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Antibacterial screening of *Phoenix dactylifera* L. (Date palm) seed extracts on some bacterial isolates associated with dental caries

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Abstract

Dental diseases refer to conditions of deterioration and disintegration of the oral cavity due to the activities of pathogenic microbes. This study was carried out to determine the antibacterial activity of aqueous and ethanol extracts of *Phoenix dactylifera* L. (Date palm) seed on selected bacteria isolates associated with dental caries namely *Staphylococcus aureus, Streptococcus mutans* and *Lactobacillus casei*. Bioactive components of the seed were extracted using water and ethanol as solvents. The antibacterial activity of the extracts was examined by agar well diffusion method. The minimum inhibitory and bactericidal concentrations (MIC and MBC) were also determined using standard methods. All extracts were active against the tested isolates at the concentrations examined. *L. casei* was the most susceptible organism followed by *S. mutans*. *S. aureus* was the least susceptible. Ethanolic extract was more effective than aqueous extract at all the concentrations tested in the study. The MIC of aqueous and ethanolic extracts was 20 mg/ml while the MBC was 80 mg/ml respectively against all the bacterial isolates tested. It can be concluded that the aqueous and ethanolic extracts of the seed possess antibacterial potential against all the test organisms and could be useful in treatment of dental diseases and other related diseases caused by the bacteria isolates tested in the study.

Keywords: **Dental, Date palm, Antibacterial action, Inhibitory, bactericidal**

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INTRODUCTION

The microbial biota of the oral/buccal cavity is enormous ranging from beneficial to pathogenic organisms. The microbial diversity of the human oral cavity has been demonstrated, through proteomics and 16SrRNA, to be dominated majorly by strains of *Streptoccocus mutans*, and other streptococci including *S. sanguis*, *S. mitis*, and *S. salivarius* in addition to *Lactobacilli* and *Veillonell*, Gram-negative anaerobic bacteria

such as *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Prevotella intermedia* which are known to be periodontal pathogens (Paster*et al.,* 2001) and *Candida albicans* among others (Dewhirst *et al.,* 2010). A balance in population of mutualist versus pathogenic microbes is necessary to maintain a balanced ecosystem required for a healthy mouth. When there is a disturbance in the microbial community even non-pathogenic organisms could become opportunistic pathogens Dental disease occurs as a resulting effect of the disturbance of the equilibrium of this complex ecosystem, this disturbance results in a population shift leading to over representation of pathogenic species and normal biota becoming opportunistic pathogens contributing to the onset and progression of oral diseases such as caries and periodontal disease (Kuboniwa *et al.,* 2012; Adolph *et al.,* 2017). Oral infections caused by microorganisms though not lethal could be frustrating and have been one of a major concern to the world (NIHCD, 2001; Bagramia *et al.,* 2009). The effects of oral disease include shameful discoloration and decay of the gum, foul smell, loss of teeth, cost of the treatment of oral infections and aesthetic among others (Adolph *et al.,* 2017). Untreated or partially treated oral infection degenerate to chronic dental disease and could be very dangerous, essentially among immunocompromised patients (Haque *et al*., 2019). Oral pathogens are not exempted from the ability of a microbe to resist the effects of medication previously used to treat them; these resistant microbes are more difficult to treat, requiring alternative medications or higher doses, both of which may be more expensive or more toxic thus necessitating a need for an alternative therapy (Bagramia *et al.,* 2009; Bonecjer *et al.,* 2013). *Staphylococcus aureus* is one of the human normal micro biota, a commensal but could easily become opportunistic pathogen (Masalha *et al.,* 2001).The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S*. *aureus* (MRSA) is a worldwide problem in clinical medicine (Garoy *et al*., 2019**).** Despite much research and development, *S. aureus* is still a common opportunistic pathogen involved in most human infections. *Streptococcus mutans* is a facultatively anaerobic, Gram-positive coccus commonly found in the human oral cavity and is a significant contributor to tooth decay along with the closely related species *Streptococcus sobrinus* (Ryan and Ray, 2004). *Lactobacillus casei* is a species found in the human intestine and mouth. This particular species of *Lactobacillus* is documented to have a wide pH and temperature range; a producer of the enzyme amylase and lactic acid, increase acidity can weaken the gingiva leading to periodontal diseases. Previous reports have shown that microorganism commonly associated with dental caries is *S. mutans*. However, caries could develop in the absence of *S. mutans* and a wide variety of microorganisms such as *Lactobacillus acidophilus* and various species of *Staphylococcus* could aid in caries initiation and progression (Kaur *et al*., 2015).

