Microbiological Assessment of *Moringa Oleifera Leaves* Extracts against Common Cariogenic Microorganisms

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Abstract

To assess the antimicrobial efficacy of leaf extracts of Moringa Oleifera using different extraction methods.

Ethanol, Acetone and water were used as solvents for preparation of leaf extracts of Moringa Oleifera. These extracts were tested for their antimicrobial efficacy against two commonly occurring cariogenic microorganisms, streptococcus mutans and lactobacillus. The antimicrobial potential was assessed using disc and well diffusion methods. Statistical analysis was done using one way Anova test to compare the inhibition zone diameter and t-test to compare between groups. The p value had to be ≤ 0.05 to be considered as statistically significant.

Ethanol and Acetone extracts did show comparable antimicrobial effects but the results of Acetone were statistically significant (P ≤ 0.05). The aqueous (water) extracts did not show any antimicrobial effect.

The leaf extracts of Moringa Oleifera showed significant antimicrobial potency against commonly occurring cariogenic microorganisms like streptococcus mutans and lactobacillus.

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Introduction

Dental caries is one of the most common chronic infections in humans especially more recently. This infection results in destruction of the calcified structure and a localized demineralization of hard tissues of the teeth.[1] It continues to infect a wide population of the world and treatment is more expensive and painful when compared to possible preventive methods.

Dental caries is usually found to be caused by a disproportionate accumulation of bacterial colonies on the tooth surfaces.[2] The microorganisms most commonly implicated in the cause of dental caries include Streptococcus mutans and lactobacillus.[2] Streptococcus mutans is known as the initiator of dental caries whereas lactobacillus is known as the progressor of dental caries.[3] It is advisable that a chronic infection like dental caries should be prevented from occurring thereby reducing the economic and personal burden. Therefore, prevention of dental caries is of vital importance and one of the methods which is been explored recently include the use of herbal or plant extracts.[4] In the present day a lot of importance is stressed upon the use of plant extracts to prevent diseases from occurring.[4,5] This is moreover stressed upon because of the lack of possible side effects.[5]

Herbal plant extracts are one of the most discussed possibilities as they contain compounds with significant antimicrobial effects.[6] Moringa oleifera also known as the drumstick tree or horseradish tree or Ben tree is one of the most important deciduous trees in the world. It is worth noting that all parts of this deciduous tree are of significance and can be used for food and also has curative characteristics.[7, 8] Lot of researches have been performed to understand the biologically active substances belonging to the various parts of this plant[9,10]. Owing to its antibacterial effects, Moringa Oleifera has been

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used in various health and body care essentials.[11] Due to many medicinal and health properties of Moringa and its various parts including seeds, stem, roots and flowers which are known to be rich in various bioactive compounds, makes it suitable and useful for various phytomedicinal preparation and pharmacological agents.[7] Studies have reported that Moringa leaves have anti-inflammatory, antibacterial, and anti-cancer efficacy.[7] It will be interesting to understand the antimicrobial effects of the leaves of Moringa Oleifera plant using different extraction methods which can help in the formulation of natural remedies which can be incorporated into oral hygiene aids like toothpastes and mouthwashes. Hence, this study was designed.

Materials and methods

Collection of Moringa Oleifera plant and its extraction:

The fresh leaves of Moringa Oleifera was collected from a local market in the United Arab Emirates. It was ensured that the plant leaves collected were healthy and not infected. The leaves of the plant were washed with running distilled water and air-dried at room temperature, the leaves were separated eventually. Moringa leaves were cut into small pieces to make it suitable for grinding. Fifty grams of the prepared Moringa powder from the leaves was soaked for 72 hours in 500 ml each of ethanol, 95%, acetone and distilled water solvents at room temperature.

