

Antimicrobial Efficacy of *Moringa oleifera* and *Sesbania grandiflora* against *Streptococcus Mutans* and *Candida Albicans*: An *In vitro* Study

P. Sivakami^{1*}, B. Siva¹, Madhulika Naidu¹, M. Anitha¹ and A. Vishalini¹

¹Ultra Nagar, Madurai-Chennai Highway, Madurai-625104 and Best Dental Science College, India.

Author's contribution

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

AIM: To evaluate the antibacterial and antifungal efficacy of *Moringa oleifera* and *Sesbania grandiflora* against *S. mutans* and *C. albicans*.

Settings and Design: The study design involves an in-vitro study.

Subjects and Methods: The microbial strains *Streptococcus mutans* and *Candida albicans* was inoculated in PDA-Himedia and SDA Media respectively. About 10 g of sample leaves powder was soaked in 99.9% ethyl alcohol and filtered using filter paper and crude extract was obtained. The crude extract was dissolved in ethanol and made into different concentrations of about 12.5 µl, 25 µl, 50 µl, 100 µl and 200 µl. Agar well diffusion method was followed for determination of antimicrobial activity. Agar wells (6mm diameter) were made and different concentrations of plant sample as 12.5 µl, 25 µl, 50 µl, 100 µl, 200 µl and respective standard 30 µl were added using sterilized dropping pipettes into the wells. The bacterial plates were incubated at 37°C for 24 h and fungal plates were incubated at 37°C for 48 h.

Statistical Analysis Used: Statistical analysis was performed using IBM software SPSS version 20 at one-way ANOVA.

Conclusion: The *Moringa oleifera* and *Sesbania grandiflora* leaves extract has an effective antimicrobial efficacy against *S. mutans* and *C. albicans* and its antimicrobial efficacy approximates that of standard ampicillin and clotrimazole. *Moringa oleifera* and *Sesbania grandiflora* leaves extract can be used as an antimicrobial agent in clinical trials.

*Corresponding author: Email: sivakamidentist@gmail.com;

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1. INTRODUCTION

Antimicrobial resistance has become an emerging global problem. Strategies to improve the situation include research in finding innovative and new antimicrobials. The chemotherapeutic agents and antibiotics have been of value in controlling many infections. But they depend on judicious use to minimize the incidence of resistance to antibiotics. Antibiotics and chemotherapeutic agents have been used to treat many infectious diseases but none of the available drugs is free from limitations and side effects. Another growing problem is the development of multi-drug resistant strains of bacteria. The indiscriminate use of antimicrobial drugs leads to development of resistance to many antibiotics that create a big problem in the treatment of infectious diseases. There is a need to look for the alternatives due to increase in resistance of many microorganisms to the currently used antimicrobials in addition to many side effects.

The therapeutic options are medicinal plants since they contained various chemical compounds with antimicrobial effects. In developing countries, the use of medicinal plants for treatment of infection is considered the affordable and safer natural treatment modality due to the expensive cost of antibiotics. Plants are the safe and cheapest alternative source of antimicrobials. One of such plants with medicinal value is *Moringa oleifera* and *Sesbania grandiflora*.

Moringa oleifera belongs to the family Moringaceae. The different parts of this plant like leaves, stem bark, root bark, flowers, fruits and seeds are used in the indigenous systems of medicine for the treatment of variety of human ailments. The "Moringa" tree is considered one of the world's most useful trees, as almost every part of the Moringa tree can be used for food or has some other beneficial properties. In India and other parts of the country the fruit called as *drumstick* [1]. The leaves contain more Vitamin A compared to carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas, and that the protein quality of Moringa leaves rivals that of milk and eggs [2].

Sesbania grandiflora also known as agathi keera belongs to the family Leguminosae. It is a fast

growing tree and is widely distributed in India. The leaves are bitter in taste and are rich in vitamin C, calcium, sterols, saponin, quercetin, myricetin, and kaempferol. Its leaves and flowers are utilized as food whereas its stem has been long used as a traditional medicine for treatment of ulcers in the oral cavity [3]. Earlier studies had shown the successful candidature of flower extract of *Sesbania grandiflora* for antibacterial potential [4].

The oral micro biota consists of a variety of group of organisms including bacteria, fungi, mycoplasma, protozoa and occasionally viruses [5]. Bacteria are the most predominant group of commensals in the oral cavity. The oral micro flora have been considered as harmless or having a low order of virulence. However, under suitable conditions, the increase in the numbers of certain species of the oral flora is the reason to cause dental caries and periodontal disease [6]. It is known that oral micro-organisms especially *Streptococcus mutans* are almost certainly associated with dental caries [7].