The challenge of antimicrobial resistance has recently been of global concern. The rise in antimicrobial resistance and tolerance to existing drugs by microorganisms has created a decreased in the efficacy of some of the drugs presently in use (WHO, 2014; O'neil, 2016). The findings of Hague *et al*. (2019) show antibiotic resistance by *S. mutans*, *S. aureus* and *Lactobacillus* spp. in treatment of dental caries. Antibiotic resistance has substantially increased in the recent years and is posing an ever-increasing therapeutic problem (*Cassir et al., 2014).* The cost of purchase, acceptability and completion of regimen are other challenges to the use of synthetic antimicrobials among the local populace. Microbes' resistance to multiple antimicrobials is called multidrug resistance (MDR); or sometimes superbugs. This has however birthed a change in trend from synthetic drugs to the natural drugs either from plants, animals, or microbes to control the microbial and also physiological diseases. Natural products are constantly being screened for possible pharmacological value particularly for their anti-inflammatory (Qadir, 2009), anti-fertility, cytotoxic, antimicrobial (Amin *et al.,* 2012), antioxidant and anti-diarrheal (Janbaz *et al.,* 2013) properties.

Plants are being used medicinally in different countries and are a source of many potent and important drugs. A broad range of medicinal plant parts with different medicinal properties are used directly or as extract for raw drugs. The various parts used include root, stem, seed, flower, fruit, and twig exudates. While some of these raw drugs are collected in small quantities by traditional healers for local therapeutic use, several other raw drugs are collected extensively and traded in the market as raw materials for many herbal industries for productions of drugs in form of concoction and capsules (Sujatha, 2005). In developing countries, herbal medicine is still the mainstay primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available

(Fentahun *et al.,* 2017). Fortunately, they are more acceptable to the indigenes, easily assessible and affordable (Fentahun *et al.,* 2017). *Phoenix dactylifera L.* is commonly available in Nigeria and is often consumed in the dried form though it can be eaten fresh (Adedayo *et al.,* 2016). It is usually reported to contain natural sucrose hence the sugary taste. Most often the seed is discarded after consuming the mesocarp. Being a medically beneficial plant, it has been successfully studied for its antioxidant, antiviral, antifungal, anti-hyperlipidemic and hepatoprotective properties amongst others **(**Sundar *et al*., 2017**).** The seed has been reported to have the ability to increase the functionality of the immune system as well as lower the risk of cancer and cardiovascular conditions due to its high content of phenolics and nutrients such as fiber, fat, moisture, protein, ash, and vitamins. Traditionally, the date seed is used in the treatment of toothaches and also as remedies for liver diseases, diabetes, and gastrointestinal tract disorders **(**Adeosun *et al.,* 2016; Sundar *et al*., 2017).

