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In vitro Evaluation of Antimicrobial Activity of *Opuntia ficus-indica* Seed Oil

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Authors' contributions

This work was carried out in collaboration among all authors. Author NFRM performed the analysis, literature search and manuscript writing. Author RVG designed the study and manuscript drafting. Author PSG provide guidance for undergoing research, data verification, manuscript correction. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Opuntia ficus-indica* is a well adapted cactus to extreme climatic environments. In the usual diet and food industry, it is an interesting source of food ingredients. In general, the protective role of prickly pears has been due to their antioxidant components, phenolic compounds, and some minor components present in the content of seed oil and seed protein and the oil from cactus pear seed has been found with high levels of unsaturated fatty acids ,with antioxidant and antimicrobial activity as well as cardioprotective, antithrombotic, anti-inflammatory, antiarrhythmic, hypolipidemic, and antihyperglycemic effect.
Aim: To evaluate the *in vitro* antimicrobial activity of *Opuntia ficus-indica* seed oil against *Streptococcus mutans.*
Materials and Methods: Broth microdilution assay was performed by following modifications: Brain heart infusion (BHI) broth was prepared and sterilise at 121c for 15lbs, the 100 microlitre of streptococcus mutans inoculated at freshly prepared broth and incubated at 37c for 24 hours. Then BHI broth prepared with sucrose and serial two fold dilution were done with 160microlitre broth and

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40 microlitre prickly pear seed oil. Then 20 microlitre of culture was added. Column 11 contains BHI using sterile micro pipettes and the plates were incubated at 30 c for 18 hours. The growth of bacteria was visualised and lowest concentration with no visible growth was recorded as MIC.

Results: This study results shows that, by broth microdilution method at concentration ranging from 10%-0.039% v/v, *Opuntia ficus-indica* seed oil potentially inhibits the growth of streptococcus mutans at the concentration of 0.03% (v/v).

Conclusion: From the study, the results demonstrated that *Opuntia ficus-indica* seed oil shows noticeable antimicrobial activity against *streptococcus mutans.* Use of essential oils against oral pathogens can be an alternative to other antimicrobial agents which could be cheap and effective modules used in control of bacteria which are responsible for oral infections.

Keywords: antimicrobial; Opuntia ficus-indica seed oil; Streptococcus mutans; essential oils.

1. INTRODUCTION

Opuntia ficus-indica is a well adapted cactus to extreme climatic environments.This cactus belongs to the family Cactaceae that originates in arid and semi-arid areas [1]. 1500 cactus species belong to the Opuntia family and are found in Europe, the Mediterranean, Africa, the southwestern United States, northern Mexico and other regions. It is regarded as a likely replacement crop for dry regions because cactus pear can survive sustained drought [2]. A multi-seeded berry with a thick peel, including a flavoured seedy pulp, is a cactus pear fruit. Cactus pear fruit, while used as forage, is mainly eaten as a fresh product. Many Opuntia species grow fruits which are edible and heavily flavoured. In the usual diet and food industry, it is an interesting source of food ingredients. In general, the protective role of prickly pears has been due to their antioxidant components, phenolic compounds, and some minor components present in the content of seed oil and seed protein [3]. The nutritional significance of cactus pear fruit is primarily due to the ascorbic acid, fibers and free amino acid. Essential oils play an important role in controlling and curing many of the bacterial diseases [4]. Some of the essential oils confer antimicrobial activity by damaging the cell membrane, cell wall or by bringing about cell lysis and leakage of cell content cactus seed oil contains essentially unsaturated fatty acids (approx. 76%) and a substantial level of vitamins that are fat-soluble. Seeds contribute 10% to 15% to the pulp weight [5,6]. Oil obtained from the seed represents 7–15% of the whole seed weight which is characterized by high levels of linoleic and oleic acids and other components such as phenols, all of which have a benefit on human health [7]. The oil from cactus pear seed has been found to have an appreciable amount of oil with high levels of unsaturated fatty acids, with antioxidant and antimicrobial activity as well as cardioprotective, antithrombotic, anti-inflammatory, antiarrhythmic, hypolipidemic, and antihyperglycemic effect [8,9].