The present study is aimed to evaluate the antibacterial and antifungal efficacy of *Moringa oleifera* and *Sesbania grandiflora* against *S. mutans* and *C. albicans*.

2. MATERIALS AND METHODS

2.1 Determination of Antimicrobial Activity

The antimicrobial activity was performed by well diffusion method followed by Antara sen and Amla batra (2012).

2.2 Preparation of Media

2.2.1 Sabouraud dextrose agar (SDA) media for Candida Albicans

Ingredients gm/L

2.2.2 Composition of media

Dextrose: 40 g
Peptone: 10 g
Agar: 15.0 g

2.2.3 Preparation of medium

Suspend 65.0 grams of nutrient agar in 1000 ml distilled water. Heat to boiling and dissolve the

medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

2.2.4 Potato dextrose agar (PDA-Himedia) media for streptococcus mutans

Ingredients gm/L

2.2.5 Composition of Media

Potatoes infusion from: 200.00 g

Dextrose: 20.00 g

Agar: 15.00 g

2.2.6 Preparation of medium

Suspend 39.0 grams of PDA in 1000 ml distilled water. Heat to boiling and dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before dispensing in specific work, when pH 3.5 is required; acidify the medium with sterile 10% tartaric acid. The amount of acid required for 100 ml of sterile cooled medium is approximately 1 ml. Do not heat the medium after addition of acid.

2.3 Microorganisms

The microbial strains employed in the biological assays were Gram-positive bacteria: *Streptococcus mutans* (MTCC 497) and *Candida albicans* (MTCC 183). Microbial strains are obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India.

2.3.1 Preparation of 24 hours pure culture

A loop full of each of the microorganisms was suspended in about 10ml of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37°C for 24 to 48 hours. After completion of incubation period, when growth was observed the tubes were kept into 2-8°C until use.

2.3.2 Preparation of the extract

The moringa and Sesbania leaves are bought from local market and sun dried. The dried leaves are grinded to fine powder. About 10 g of sample leaves powder (*Moringa oleifera* and *Sesbania grandiflora*) was soaked in 99.9% ethyl alcohol for 4 days and filtered using Whatman filter paper. The obtained filtrate was subjected to rotary evaporator at a temperature of 70°C and 120 rpm, and crude extract was obtained. The weight of crude extract was calculated by measuring the difference between the weights of

beaker before and after the collection of extract. The final material (crude extract) was dissolved in ethanol and made into different concentrations of about 12.5 µl, 25 µl, 50 µl, 100 µl and 200 µl. Ethanol group was considered negative control in this study to investigate the presence of any antibacterial and antifungal property for ethanol because the crude extract was dissolved in ethanol. The final crude extract was subjected to antibacterial and antifungal susceptibility test.

2.3.3 Preparation of samples solutions for the experiment

The sample were weighed (10mg/1ml) and dissolved in ethanol to prepare appropriate dilution to get required concentrations of about 12.5 µl (12.5 µg/ml), 25 µl (25 µg/ml), 50 µl (50 µg/ml), 100 µl (100 µg/ml) and 200 µl (200 µg/ml). Standard solution for *C.albicans* is Clotrimazole (25 mg/ml distilled water) and for *S.mutans* is Ampicillin (25 mg/ml distilled water). They were kept under refrigerated condition unless they were used for the experiment.

2.3.4 Agar well – diffusion method

Agar well diffusion method was followed for determination of antimicrobial activity. The bent glass rod is sterilized and used to spread the microbe-containing (10^7) liquid uniformly on the Sabouraud Dextrose Agar (SDA) and Potato dextrose agar (PDA) medium plates using 24 to 48 hours culture of respective microbial strains. Agar wells (6mm diameter) were made in each of these plates using sterile cork borer. Different concentrations of plant sample as 12.5 µl, 25 µl, 50 µl, 100 µl, 200 µl and respective standard 30 µl were added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of pre-incubation diffusion. The bacterial plates were incubated at 37°C for 24 h and fungal plates were incubated at 37°C for 48 h. Each sample was tested in triplicate. Results were recorded as the presence or absence of inhibition zone. The inhibitory zone around the well indicated absence of tested organism. The diameters of the zones were measured using diameter measurement scale. Triplicates were maintained and the average values were recorded for antimicrobial activity.

