An Antimicrobial Activity of *Moringa Oleifera* Extract in Comparison to Chlorhexidine Gluconate (*In vitro* study)

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ABSTRACT

Background: Oral diseases persist to be a major health problem all over the world. Various bacteria and fungi are found to be the possible pathogensresponsible for the oral diseases. *Moringa oleifera* it is an extraordinary nutritious vegetable tree with many different uses. These leaves have high medicinal value. In the present study, antibacterial and antifungal activities of aqueous extracts of plant *Moringa oleifera* in comparison to chlorohexidene gluconate and deionized water were determined.

Materials and methods: The leaves of plant of *Moringa oleifera* were collected from College of Pharmacy; Baghdad, Iraq. Tested microorganism (bacterial and fungal) was isolated from different clinical specimens. *In-vitro*antimicrobial activity was performed by agar well diffusion method on Muller Hinton agar medium.

Results: The water extract of *Moringa oleifera*showed antibacterial *effect* on the tested organisms: *Staphylococcus aureus, Streptococcus spp.* and *Enterococcus faecalis.* Aqueous extract showed maximum zone of inhibition against *S.aureus*.

Conclusion: *Moringa olifera* can be used as safe and cheap plant antimicrobial agent.

Key words: *Moringa oleifera*, Antimicrobial effect, Chlorohexidene gluconate. (J Bagh Coll Dentistry 2016; 28(1):183-187).

INTRODUCTION

Moringa oleifera commonly referred as Moringa only. It is an extraordinary nutritious vegetable tree with many different uses. These leaves have high medicinal value [(1)]. Moringa *oleifera* found in any tropical and subtropical country with strange environmental features, like dry to moist tropical or subtropical weather, with annual precipitation of 760 to 2500 mm and temperature 18 and 28 °C. It cultivates in any type of soil, but dense clay and saturated with water, and the pH range between 4.5 and 8, at an elevation up to 2000 m is more preferable environment [(2)]. Conventional medicines turn into a chief source of main health to majority of people in most developed country, particularly in Africa due to cheapness and feasibility of antibiotic furthermore antibiotic resistance and side effect of them [(3)]. Many epidemiological studies have indicated that *M. oleifera* leaves are a well source of nutrition and exhibit anti-tumor, anti-inflammatory, anti-ulcer, anti-atherosclerotic and anti-convulsing activities [(4-6)]. Olden Egyptians consumed *Moringa oleifera* oil for its improving worth and dermatological ground work [(7)].Today, *Moringa oleifera* and its byproducts are dispensed chiefly in Middle East, Asian and African countries [(8)], and are still dispersion to other regions.

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Chlorhexidine gluconate (CHX) is cationic biguanide that act on cell wall of microorganism by adsorbing on it resulting in leakage of intracellular components. Furthermore, because of its cationic structure, chlorhexidine has the unique property of substantively. At low concentration it is bacteriostatic and at a high concentration it is bactericidal [(9)]. Chlorhexidine gluconate was tested against common oral pathogens like *Streptococcus mutans* and *Enterococcus faecalis* and showed considerable antibacterial activities especially against *Staphylococcus aureus* [(10)].

The leaves of *Moringa oleifera* are highly nutritious, it considered a considerable source of beta-carotene, protein, vitamin C, potassium and iron [(11)]. *Moringa* leaves contains phytochemical having potent anticancer and hypotensive activity and are considered full of medicinal properties [(12)]. The whole *Moringa oleifera* plant is used in the treatment of psychosis, eye diseases and fever [(13)].

This study aimed to show antimicrobialactivity ofwater extracts of the Moringa *oleifera* against different oral and other pathological microorganism in comparison to Chlorhexidine gluconate.

MATERIALS AND METHODS

Collection of Plant Material

The leaves of plant of *Moringa oleifera* were collected from college of pharmacy, Baghdad, Iraq (figure 1). It was ensured that the plant was healthy and uninfected. The leaves were washed

under running tap water followed by distilled water to eliminate dust and other foreign particles and shade dried for 5 days to remove water. The dried leaves and stems were powdered and stored in air tight containers until use.

Figure 1: Leaves of *Moringa Oleifera* Plant

Preparation of Extract (Aqueous Extraction)

Twenty grams of dried powered plant material (leaves and stems) were soaked separately in 200 ml double distilled water (DDW), kept on a rotary shaker for 24 hours. Then after, these were kept at slow heat for 8 h and then filtered through eight layers of muslin cloth. The resultant liquid was subsequently centrifuged at rate 7000 rpm for 15 minutes.