Dental caries is a disease caused by the interactions between bacteria, foods, and saliva within the dental plaque and its major causative germ is known as *S. mutans,* among the germs present in the dental plaques [10]. *S.mutans*, as gram-positive and facultative anaerobic bacteria, generates acid and acidifies the environment within the dental plaque since it is acid-resistant. A study revealed that ethyl acetate is the most effective, in his study on the antimicrobial activity of *Opuntia ficus-indica var.* in the oral cavity depending on the solvent [11]. Essential oils of plant origin are widely used to treat many infections caused by both Gram positive and Gram negative bacteria. Many of the essential oils are as efficient as the currently administered antibiotics and are highly desirable to be used alone for the treatment of Pseudomonas infection. Essential oils are generally hydrophobic in nature and limit the direct testing of essential oils on broth culture [12]. In a previous study, the extract from clove was strongly active against *B. subtilis, P.aeruginosa and C. Albicans* [13] whereas Cinnamon oil inhibited the growth of *B. subtilis* and *C. albicans* only. Moreover, volatile essential oils have shown strong antibacterial activity against clinical pathogens [14,15] Our team has extensive knowledge and research experience that has translated into high quality publications [16-40]. Therefore, this research aimed to identify the possibility of natural herbal extracts as alternative preventing agents of dental caries by opuntia ficus indica seed oil based on the minimum growth inhibitory concentration. The goal of this research was to evaluate the antimicrobial activity of *Opuntia ficus indica* seed oil against *Streptococcus mutans*.

2. MATERIALS AND METHODS

2.1 Minimum Inhibitory Concentration Assay

The minimum inhibitory concentration(MIC) assay was performed as described previously [41] with slight modifications. BHI(Brain Heart Infusion) broth was prepared and 100µL of *Streptococcus mutans* culture was inoculated into freshly prepared BHI broth and incubated at 37^0 C for 24 h. Then BHI broth prepared and supplemented with sucrose and serial two fold dilution were done with 160µL broth and 40 µL of essential oil was loaded into each well. Then 20 µL of *Streptococcus mutans* culture was added. Column 1-10 contains serial two fold dilution(0.039%-10%v/v) of oils mixed with broth and test organisms. Column 11 contains BHI broth (sterility control). Column 12 contains control. The microtiter plate was incubated at 37^0 C for 24 h. The growth of bacteria was visualised by adding the TTC(Trimethyl Tetrazolium Chloride) and no visible growth was recorded as MIC with respect to control.

3. RESULTS

In this study, results showed that, using microdilution method at concentration ranging from 10%- 0.039% v/v , *Opuntia ficus indica* seed oil has potentially inhibited the growth of *S. mutans* at the concentration of 0.039%(v/v)

