

Assessment of antimicrobial effect of *Phoenix dactylifera* against *Porphyromonas gingivalis*, *Streptococcus mutans* and *Lactobacillus acidophilus* - In Vitro Study

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ABSTRACT

Aim: The aim of the study is to assess the antimicrobial efficacy of seeds of *Phoenix dactylifera* against *Porphyromonas gingivalis*, *Streptococcus mutans* and *Lactobacillus acidophilus*.

Materials and methods: Different concentrations of ethanolic and aqueous extract was prepared at 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml. Using the Muller Hinton agar (MHA) medium and the well diffusion method, the antibacterial activity of the material was assessed through zone of inhibition.

Results: On Comparison ethanol extract was to be found better on comparison with the aqueous extract with better zone of inhibition.

Conclusion: The seeds of *Phoenix dactylifera* has potent antibacterial activity.

Keywords: *Porphyromonas gingivalis*, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Phoenix dactylifera*.

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INTRODUCTION

The oral cavity has a vast microbial biota that includes both harmful and helpful organisms. Proteomics has shown the diversity of microorganisms in the human oral cavity and revealed that strains of *Lactobacillus acidophilus* and *Streptococcus mutans* predominate. Periodontal pathogens include anaerobic gram-negative bacteria which predominantly includes *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum*¹. A balanced ecology required for a healthy mouth depends on the population balance between pathogenic and mutualist bacteria. Even non-pathogenic organisms have the potential to become into opportunistic pathogens when there is a change in the microbial population.

The imbalance of this intricate ecosystem leads to a population shift that causes an overrepresentation of pathogenic species and transforms the normal biota into opportunistic pathogens, which in turn causes dental disease. These pathogens are responsible for the development and progression of oral diseases like caries and periodontal disease². Oral infections that are left untreated or just partially treated can progress to

chronic dental disease and pose a serious risk, particularly to people with impaired immune systems³.

Antimicrobial resistance has been a global problem in recent times. The increasing resistance of bacteria to antibiotics and their tolerance to them has resulted in a reduction in the effectiveness of certain medications that are now prescribed. Other obstacles to the local population using synthetic antimicrobials include cost of purchasing, acceptability, and regimen completion. Multidrug resistance (MDR) is the resistance of microbes to several antimicrobials. However, this has led to a shift in the treatment of microbiological and physiological disorders from synthetic medications to natural therapies derived from plants, animals, or microbes⁴.

Although it can be eaten fresh, *Phoenix dactylifera*, or date palm, is most commonly consumed in its dried state⁵. The sweet flavour is reportedly due to natural sucrose. After the mesocarp is consumed, the seed is often thrown away. The date seed has historically been used to treat liver conditions, diabetes, and gastrointestinal issues in addition to toothaches. It has

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been discovered that date seeds contain tannin, which has anti-inflammatory qualities⁶.

The existing literature shows the antimicrobial efficacy of this date seed against uncommon oral pathogen. Dearth of literature exists on assessing the antimicrobial efficacy of this date seed against common oral pathogen. Hence the aim of the study is to assess the antimicrobial efficacy of seeds of *Phoenix dactylifera* against *Porphyromonas gingivalis*, *Streptococcus mutans* and *Lactobacillus acidophilus*.

MATERIALS AND METHODS

PREPARATION OF PLANT EXTRACT

The study was done after obtaining approval from Institutional Ethical Committee (IEC-PDCH 5/12-2023). In the microbiology lab, the fruit was manually removed from its seeds, cleaned with distilled water, and allowed to air dry. They underwent additional mechanical processing in a grinder to become powder. Fifty grams of ground seeds of *Phoenix dactylifera* was weighed into conical flasks, to which 500 millilitres of ethanol (ethanol to water in a 1:1 ratio) was added and prepared. In a same way an aqueous solvent was also prepared. Extraction was carried out using a mechanical shaker at 130 revolutions per minute (rpm). Whatman No. 1 filter paper was used to filter the extract. A rotary evaporator was used to evaporate the resulting filtrate until it was completely dry. The yield was calculated to determine the dry extracts after they had evaporated.

Yield= Final beaker weight- Initial beaker weight

Yield of ethanol extracts = 3.23g

Yield of aqueous extracts = 2.8g

PREPARATION OF INOCULUM:

Stock cultures were kept on nutrient agar slants at 4°C. In order to prepare active cultures for research, a loop full of cells from the stock cultures was transferred to test tubes containing nutrient broth for bacteria, which were then cultured for 24 hours at 37° C. The agar well diffusion method was used to carry out the assay.