3. RESULTS

The results were obtained based on the zones of inhibition produced by *Moringa oleifera* and *Sesbania grandiflora* extract, ethanol, ampicillin

and clotrimazole. The widths of the zones of inhibition in each group have shown increase with increase in their concentrations, as tabulated in Table 1. The width of zones of inhibition of *Moringa oleifera* and *Sesbania grandiflora* approximated with that of ampicillin at concentration of 200 μ l as depicted in Fig. 1 and with that of clotrimazole at concentration of 200 μ l as depicted in Fig. 2. This proves that *Moringa oleifera* and *Sesbania grandiflora* has almost equal antibacterial and antifungal efficacy as ampicillin and clotrimazole respectively.

4. DISCUSSION

The Indian subcontinent is well known for its richness in the presence of numerous plants with

medicinal properties [8]. The reasons behind the need for the discovery of natural products with therapeutic properties are the adverse effects caused by synthetic drugs and the increasing resistance of microorganisms to the antimicrobials prescribed currently [9].

According to Nadia K. J. Al-Dawah et al.,(2014), the chemical constituents found are galactomannans, linoleic acid, beta-sitosterol and carbohydrates [10]. The major contributors of phenolic substances in *S.grandiflora* are simple phenolics acids [11]. Apart from this the other bioactive compounds reported in this plant are saponins. This is responsible for the antimicrobial activity of *Sesbania grandiflora* [12].

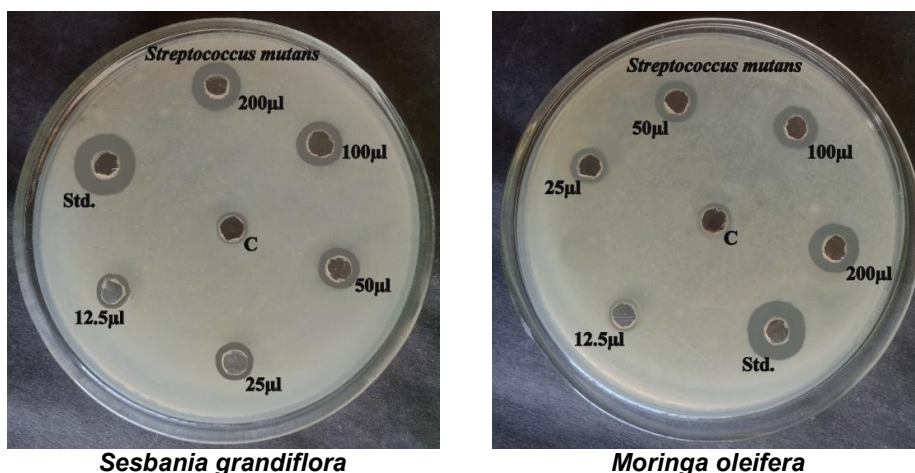


Fig. 1. Zones of inhibition of *Moringa oleifera* and *Sesbania grandiflora*, ethanol and ampicillin at different concentrations against *Streptococcus mutans*

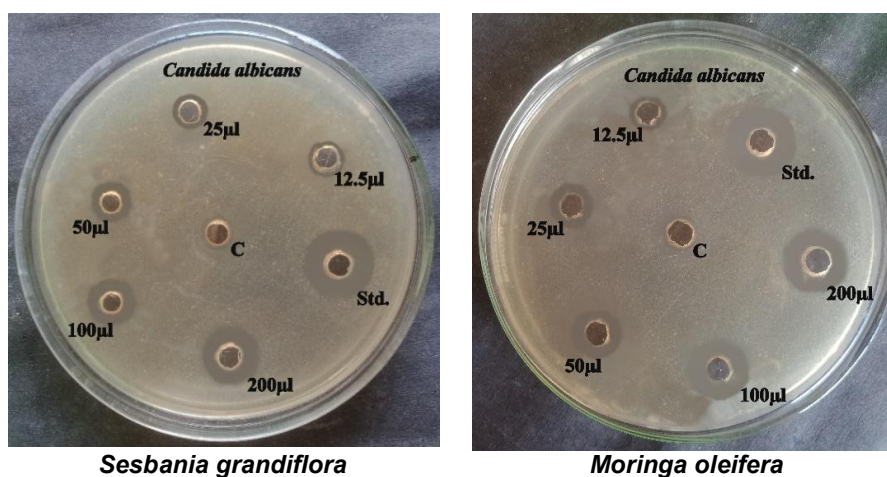


Fig. 2. Zones of inhibition of *Moringa oleifera* and *Sesbania grandiflora*, ethanol and clotrimazole at different concentrations against *Candida albicans*