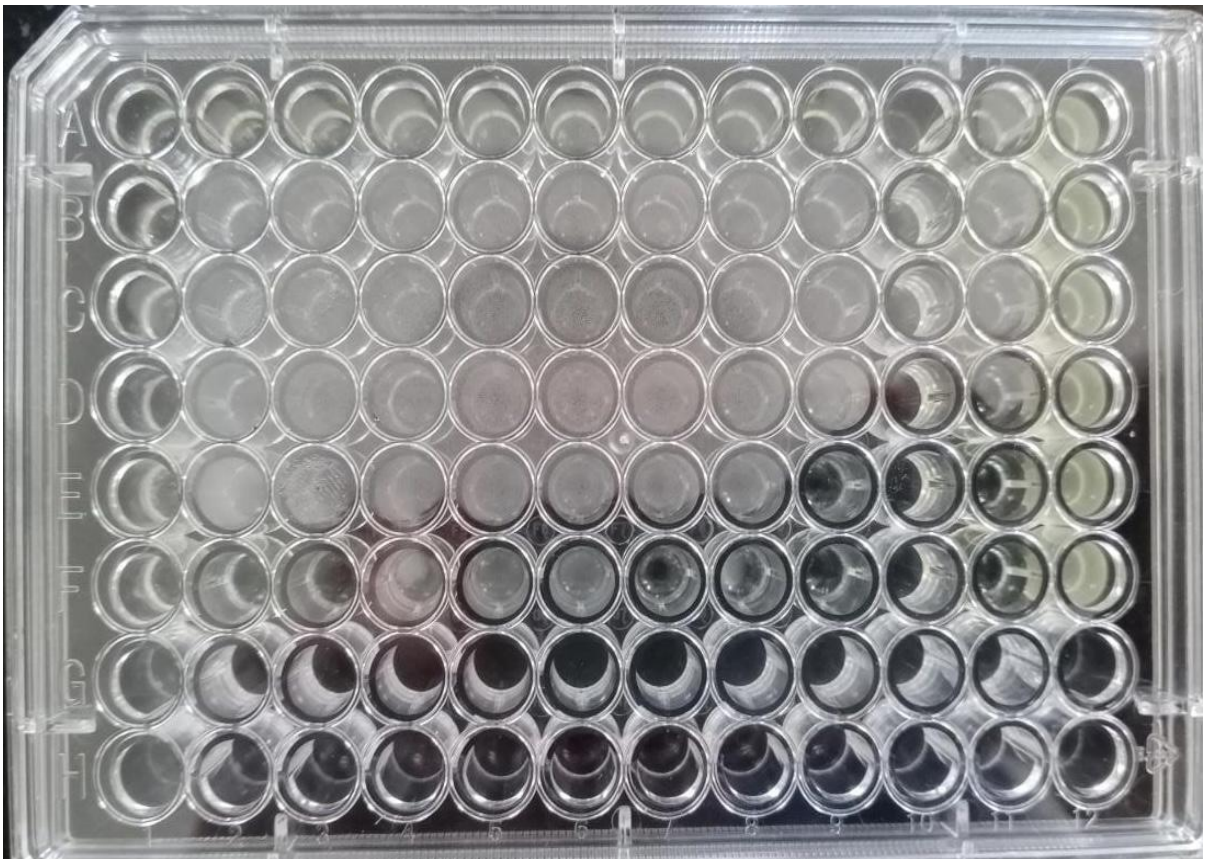

Fig. 1. Determination of minimum inhibitory concentration of *Opuntia ficus-indica* seed oil by serial two fold dilution method

Table 1. Minimum Inhibitory concentration of essential oil against *Streptococcus mutans.* At the lower concentration(0.039%,v/v) of EO inhibited the bacterial growth

S. No	Essential Oil	*Streptococcus mutans*									
1.	*Opuntia ficus-indica*	10% MIC	5% MIC	2.5% MIC	1.25% MIC	0.625% MIC	0.312% MIC	0.156% MIC	0.078% MIC	0.039% MIC	0.019% Growth

4. DISCUSSION

The data showed that the *O. ficus-indica* seeds oil has an interesting capability to inhibit the growth of different pathogens, confirming previous studies which demonstrated the effectiveness of this oil in inhibiting the growth of different Gram-positive and Gram-negative negative pathogens [42]. The action of *Opuntia ficus-indica* seed oil was effective also in blocking at 64.97% the metabolism of *L. monocytogenes* cells, which therefore had conversely demonstrated to be more resistant compared to the other bacteria. Our result suggested that, conversely to a general higher resistance exhibited by the Gram-negative bacteria to biocides [43], the Gram-positive strain used in these experiments, that is *L. monocytogenes,* could be slightly more resistant, for the same volume of oil used, during the biofilm formation phase, but not from a metabolic point of view [44]. Related to this, a study demonstrated that the antimicrobial activity of cactus pear seed oil was more effective against fungi compared to bacteria cultures which shows there is a link between the oil chemical contents and the antimicrobial activity. The membrane disruption could be one mechanism of action by inactivating microbial adhesion, enzymes, and protein transport [45]. In general, gram-negative bacteria have an effective outer membrane that restricts the penetration of amphipathic compounds and has a mechanism to extrude toxins [46]. Due to this, antimicrobial activity was not detected for *Salmonella Typhi*, which is a gram-negative bacterium. This may explain the apparent antimicrobial ineffectiveness of the oils against the permeability barrier in addition to the presence of multidrug resistance encoding plasmids [47]. A study shows that *Saccharomyces cerevisiae* was highly inhibited (38–40 mm) by the extracted oils but grew in presence of the antimicrobial agents. Similar results were observed for *Candida albicans*, although inhibition zones were smaller and similar for oils. These observations demonstrate that certain compounds in the cactus pear seed oil have antimicrobial activity. Other researchers also reported similar observations for cactus pear fruit cv. *Opuntia stricta* [48] and for other plants as fennel (*Foeniculum vulgare* L.) and chamomile (*Matricaria chamomilla* L.). Differences in the levels of antimicrobial activity may be partially attributed to variable chemical composition of the oils [49] Studies have also been conducted to assess the antifungal effectiveness of these essential oils against candida wherein all the oils used in the study have demonstrated varying amounts of antifungal effectiveness [50].

Although a number of publications have focused on the isolation and identification of bio-active compounds, it is important to keep in mind that a single compound may not be responsible for the observed activity but rather a combination of compounds interacting in an additive or synergistic manner. So further studies need to be carried out to identify and isolate the active compounds which are responsible for antimicrobial activity and invivo studies need to be conducted as certain for safety and acceptability of products in *Opuntia ficus-indica* seed oil.

5. CONCLUSION

Use of essential oils against oral pathogens can be an alternative to other antimicrobial agents which can after cheap and effective modules used in control of bacteria which are responsible for oral infections. Thus the results demonstrated that *Opuntia ficus-indica* seed oil shows noticeable antimicrobial activity against *Streptococcus mutans.*

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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