Report from Sundar *et al*. (2017); Metoui *et al*. (2019) show that the date seed has high tannin content and could be used medicinally. The potential of different parts of the date plant against many ailments has been variously documented, it include antibacterial action on pathogenic bacteria, use as a detersive and astringent in intestinal troubles, ability to relieve fever, gonorrhea, edema, liver and abdominal disorder as a treatment for sore throat, colds, bronchial catarrh in the form of an infusion, decoction, syrup or paste, use in counteracting alcohol intoxication, the gum is used as diuretic and demulcent in treating diarrhea and genito-urinary ailments, the root is used against toothache (Jassim and Naji, 2007; Al–Taher, 2008; Kumar *et al.,* 2010; Saddiq and Bawazir, 2010; Perveen *et al*., 2012; Tauqeer *et al.,* 2014; Adeosun *et al.,* 2016;; Alrajhi *et al*., 2019; Metoui *et al*., 2019). Literature is however scanty on its antibacterial properties as regards the micro floral of the human dental cavityand its potential as an alternative to controlling dental pathogens.The aim of this research is to investigate the antibacterial activity of *Phoenix dactylifera L.* (Date palm) seeds on some common bacteria associated with dental caries.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Dried *Phoenix dactylifera L* (Date palm) fruits were purchased from Oja-tuntun market in Ilorin, Kwara State. The date seed was identified and authenticated as *Phoenix dactylifera L*. seed at the Plant and Environmental Biology Unit of the Department of Biosciences and Biotechnology in Kwara State University Malete, Kwara State, Nigeria.

Collection and Maintenance of Bacteria Associated with Dental Caries

Test organisms were collected from the Microbiology laboratory section of the University of Ilorin Teaching Hospital Ilorin, Kwara State. The isolates were characterized and identified as *Staphylococcus aureus, Streptococcus mutans* and *Lactobacillus casei.* All test organisms were obtained as pure isolates and was aseptically maintained on slants stored at 4^{o}C. Isolates were reactivated prior to use by culturing in Mueller Hinton broth incubated for 6-8 hours to assume exponential phase of growth

Preparation of the Aqueous and Ethanolic Plant Extract

The fruits were separated from its seeds manually, cleansed with distilled water and air dried at 28 ±2^{o}C in the Microbiology laboratory of Kwara State University Malete, Kwara State. They were further processed mechanically into powder using a grinder (Sonik, Japan). The sample was stored in airtight container for further research. Fifty grams of the grinded seed of *Phoenix dactylifera* was weighed into conical flasks and 500 ml of extractant was added and mixed properly. Extraction was done on mechanical shaker at 130 revolutions per minute (rpm) for 48 hours at 28 ± 2 ^{o}C. The extract was filtered using a Whatman No. 1 filter paper. The filtrate obtained was evaporated to dryness using a rotary evaporator. The semi solid extract obtained was used for the analysis. DMSO was used to reconstitute the extract. Note that ethanol was completely evaporated from the extract before use to avoid interference.

Antibacterial Assay

The Muller Hinton broth cultures of test organisms were standardized by adjusting them to turbidity corresponding to 0.5 McFarland standard and used as inoculum as adopted by Egwari and Abah (2011). Agar well diffusion method described by Akinbosun and Edionwe (2015) was used to determine the

antibacterial activity of the ethanol and aqueous extracts of date palm seeds on Mueller Hilton Agar plates. Sterile cotton swabs were immersed in the standardized broth culture suspension and evenly streaked over the entire surface of the plates to obtain uniform inoculums. Six wells per plate were made with sterile cork borer of 6 mm in diameter. The wells were filled with 0.1 ml of each of the reconstituted extracts at 20, 40, 60, 80 and 100 mg/ml concentrations of the extracts with the aid of micropipette; DMSO was added to the sixth well appropriately to act as negative control while Chloramphenicol (5 µg) was used as positive control. The plates were allowed to sit on the sterile workbench for one hour to allow diffusion of extracts before incubating at 37 ^{o} C for 24 hours. The antibacterial activity was interpreted based on the size of the diameter of zone of inhibition measured to the nearest (mm) as observed from the clear zone surrounding the wells.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the aqueous and ethanol extracts was determined by broth dilution method (Andrews, 2001). To test tubes containing 9 ml of sterile Mueller Hilton broth were added 1ml of the extracts at various concentration, a control tube was set. The tubes were seeded with 0.1 ml of standardized inoculums. The broths were incubated at 37^{o} C for 24 hours after which visible turbidity was checked. The tube with the least concentration that showed no growth compared to control was taken as the MIC (Andrews, 2001; Adegoke *et al.,* 2010).