Table 1. Zones of inhibition of *Moringa oleifera* and *Sesbania grandiflora*, ethanol, ampicillin and clotrimazole at different concentrations

Samples	Control	12.5 µl	25 µl	50 µl	100 µl	200 µl	Standard (30 µl)
Bacteria (<i>Streptococcus mutans</i>) (mm)							
<i>Sesbania grandiflora</i>	0.10±0.01	0.40±0.02	1.20±0.08	2.60±0.18	4.30±0.30	6.50±0.45	7.20±0.50
<i>Moringa oleifera</i>	0.10±0.10	0.50±0.03	1.40±0.09	2.90±0.20	4.60±0.32	6.80±0.47	7.20±0.50
Fungal (<i>Candida albicans</i>) (mm)							
<i>Sesbania grandiflora</i>	0.10±0.01	0.20±0.02	0.80±0.05	2.30±0.16	3.90±0.27	6.10±0.42	6.80±0.47
<i>Moringa oleifera</i>	0.10±0.01	0.30±0.02	1.10±0.07	3.00±0.21	4.20±0.29	6.80±0.48	7.00±0.49

Values expressed as Mean ± SD for triplicates; Bacteria Standard: Ampicillin; Fungal Standard : Clotrimazole; Control: Ethanol

According to Teklit Gebregiorgis Amabye et al., (2016), alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications and recreational drugs [13]. Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes [14]. Plant terpenoids are used extensively for their aromatic qualities [15]. They play a role in traditional herbal remedies and are under investigation for Antibacterial, Anti-neoplastic and other Pharmaceutical functions. Tannins have shown potential Antiviral, Antibacterial and Antiparasitic effects [16].

The antimicrobial activities of *Moringa oleifera* and *Sesbania grandiflora* leaves were tested against *S. mutans* and *C. albicans* in this study. These microorganisms are associated with dental caries and oral candidiasis [17].

Moringa oleifera leaves shows antifungal activity against *C. albicans* which is contrast to Elgamily study which reports none of the extracts of *Moringa oleifera* showed any antifungal activity against *C. albicans* [18].

The findings of this study, however coincide to (Mahdi et al.) [19] who reported that ethanol and acetone leaf extracts of *Moringa oleifera* showed antifungal activity against *C. albicans*.

In the present study, the *Moringa oleifera* extract of has the effect on *S. mutans* in all concentrations. This finding is in agreement with Koteswara Rao's study. *Moringa oleifera* has proved to be effective against *S. mutans* and *C. albicans*, which is in accordance with the study conducted by Devendra et, al. According to Hanaa Elgamily, the different extracts of different parts of *Moringa* showed an antibacterial effect against *Staphylococcus aureus* and *Streptococcus mutans* growth [20].

The results of our study also were in agreement with Rao et al [21] study. Methanol bark extract showed promising antibacterial activity to most of the test pathogens. Similar result was observed in the study of Rao et al [21] who investigated antibacterial activity of methanolic extract of *M. oleifera* by using well diffusion technique and reported that the most significant activity of this plant was seen against *S. aureus*. While working on same plant species Devi et al [22] investigated the antibacterial activity of

methanolic extract of bark by agar well diffusion method against *Bacillus spp.* and *S. aureus*.

In the present study, the *Moringa oleifera* and *Sesbania grandiflora* leaves extract had MIC against *S. mutans* and *C. albicans* at all concentrations and the antimicrobial efficacy of *Moringa oleifera* and *Sesbania grandiflora* leaves extract approximated with that of ampicillin and clotrimazole. *Moringa oleifera* and *Sesbania grandiflora* leaves extract can be used as an antimicrobial agent in clinical trials. This study is limited as it is an *in-vitro* study. Further *in-vivo* studies should be carried out to confirm the results of this study.

5. CONCLUSION

Our results indicate that *Moringa oleifera* and *Sesbania grandiflora* leaves extract is an effective antimicrobial agent against *S. mutans* and *C. albicans* and its antimicrobial efficacy approximates that of standard ampicillin and clotrimazole. The discovery of a natural antimicrobial agent with beneficial qualities of biosafety and efficacy will be a great boon in the therapy of infections. Apart from providing treatment to drug-resistant patients, cost-effectiveness also plays a major role in the replacement of synthetic drugs with natural ones, especially in developing countries.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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