ANTIBACTERIAL ACTIVITY:

Using the Muller Hinton agar (MHA) medium and the well diffusion method, the antibacterial activity of the material was assessed. Following sterilisation, the media was transferred into sterile petriplates and left for one hour to harden. Utilising a sterile brush soaked with the bacterial suspension, the inoculums were uniformly scattered across the entire surface of the plates once the medium had set. A cork borer was used to create 6 different wells. Different concentrations of ethanolic and

aqueous extract was prepared at 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml. Sixty microlitre of sample was loaded into the wells containing different concentration of extract using micropipette; Dimethyl sulfoxide (DMSO) was used as the negative control (20 µl), and streptomycin (1 mg/ml) as the positive control (20 µl) in each well. These plates were incubated at 37°C for a whole day. To determine the microbial growth, the diameter of the zone of inhibition was measured.

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS software version 20 (IBM, Chicago, IL). Independent sample t test was done to find significant difference between ethanolic and aqueous extract.

RESULTS

Table 1: Comparison of antibacterial efficacy against the microorganisms through zone of inhibition.

Microorganisms		Zone of Inhibition in mm					
		1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	DMSO	Streptomycin (20 µl)
<i>Streptococcus mutans</i>	Aqueous extract	13.8 ± 0.8	-	-	-	-	20.8 ± 0.62
	Ethanol extract	18.9 ± 0.29	16.2 ± 0.6	14.3 ± 0.27	12.1 ± 0.68	-	20.6 ± 0.57
P value		<0.01	-	-	-	-	0.7
<i>Lactobacillus</i>	Aqueous	10.2 ± 1	-	-	-	-	23.9 ± 0.2

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<i>S. acidophilus</i>	Aqueous extract	23.8 ± 0.63	11.5 ± 0.56	-	-	-	-
	Ethanol extract	20.6 ± 0.75	13.2 ± 0.55	-	-	-	-
P value		0.02	-	-	-	-	0.1
<i>Porphyromonas gingivalis</i>	Aqueous extract	-	-	-	-	-	-
	Ethanol extract	-	-	-	-	-	-
P value		-	-	-	-	-	-

Comparison of antibacterial efficacy of seeds of *Phoenix dactylifera* against *Streptococcus mutans*, *Lactobacillus acidophilus*, *Porphyromonas gingivalis* shows that ethanol extract to be found better on comparison with the aqueous extract with better zone of inhibition. However the control group, streptomycin showed the highest zone of inhibition (20.6mm and 23.8 mm for *S. mutans* and *L. acidophilus* respectively). Significant difference in zone of inhibition was obtained between aqueous and ethanolic extract in *S. mutans* and *L. acidophilus* with P value <0.001 and 0.02 respectively (Table 1), (Fig. 1)

Fig 1. Zone of inhibition of aqueous and ethanolic extract of seeds of Phoenix dactylifera and control at various concentrations against Streptococcus

mutans, *Lactobacillus acidophilus* and *Porphyromonas gingivalis*.

Fig. 1A

Fig. 1B

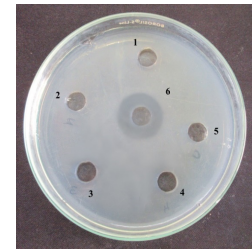
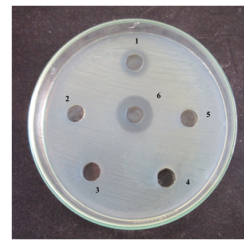


Fig. 1C

Fig. 1D

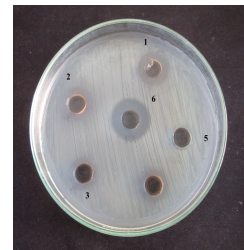
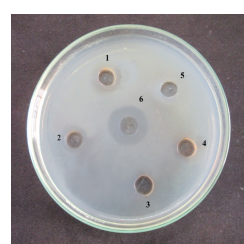