The supernatant part was collected and then concentrated by evaporation at 50°C to make the final volume one-twentieth of the original volume (10 ml). The extract was then autoclaved at 121°C and 15 lbs. pressure, and stored in sealed tubes at 4°C until use.This is preparation for the stock solution (100 % concentration). From this stock solution prepare different dilutions (20%, 40%, 60%, 80%), by using dilution law ($N_1V_1 = N_2V_2$), diluted by addition distill water to the stock solution.

Test Organisms

Tested microorganism (bacterial and fungal) was isolated from different clinical specimens; the isolation and identification of the samples was according to typical laboratory methods [14]. Isolated bacteria include: Gram negative bacteria (*Salmonella spp, Escherichiacoli and Klebsiella pneumonia*) Gram positive bacteria (*Streptococcus spp, Enterococcus faecalis, Staphylococcus aureus*). Isolated fungi include: *Candida albican*s.

Bacterial and Fungal Media (Agar Media)

Muller Hinton Agar prepared according to manufacturer's instruction which involved the suspension of 38 gm. in one liter of de-ionized water, after being completely dissolved with boiling, it was sterilized by autoclave at 15 lb. pressure for 15 minutes, then left to cool at 45-50°C, poured and left to solidify then put them in incubator at 37°C for 24 hours then stored in refrigerator until being used.

Antimicrobial Screening (*in vitro*)

The antimicrobial activity of the *Moringa oleifera,* chlorohexidene gluconate and deionized water were measured by well diffusion method[15,16]. The prepared culture plates wereinoculated with different selected strains ofbacteria and fungi using spreading method.Wells were made on the agar surface with 6 mm cork borer. The position of the wells for each extract was marked at the outside walls of plates before application of plant extracts, chlorohexidene gluconate and deionized water.

The extracts were poured into the well. Each well was filled with 100µl with corresponding extract with the help of a micropipette. The plates were incubated at 37±2 ºC for 24 hours for bacterial and 25±2 ºC for 48 hours for fungal activity. The plates were observed for the zone clearance around the wells. The resulting inhibition zones were uniformly circular. The diameters of the zones of inhibition were measured, including the diameter of the well. Inhibition zones are measured to the nearest millimeter, using a ruler, which is held on the back of the inverted petri plate.

RESULTS

This study entails the important antimicrobial activity of the *Moringa oleifera* leaf in inhibition of growth of *Staphylococcus aureus* 32 mm, *Streptococcus spp.* 30 mm which is more than inhibition zone caused by Chlorohexidene gluconate 19 and 16 for the two bacteria respectively and *Enterococcus faecalis* 11 mm *that* is less than inhibition zone of Chlorohexidene gluconate14 mm. All the three type of bacteria gram positive. While gram negative bacteria (*Salmonella spp, Escherichiacoli and Klebsiella pneumonia*) and fungi (*Candida albican*s) exhibited resistance to the water extract of

Moringaoleifera leave and have no any inhibition zone as in deionized water, on the other hand Chlorhexidine gluconate have inhibition zone with different diameter on all these bacteria and fungi.

De-ionized water considered as control negative in this study as showed in (table 1) and (figure 2). Water extractsof *Moringa oleifera* exhibit variable antibacterialactivity against bacteria; *Staphylococcus aureus* showed higher inhibition zone 32 mm (figure 3) when use crude extract which is more than chlorhexidine gluconate followed by *Streptococcus spp* as in (figure 3 and 4), and this inhibition zone proportionate with the concentration of the plant as the concentration of water extract of *Moringa oleifera* increase from 20% to 100%, the inhibition zone increase gradually,as showed in (table 2) and (figures 5 and 6).