The resultant mixtures after 72 hours of soaking were stirred using a magnetic stirrer for 24 hours to obtain a mix which could be homogenous in nature. The mixture was then stored for 48 hours at 4°C to allow for the extraction of active ingredients. After 48 hours each of the extracts were filtered using Whatman No.1filter paper and concentrated at 40°C using an evaporator to enable evaporation of the solvents used for extraction. [12]

Microorganisms used for the research

Two microorganisms Streptococcus mutans and Lactobacillus species were used to test the antimicrobial potential of the prepared Moringa plant leaves extracts. For the isolation of Streptococcus mutans the methodology previously used in a research was followed wherein dental plaque of high caries index patients was isolated by swabbing method from the oral cavity of the patients. [13]

Selective media Mitis salivarius-bacitracin agar (MSB; BD Difco, Paris) was used to isolate and grow *S. mutans*. Both pathogenic bacteria were routinely transferred into Tryptone Soya broth (TSB; Difco laboratories, Detroit, MI, USA) and incubated at 37°C for 24 hours. The diluted saliva samples were centrifuged 5 times at 5000 rpm and then vortexed. 1 microliter of the solution was manually inoculated on the prepared agar plates of Tryptone Yeast Extract Cystine (TYCSB) with 2 units of Bacitracin TYCSB supplement to isolate Streptococcus mutans. MRS (De Man, Rogosa and Sharpe) agar was used to isolate and grow Lactobacillus species. The lactobacillus was then transferred into MRS broth and was incubated at 37°C for 24 hours.

Assessment of antimicrobial activity

Disc and Well diffusion methods were used to test the antimicrobial activity of ethanol, acetone and distilled water extracts of the plant on the profile growth of Streptococcus mutans and lactobacillus species. The blood agar plates were first swabbed with each of the bacterial inoculums using a disposable cotton swab stick. Wells of 6 mm diameter each were made using a sterile borer into the agar plates containing the inoculums.

Sterile filter paper discs 4 mm in diameter were impregnated with 20 µl of each extract and placed on surface agar inoculated with pathogenic bacteria. Then, the plates were incubated at 35-37°C for 24 hours. At the end of incubation period, the antimicrobial activity of different plant parts extracts was evaluated by determining the diameter of inhibition zones (mm) around each disc of extract solution. [Figure 1(a), 1 (b) and Fig 2 (a), (b)]

Statistical Analysis

The observations were made on MS excel and then data were analysed using IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 22 and MS excel. One Way ANOVA test was used to compare between different extracts of Moringa Oleifera plant and t test for pairwise comparison. The significance level was set at $P \leq 0.05$.

Results

The three extraction methods used in the present study were Ethanol, Acetone and Water. The part of Moringa Oleifera which was used for preparing the extracts were the leaves. These extraction methods were assessed for their antimicrobial activity against streptococcus mutans and lactobacillus. The antimicrobial activity of the extracts was evaluated using the well diffusion and disc diffusion methods which are in-vitro in nature and economical. The means and standard deviations (SDs) of the Inhibition Zones (mm) using disc diffusion methods for the leave extracts of Moringa Oleifera using different extraction methods for streptococcus mutans and lactobacillus are reflected in Tables 1 & Figure 1 including Figure 1(a) and 1 (b). The means and standard deviations (SDs) of the inhibition Zones (mm) using well diffusion methods for the leave extracts of Moringa Oleifera using different extraction methods for streptococcus mutans and lactobacillus are reflected in Tables 2 & Figure 2 including Figure 2 (a) and 2 (b). Means and Standard deviations (SDs) of the inhibition zones (mm) using both disc diffusion and well diffusion methods for the leave extracts of Moringa Oleifera using different extraction methods for streptococcus mutans and Lactobacillus are shown in Table 3. When the mean inhibition zones are compared for streptococcus mutans and lactobacillus, the results show a statistically significant result ($p < 0.05$) for acetone extract when compared to ethanol extract and water.

Method of extraction	Mean	SD	F	P
Acetone	25.33	0.577	.196	0.000
Ethanol	9.33	4.041	4.000	0.116
Water	0.00	0.000	-	-

Table 1. Means and Standard deviations (SDs) of the Inhibition Zones (mm) using disc diffusion methods for the leave extracts of Moringa Oleifera using different extraction methods for Streptococcus Mutans and lactobacillus.