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by taking inoculum from test tubes that showed no growth after incubation in the MIC test and sub culturing on sterile Mueller-Hinton agar plates which were incubated at 37^{o}C for 24 hours. Lowest concentrations that showed no growth on plate was taken as the MBC (Andrews, 2001; Adegoke *et al.,* 2010).

RESULTS

Antibacterial Assay

All the extracts had their highest zone of inhibition at concentration of 100mg/ml. The sterile distilled water and DMSO that was used as controls showed no zone of inhibition against the isolates. The aqueous extracts of *Phoenix dactylifera l.* had highest inhibition on *Lactobacillus casei* (14.00 mm), followed by *Streptococcus mutans* (10.00 mm). *Staphylococcus aureus* was the least susceptible with inhibition zone of 4.00 mm (Table 1). The ethanolic extract was more effective as shown by the zone of inhibition observed (Table 2). Again *L. casei* was the most susceptible (18.00 mm) followed by *Streptococcus mutans* ((15.60 mm) and *Staphylococcus aureus* was mildly susceptible (12.00 mm).

Minimum Inhibitory Concentration (MIC) and Bactericidal concentration (MBC)

The bacterial isolates showed MICs and MBCs at 20 mg/ml and 80 mg/ml respectively for both the aqueous and ethanolic extracts of *Phoenix dactylifera L* seed (Table 3).

Table 1: Zones of inhibition of bacterial isolates associated with dental caries using aqueous extracts of *Phoenix dactylifera l.* (Date palm) seeds

Bacterial Isolates	Zones of inhibition (mm)						
	Concentration of aqueous extract (mg/ml)					Positive control: chloramphenicol (5µg)	Negative control: DMSO
	20	40	60	80	100		
S. aureus	4.00±0.00	4.50±0.28	5.00±0.28	6.00±1.27	8.00±0.42	20.00± 2.83	-
S. mutans	4.00±0.71	6.00±0.28	7.50±0.00	8.00±0.42	10.00±1.98	12.50±1.13	-
L. casie	5.00±1.41	7.00±0.00	7.60±0.99	9.50±2.97	14.00±2.83	25.00±1.13	-

Values are means of duplicate readings and standard deviation of zones of inhibition of bacterial isolates using aqueous extract of *Phoenix dactylifera* L. (ate palm) seeds; **Key**: - = Not active against bacterial isolates.

Table 2: Zones of inhibition of bacterial isolates associated with dental caries using ethanolic extracts of *Phoenix dactylifera* L. (date palm) seeds

Bacterial isolates	Zones of inhibition (mm)						
	Concentration of ethanol extract (mg/ml)					Positive control (5µg chloram-phenicol)	Negative control (DMSO).
	20	40	60	80	100		
S. aureus	4.00±0.21	6.50±1.13	8.00±0.28	9.60±2.12	12.00±0.71	20.00±2.83	-
S. mutans	4.50±1.41	7.00±0.28	9.00±1.56	11.00±0.42	15.60±2.12	22.60±1.13	-
L. casie	6.00±2.83	9.50±1.41	12.50±0.99	14.50±0.99	18.00±2.83	25.00±1.13	-

Values are means of duplicate readings and standard deviation of zones of inhibition of bacterial isolates using ethanolic extract of *Phoenix dactylifera l.* (Date palm) seeds; Key: - = Not active against bacterial isolates

Table 3: Minimum inhibitory (MIC) and bactericidal concentrations (MBC) of the aqueous and ethanolic extract of *Phoenix dactylifera* L. seeds