Table 1: The Antimicrobial Activity of the Three Agents

Bacteria and fungi	Aqueous Extraction of *Moringa oleifera* (Stock solution 100%)	Chlorhexidine Gluconate	De-ionized water
Escherichia coli	0	16 mm	0
Klebsiella pneumonia	0	13 mm	0
Salmonella spp	0	13 mm	0
Staphylococcus aureus	32 mm	19 mm	0
Enterococcus faecalis	11 mm	14 mm	0
Streptococcus spp	30 mm	16 mm	0
Candida albicans	0	12 mm	0

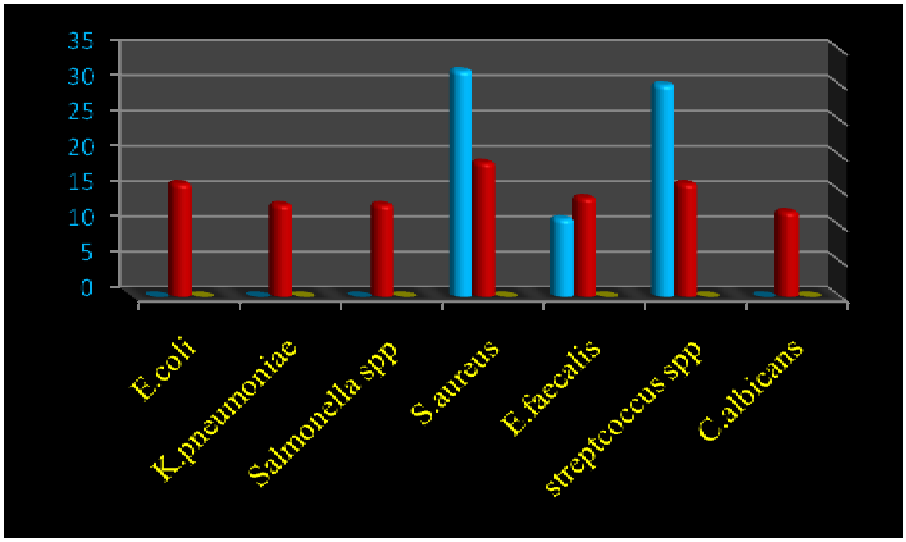


Figure 2: Antimicrobial Effect of Three Agents on Different Strain of Microorganism
The red is chlorhexidine, blue color is *moringa oleifera* and yellow color is de-ionized water.

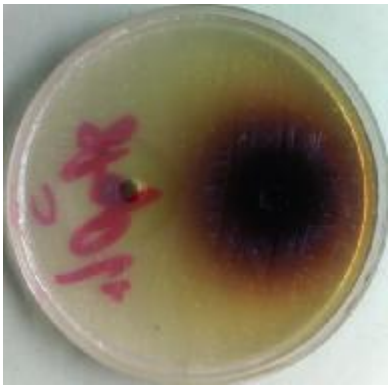


Figure 3: Crude extract of *Moringa Oleifera* and Chlorhexidine Gluconate on *Staphylococcus Aureus.*

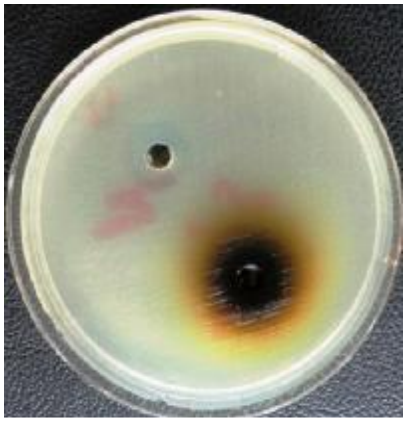
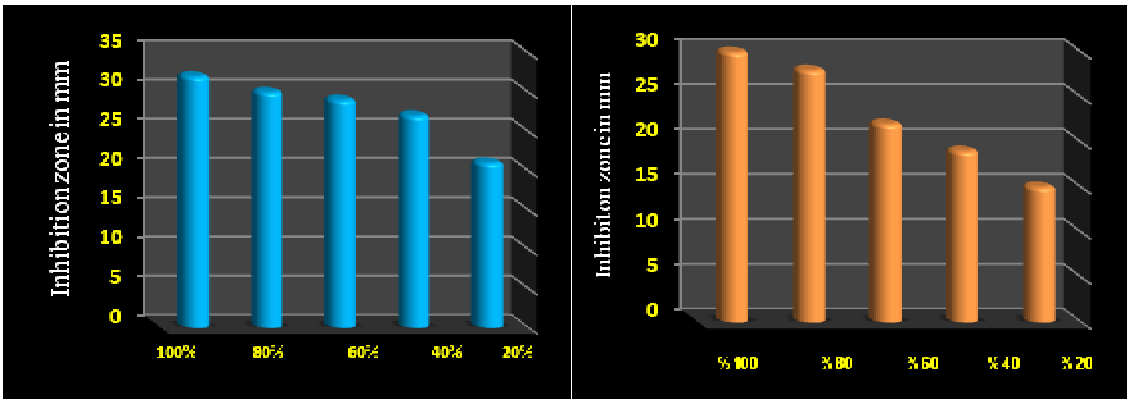