The results showed no difference between the two methods (well diffusion and disc diffusion) used for determining the antibacterial activity. It was seen in the present study that extracts prepared using acetone had maximum antibacterial activity when compared to ethanol and water. It is also seen that the aqueous (water) extracts do not have any antibacterial activity.

Method of extraction	Mean	SD	F	P
Acetone	35.66	.1154	.196	0.000
Ethanol	19.33	0.577	4.000	0.116
Water	0.00	0.000	-	-

Table 2. Means and Standard deviations (SDs) of the Inhibition Zones (mm) using well diffusion methods for the leave extracts of Moringa Oleifera using different extraction methods for Streptococcus Mutans and lactobacillus.

Method of extraction and comparison between them		Mean	SD	F	P
Acetone	Disc Diffusion	15.25	.577	.196	0.000
	Well method	35.66	.1154		
Ethanol	Disc Diffusion	9.33	4.041	4.000	0.116
	Well method	19.33	0.577		
Water	Disc Diffusion	0.00	0.000	-	-
	Well method	0.00	0.000		

Table 3. Means and Standard deviations (SDs) of the inhibition zones (mm) using both disc diffusion and well diffusion methods for the leave extracts of Moringa Oleifera using different extraction methods for Streptococcus Mutans and Lactobacillus.

Discussion

The present study was conducted to evaluate the antibacterial property of leaves extracts of Moringa oleifera plant which is a deciduous tree known for its medicinal properties.[10, 11, 12] Dental caries is a chronic condition and is a multifactorial disease. The most common microorganisms implicated in the occurrence of dental caries include streptococcus mutans and lactobacillus. Streptococcus mutans is known as the initiator and lactobacillus is the progressor of dental caries.[13, 14, 15, 16] Therefore this study was conducted to understand the antibacterial activity of the leave extracts of Moringa oleifera against these pathogenic microorganisms. In this study two organic solvents, ethanol and acetone were compared

with water. All these three were used for preparing the leave extracts.

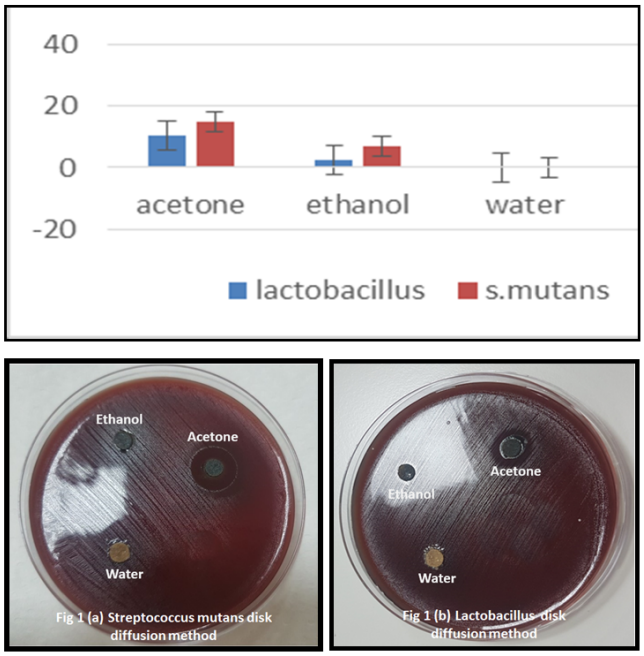

Figure1. Mean inhibition zones by disk diffusion method.

