential growth medium. Suspension of bacterial cultures of 300 microliters was used to evenly spread across the agar to create a lawn in a 150-millimeter petri dish. Bacteria tested include Bacillus brevis, Bacillus cereus, Bacillus megaterium, Bacillus subtillis, Branhamella catarrhalis, Citrobacter freundii, Enterobacter aerogenes, Enterococcus hirae, Escherichia coli, Micrococcus luteus, Mycobacterium smegmatis, Pseudomonas fluorescens, Pseudomonas stutzeri, Staphylococcus epidermidis, Staphylococcus saprophyticus, Saccharomyces cerevisiae, Streptococcus mutans. Agar plates were allowed to dry for 5-10 min. Filter discs (6mm) were impregnated with each chemical reagent. Chemical reagents used include Crest toothpaste, Colgate toothpaste, Oral-B mouthwash, O2 Rinse mouthwash, miswak toothpaste, fresh dried miswak stick, vacuumed miswak stick, and control antibiotics, trimethoprim, and bacitracin. For vacuum miswak and fresh dried sticks, the same size fragments as the filter discs were utilized. All nine discs were placed on the agar, each at least 24 mm apart. Agar plates were incubated between 24 to 48 hours according to optimal bacterial strain growth parameters (Table 1). Plates were stored at 4 degrees Celsius and disposed of following proper procedures. Inhibition zone diameters of susceptibility testing were determined. Institutional Biosafety Committee approval was obtained prior to the initiation of the study.

Extraction of Miswak

Vacuum miswak sticks were dried and crushed into powder-like format. We aimed to separate several different compounds of flavonoids. Following that, the material was extracted and stirred in 100% methanol for 4 hours. The sample was filtered to remove solid residues and concentrated under reduced pressure using a rotatory evaporator. For purity, we performed a preliminary separation technique using solvent ethyl acetate to extract the phenolic compounds–this allows liquid-liquid separation where we obtain two layers, an organic layer, and an aqueous layer. To remove possible traces of water in the organic layer, we used the drying agent, anhydrous sodium sulfate. We repeated rotatory evaporation to remove the ethyl acetate under reduced pressure. Thin Layer Chromatography (TLC) was performed using the solvent system of ethyl acetate in hexane in the ratio of 1:4 to get an understanding of the number of compounds present. UV light was then used to visualize the compounds. Column Chromatography was used as a main separation technique using various solvent ratios. We expected to separate three compounds of flavonoids–quercetin, kaempferol, and myricetin. Lastly, the retardation factor (Rf) value, a relative mobility of different compounds, was calculated to identify the position of each compound to allow us to observe the true separation.

Results

To investigate the antibacterial activity of miswak, we used three different formats, vacuum miswak, fresh dried miswak, and miswak toothpaste, which were compared to commercially available mouthwashes, Oral-B and O2 Rinse and toothpaste, Crest, and Colgate. For control, we used antibiotics Trimethoprim and Bacitracin (Figure 1). This revealed significant results, as the data indicate vacuum miswak has antibacterial properties in most bacterial strains. After TLC and Column Chromatography, we separated 3 different compounds of flavonoids (Figure 2). The Rf values were calculated to highlight the significance of this (Table 2). The Rf value is a ratio that measures the distance that each compound traveled to the solvent distance traveled. High Rf values indicate less polar molecules while low Rf values indicate more polar molecules.

Discussion

This study investigated the antimicrobial and chemical properties of Salvadora persica (miswak). While previous studies have explored the properties of miswak, chemical and physical properties have not been evaluated together in one study.

From the susceptibility test, we found that vacuum miswak and miswak toothpaste had high antimicrobial activity closely compared to bacitracin. On the other hand, Crest toothpaste and Oxygen mouthwash had the highest antibacterial activity in commercial products. This was similar to the 4 species that could inhabit the oral cavity. The fresh miswak and Oral B mouthwash had the lowest antimicrobial effect. Overall, the vacuum miswak had the greatest antimicrobial effect among the miswak materials, and Crest had the greatest antimicrobial effect among commercial dental hygiene products (Figure 1). In addition, extracting vacuum miswak, revealed 3 different compounds separated with different Rf values (Table 2). The compounds that we expected were flavonoids, especially quercetin, kaempferol, and myricetin. Flavonoids are polyphenolic compounds with antioxidants. They are known to alter bacterial activity by inhibiting their metabolism, cell membrane, and the synthesis of their genetic material [14]. Our results support these findings. Although there was a limitation to the time where we could have separated the other compounds we mentioned above, we were able to completely extract flavonoids.