Figure 4: Crude Extract of *Moringa Oleifera* and Chlorhexidine Gluconate on *Streptococcus spp.*

Table 2: Inhibition Zones of Different Concentration of *Moringa Oleifera* on *Staphylococcus Aureus*

Different concentration of *Moringa oleifera*	Inhibition zones in mm on *Staphylococcus aureus*	Inhibition zones in mm on *Streptococcus spp.*
100 %	32 mm	30 mm
80 %	30 mm	28 mm
60 %	29 mm	22 mm
40 %	27 mm	19 mm
20 %	21 mm	15 mm



A **B**

Figure 5: Inhibition zones of Different Concentrations of *Moringa Oleifera* (20%, 40%, 60%, 80%and 100%) on *Staphylococcus Aureus* and *Streptococcus spp.*

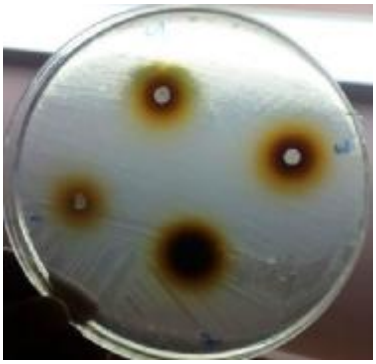


Figure 6: Inhibition Zones of Different Concentrations of *Moringa Oleifera* (20%, 40%, 60%, and 80%) on *Staphylococcus Aureus.*

DISCUSSION

The antibacterial activity of the aqueous extract of leaves of the plant *Moringa oleifera*was assayed *in vitro* by agar well diffusion method against six potentially pathogenic bacterial species with only one fungal species : three gram positive bacteria which are *Staphylococcus aureus, Enterococcus faecali*and *Streptococcus spp.* Three grams negative which are *Escherichia coli, Klebsiella pneumonia* and *Salmonella spp.* The only fungal species is *Candida albicans*.

All these microorganism have a causative role in the pathogenesis of many oral diseases and other diseases this agreed with Kakehashi *et al*.,[17]. Gomes et *al*., show that the facultative microorganisms such as *Enterococcusfaecalis, Staphylococcus aureus,* etc., which are considered by many to be the most resistant species in the oral cavity [18]. Numerous researchers stated that *Moringa oleifera* leave have antimicrobial action of water extract against multiple pathogens, several of them agree with the result of this study but some had slight different as a result of difference in genes of bacteria that make bacteria to be resistance to antimicrobial activity. Similarly to Priya *et al*., who evaluated the antibacterial activity of the aqueous leaf extracts

of *Moringa oleifera* on pathogenic strain of bacteria like *Staphylococcus aureus, Escherichia coli, Klebsiellapneumoniae, and Shigella spp.* [19].

The causes for the different may retain to concentration of the plant moringa oleifera or which part from the plant used or may be due to difference in bacterial species. Thilza *et al* assessed the antimicrobial action of *Moringa oleifera* leave extract on *Staphylococcus aureus, Escherichia coli, Pseudomonasaeruginosa* and *Staphylococcus albus,* and they discovered that only *Escherichia coli* among tested bacteria exhibited inhibition zone [20]. While Vinoth *et al.,* examined *Moringa* leave water extract for antibacterial action, *Staphylococcus aureus* only from tested bacteria exhibited sensitivity *while* no activity was recognized for *Escherichia coli, salmonella spp. andKlebsiella, pneumonia* [21]. These results coincide completely with results of this study. This study revealed also that the chlorhexidene gluconate more effective against most of the pathogenic microorganisms except for the *Staphylococcus aureus* and *Streptococcus spp.* in which chlorhexidine gluconate less effective. This agrees with Kanazwa and Ueda, 2004 who suggested that chlorhexidine is important antiseptic agent or disinfectant for clinical isolates of various bacterial pathogens [22], while no activity was found with deionized water for any microorganism.

As a conclusion; conflict additional increase of antibiotic resistant pathogens. It may be concluded from this study that the water extract of *Moringa oleifera* is active against the tested gram positive bacteria especially *Staphylococcus aureus,* the results confirm the use of the plant Moringa *oleifera* showed promising antibacterial activities and it can play a role in the therapy of infection diseases.Further *in vivo* studies and other studies are essential to verify its efficacy in clinical practice.

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