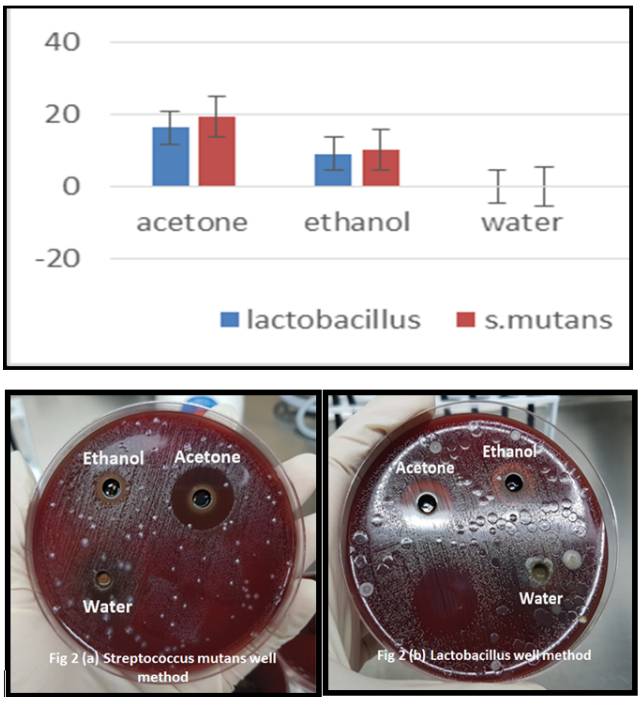

Figure 2. Mean inhibition zones by well diffusion method.

It was found in previous studies that the extracts of leaves of Moringa Oleifera have a greater antimicrobial activity when compared to the other parts of the plant, therefore in this study only leave extracts were used.[17, 18, 19] It was suggested that the leaves of Moringa Oleifera have bio-active substances which are antibacterial in nature and can act against several microorganisms. The leaves of Moringa Oleifera are found to contain phytochemical substances like Saponins, Tannins and Flavonoids which exhibit antibacterial properties.[20, 21, 22] The phytochemical substances found within the leaves of Moringa Oleifera also include steroids and alkaloids. All these substances found exhibit strong antimicrobial properties. [22, 23] Tannins are an important constituent, they inhibit cell protein synthesis by forming complexes with proline-rich proteins and these complexes are irreversible in nature. Antimicrobial properties of Tannins include mechanisms like enzyme inhibition, iron deprivation and oxidative phosphorylation. These mechanisms allow Tannins to exhibit broad spectrum antimicrobial properties. Steroids within the plants have also shown antibacterial effects. The alkaloids in Moringa Oleifera have shown analgesic properties as well.[23] All these properties of the constituents of Moringa Oleifera make it a suitable herbal remedy even in dental practice.

The results of the present study reflected that there is a definite antimicrobial activity when the extracts are prepared using the organic solvents including ethanol and acetone. The water extracts did not show any antimicrobial activity at all (Tables 1-3). The findings in relation to the water extracts is similar to other studies published earlier which suggested minimum or no antimicrobial activities of water extracts. [22, 23] The antimicrobial activity of the organic solvents (ethanol and acetone) were compared and it was found that acetone had more antimicrobial activity when compared with ethanol. Though the results of acetone and ethanol were comparable but statistically significant results were found for acetone when compared with ethanol as seen in Tables 1-3 and Figures 1-2. The findings were same using both disc diffusion and well diffusion methods.

The results of the present study suggest that the leaf extracts of Moringa Oleifera can be used for its antibacterial properties. How the leaf extracts can be added into dental products need to be experimented. Some studies in the past have done these experiments by incorporating the leaf extracts of Moringa Oleifera into

toothpastes and mouthwashes and the results showed promising antimicrobial activities of these oral hygiene aids.[24] It however needs to be proven in a large scale before commercializing it. As for the results of this study, it can be suggested that the leaf extracts of Moringa Oleifera has antimicrobial properties and can be used in dental products after appropriate researches and approvals. However, when extracts of plants are used for therapeutic purposes the safety and toxicity will need to be considered.

Conclusions

With the results of the present study, it can be concluded that:

1. The leaf extracts of Moringa Oleifera has antibacterial activity.
2. The acetone and ethanol extracts both are antibacterial in nature though a statistically significant result was achieved with acetone.
3. Future studies need to be done to understand how these extracts can be successfully added into oral hygiene aids like toothpastes and mouthwashes.

Declaration of Interest

The authors report no conflict of interest.

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