Our findings align with previous findings and confirm the potential miswak offers in its antimicrobial properties. The comparison of different formats of miswak revealed the significance of processing the branches of Salvadora persica. This study also suggests that there may be additional unidentified compounds that play a role in miswak's antibacterial properties.

During this study, we came across several limitations including methodological and external factors. We had a few contaminations of the bacterial cultures, which were not included as part of our results, and a longer process of extracting the miswak than we anticipated. In the future, we hope to continue further extraction of other compounds and process Nuclear Magnetic Resonance (NMR) and Ultraviolet-Visible spectroscopy to specifically identify the structure of each compound we extracted. Moreover, to get a thorough quantitative analysis, we aspire to measure the Minimum Inhibitory Concentration (MIC) by repeating the susceptibility test with different concentrations of the chemical reagents. Testing a greater

number of bacterial strains would increase confidence and may help indicate linearity of results.

Conclusion

In conclusion, we aimed to evaluate Salvadora persica (miswak) to comprehend both its antimicrobial and chemical properties. A susceptibility test was performed on several bacterial strains and TLC and Column chromatography were conducted to extract miswak. Our results provide evidence that miswak has an antimicrobial effect on these bacterial species and warrants further study.

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Bacterial Strain	Incubation Time (hr)	Temperature (◦C)
Pseudomonas fluorescens	48	27
Staphylococcus epidermidis	48	37
Bacillus cereus	24	40
Branhamella catarrhalis	48	37
Micrococcus luteus	48	37
Bacillus brevis	48	37
Bacillus subtillis	24	37
Staphylococcus saprophyticus	24	37
Citrobacter freundii	48	37
Escheroli coli	24	37
Bacillus megaterium	24	37
Enterobacter aerogenes	24	37
Streptococcus mutans	48	37
Mycobacterium smegmatis	48	37
Enterococcus hirae	24	37

Table 1. Optimal temperature and time incubated for each bacterial strain.

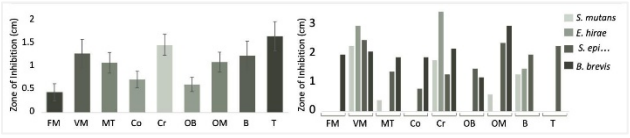

Figure 1. Antimicrobial activity elucidated by disc diffusion assay of commonly used oral hygiene products and miswak. Left: Disc diffusion assay of oral hygiene products and antibiotics. The error bar represents the average standard deviation of 15 bacterial species tested. Right: A pictorial representation of sensitivities of four selected bacterial species, all capable of oral cavity invasion. Fresh Miswak (FM), Vacuum Miswak (VM), Miswak Toothbrush (MT), Colgate (Co), Crest (Cr), Oral B (OB), Oxygen Mouthwash (OM), Bacitracin (B), Trimethoprim (T).

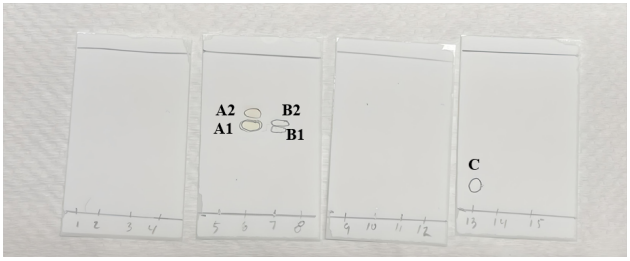

Figure 2. Chemical separation of compounds extracted from miswak on Thin Layer Chromatography (TLC) plate. Each TLC plate has been processed in a mobile phase with different concentrations of ethyl acetate in hexane. From left to right, plate 1; 20% ethyl acetate, plate 2; 30% ethyl acetate, plate 3; 40% ethyl acetate, and plate 4; 50% ethyl acetate. A1 and A2 are non-compound spots that were faintly observed under the UV light, possibly fragments of the outer layer of miswak. B1 and B2 are two flavonoid compounds separated, and C is the third flavonoid compound separated.

Compound Separation	Retardation factor (Rf) value (cm)
A1	0.55
A2	0.63
B1	0.53
B2	0.57
C	0.15

Table 2. Rf values calculated for each compound separation. A ratio measurement of the distance that each compound traveled to the distance that the solvent traveled.

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