Fig. 1E

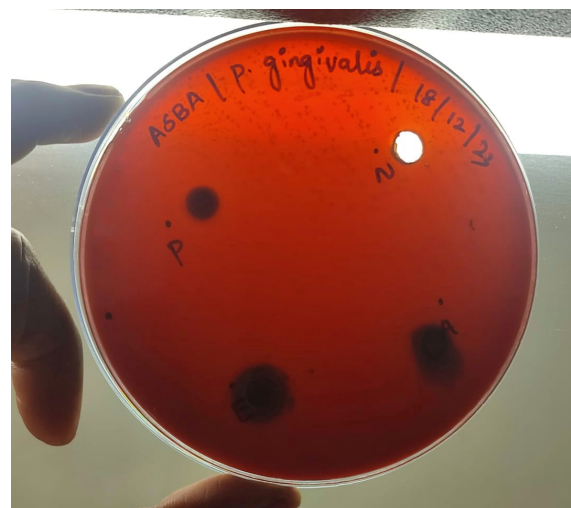


Fig. 1A: Zone of inhibition of aqueous extract against *Streptococcus mutans*

Fig. 1B: Zone of inhibition of aqueous extract against *Lactobacillus acidophilus*

Fig. 1C: Zone of inhibition of Ethanolic extract against *Lactobacillus acidophilus*

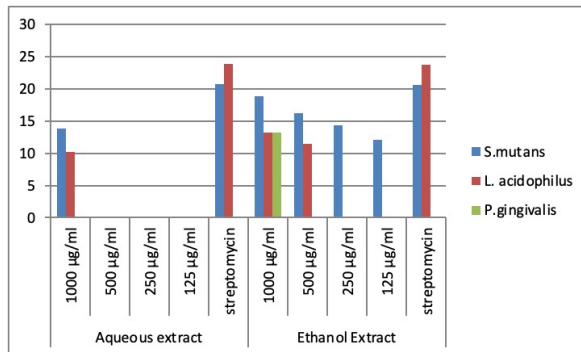
Fig. 1D: Zone of inhibition of Ethanolic extract against *Streptococcus mutans*

Fig. 1E: Zone of inhibition of aqueous and ethanolic extract against *Porphyromonas gingivalis*

Graph1: Comparison of Zone of inhibition of aqueous and ethanolic extract of Phoenix dactylifera against Streptococcus mutans,

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Lactobacillus acidophilus and *Porphyromonas gingivalis*.



DISCUSSION

Dental caries and Periodontal diseases are the most common oral diseases. *Streptococcus mutans* initiates dental caries whereas *Lactobacillus acidophilus* aids in progression of it. Majority of the periodontal diseases are caused by *P. gingivalis*. Hence the control of these microorganisms with lesser side effects to human tissue becomes the need of the hour. Hence the present in vitro study was conducted with an aim of comparing the antimicrobial efficacy of seeds of *Phoenix dactylifera* against *Porphyromonas gingivalis*, *Streptococcus mutans* and *Lactobacillus acidophilus*. Some important published studies support our findings. Abdulmajeed N et al⁷ and Perveen Ket al⁸ Abdallah E et al⁹ conducted similar studies using pathogens other than the tested microorganism in the present study which includes gram-positive and gram-negative bacteria including *S. pneumoniae*, *E. coli*, *K. pneumoniae* and *P. aeruginosa*, *S. flexneri*, *S. aureus*, *E. coli*, *S. pyogenes*, *B. subtilis* and *S. aureus*, *S. epidermidis*, *E. faecalis*, *B. cereus*, *K. pneumoniae*, *E. coli* and *C. albicans* respectively. However in these studies the extract was obtained from the date leaves.

The phytochemicals derived from the seeds include phenolic compounds, essential oils, antioxidants, proteins which synergistically act as biocontrol agents.¹⁰ Because of their damaging effects on the cell wall and plasma membrane, inhibition of enzymes or metabolic inactivation of microbes, and destruction of DNA molecules, antibacterial activity has been observed.¹¹

In the current study, Comparison of antibacterial efficacy of seeds of *Phoenix dactylifera* against *Streptococcus mutans*, *Lactobacillus acidophilus*, *Porphyromonas gingivalis* shows that ethanol extract to be found better on comparison with the aqueous extract with better zone of inhibition. The efficacy of the extracts was found to be concentration dependent

as widest zones of inhibition were observed at higher concentrations of extracts used. Given their distinct polarity, the potency of the ethanolic extract may indicate that the active components in the seed were more soluble in ethanol than in water.¹²

Limitation of the study is that as it is an in vitro study, laboratory test was done using single sample in all the groups. Future studies shall involve addition of more samples along with the assessment of cytotoxicity of the extract to be used in human samples.

CONCLUSION

Based on the observed results of this study, it can be concluded that the seeds of *Phoenix dactylifera* have potential for antibacterial activity. Among the tested bacteria *S. mutans* showed highest zone of inhibition. The information provided should raise awareness among food technologists and consumers about the advantages of incorporating this ancient plant as a possible antioxidant source into modern diets and also render future use of this extract in the field of dentistry.

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