	Aqueous extracts/MIC/MBC (mg/ml)		Ethanolic extracts/MIC/MBC (mg/ml)	
Bacterial Isolates	MIC	MBC	MIC	MBC
Lactobacillus casie	20	80	20	80
Streptococcus mutans	20	80	20	80
Staphylococcus aureus	20	80	20	80

DISCUSSION

The results obtained from this study showed that the growths of the tested organisms (*S. aureus, S. mutans* and *L. casei)* were inhibited at all the concentrations examined in both the aqueous and ethanolic extracts (Tables 1 and 2). However, *Lactobacillus casei* was the most susceptible among the tested organisms to the extracts. The susceptibility of these organisms could probably be traced to thepotency of the seed extracts. This observation is in line with the earlier study on *P. dactylifera* fruit extract on some Gram-positive organisms (Nizar *et al.,* 1999; Adeosun *et al.,* 2016; Sundar *et al*., 2017; Metoui *et al*., 2019; Alrajhi *et al*., 2019). The susceptibility of *S. mutans to* the two extracts gave credence to the study carried out by Perveen *et al.* (2012) who concluded that the seed extract of *Phoenix dactylifera* has promising antibacterial activity against *Streptococcus* spp. The moderately susceptible organism according to this research was *Staphylococcus aureus* as indicated by the diameter of the zones of inhibition. This response of *S. aureus* could be traceable to the strong resistance ability of the organism to many antimicrobials including synthetic antibiotics. *S. aureus* has been an organism of concern to public health as a result of its multi drug resistance status; thus, making it difficult to treat infections caused by the organism (Garoy *et al*., 2019**).**The antimicrobial activity of the seed extracts of *Phoenix dactylifera l* may be attributed to the presence of a sour astringent substance (tannin) and other phytochemicals reported by Biswas *et al.* (2002);Sundar *et al*. (2017); Metoui *et al*. (2019) in their works to be the main bioactive ingredient in date seed.

However, the efficacy of the extracts was found to be concentration dependent as widest zones of inhibition were observed at highest concentrations of extracts used. The MIC of all the organisms were at the lowest concentration tested while the MBC was at 80 mg/ml in ethanolic and aqueous extracts. The antimicrobial potency of any agent is directly dependent on the concentration. Microbial growth might only be inhibited at lower concentration while an organism may even utilize an over diluted agent for its growth and metabolism. It is important to state here that the Clinical and Laboratory Standard Institute method for interpreting MIC was not applied here owing to the fact that the study was done with crude extracts while the CLSI system is applicable to purified active ingredients. Furthermore, it was observed that the ethanolic extracts of the date palm seed were more effective against the bacterial isolates associated with dental caries compared to the aqueous extract. The potency of the ethanolic extract could probably be an indication that the active ingredients in the seed were more soluble in ethanol than water since they have different polarity though both of them are polar solvents. Previous study has emphasized that antimicrobial property of plants extracts varies with different solvents used as extractant (Egwari and Abah, 2011). Moreover, antibacterial activity of a plant extract is due to the synergetic effect of the various bioactive compounds present or the action of a particular major ingredient with little or no contribution from some others. The biological active compounds from any herb mainly depend on the solvent used in extraction and the process (Pichersky *et al.,* 2012). Conclusively *Phoenix dactylifera l* seed extracts can be considered a promising source of bioactive substances effective against caries-related microorganisms, particularly *S. mutans, Lactobacilus casei* and *S. aureus.* Chewing of the seed frequently might be a preventive measure against dental and periodontal diseases. More works including clinical trials are required to validate this.

CONCLUSION

Since this is a preliminary study, it is hereby recommended that further research should be done towards isolating, purifying, and standardizing the active antimicrobial constituents of *P. dactylifera* seed for use in herbal concoctions for local use solely or in combination with other herbs. Also, more work should be carried out to determine the best solvent for its extraction and possible toxicity of the active constituents of *P. dactylifera* seed on human subjects.

Conflict of interest

Authors have no conflict of interest to declare

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