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A COMPARATIVE STUDY OF THE *IN VITRO* ANTIBACTERIAL ACTIVITY OF ENDOCARP, DATE PALM TISSUE AND DATE PALM TISSUE WITH ENDOCARP TOGETHER AGAINST SOME GRAM NEGATIVE AND GRAM POSITIVE PATHOGENIC BACTERIA

R Al Qroom and W.M Al Momani*

Department of Allied Medical Sciences, Al Balqa' Applied University, P.O. Box 19117, Al-Salt, Jordan

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Correspondence to Author:

W. M Al Momani

Department of Allied Medical Sciences,
Al Balqa' Applied University, Al-Salt,
Jordan.

Email: waleed.almomani@bau.edu.jo

ABSTRACT: The antibacterial activities of water extracts of the three major parts of the date palm fruit were compared against *Escherichia coli*; *Klebsiella pneumoniae*; *Salmonella enteritidis*; *Pseudomonas aeruginosa*; *Staphylococcus aureus* using the agar well diffusion method. Endocarp extract showed a wide range of activity against all Gram negative bacteria while it exhibited a low activity against Gram positive bacteria. The date tissue and endocarp didn't show any activity against *Klebsiella pneumoniae* with a low or no activity against *Pseudomonas aeruginosa*. Date tissue extract alone didn't exhibit any activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. All types of extract were active against *Escherichia coli*. A consistently lower susceptibility of *Klebsiella pneumoniae* and *P. aeruginosa* indicated its capability for developing resistance against various antibacterial substances. Antibacterial activities displayed by all extracts especially the endocarp also signified their remarkable potential for exploration of effective natural antibacterial agents against common pathogenic bacteria.

INTRODUCTION: The date palm fruit is a drupe exhibiting a high diversity in texture, shape, color and chemical composition depending on the genotype, environment, season and cultural practices.

The beneficial health and nutritional values of date palm for human and animal consumption have been claimed for centuries¹. The fruit of the date palm contains tannin, which makes it an effective astringent.

Dates have been used as a deterrent and astringent in intestinal troubles, treatment for sore throats, colds, bronchial catarrh, fevers, gonorrhoea, edema liver and abdominal troubles and to counteract alcohol intoxication².

The various parts of this plant are widely used in traditional medicine for the treatment of various disorders which include memory disturbances, fever, inflammation, paralysis, loss of consciousness and nervous disorders³.

Some plants represent a rich source of antimicrobial agents. Approximately 20% of the plants found in the world have been subjected to pharmacological or biological test, and a substantial number of new antibiotics introduced in the market are obtained from natural or semi-synthetic resources⁴.

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A wide range of medicinal plant parts is used as extract for raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, and fruit and twigs exudates. While some of these raw drugs are collected in smaller quantities by folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as raw material for many herbal industries⁵. Many pharmaceutical companies show interest in plant-derived drugs mainly due to the current widespread believe that 'Green Medicine' is safe and more dependable than the costly synthetic drugs which may have adverse side effects. As per the World Health Organization (WHO) report, 80% of the world population presently uses herbal medicine for some aspect of primary health care⁶. About 42% of 25 top selling drugs marketed worldwide are either directly obtained from natural sources or entities derived from plant products⁷.

Infectious diseases account for high proportion of health problems in the developing countries⁸. Antimicrobial resistance is a global concern that has significant impacts on human health and well-being⁹. In recent years a growing body of literature has highlighted the dynamic nature of antimicrobial resistance in community pathogens, and the pressing need for surveillance at the local level to guide healthcare practitioners on antimicrobial choices and clinical decision-making. Acquired resistance to antibiotics is currently steadily increasing in microorganisms. Therefore, much attention is being paid to look for a novel biologically active compound, which exhibit a broad spectrum of biological activity¹⁰.

In this study the endocarp (the membrane covering the seed) water extract as well as other parts of the date palm fruit were evaluated separately for their *in vitro* antibacterial activity against Gram negative and Gram positive pathogenic bacteria.

MATERIALS AND METHODS:

Collection of plant material: Three different species of palm dates were collected, namely majhool (Jordan), Saqei (Saudi Arabia) and Dejlat Noor (Algeria). Endocarp was taken off from the seeds in aseptic technique and separated accordingly. Date palm tissue only was collected

and the whole date fruit with endocarp was also collected separately for the three date palm types.

Extraction of plant material: 10 g of air-dried powder of endocarp; date tissue and endocarp and date tissue together separately were added to 100 ml of distilled water and boiled on slow heat for 2 hrs. The mixture was filtered through eight layers of muslin cloth and centrifuged at 5000g for 10 min. The supernatant was collected, pooled and concentrated to a final volume of one-fourth of the original volume of solvent used (which was 100 ml)¹¹. It was then sterilized using 0.22 μ m Millipore filter membrane and stored at 4°C

Bacterial species: The clinical isolates used in this study were obtained from the Central Laboratories, Jordan Ministry of Health. These clinical isolates were *Escherichia coli*; *Klebsiella pneumonia*; *Salmonella enteritidis*; *Pseudomonas aeruginosa*; *Staphylococcus aureus*

Media preparation and antibacterial activity: Antibacterial activity was determined using the agar well diffusion method¹². Autoclave sterilized 20 ml aliquots of Mueller–Hinton agar were poured into 90 mm petri-dishes. Once these plates were cooled a bacterial lawn was prepared by spreading 100 μ l from the above prepared bacterial suspension using sterile swabs. Wells of 6 mm in diameter were punched into the agar and filled with 100 μ l of the antimicrobial under investigation at a concentration of 1 μ g/ml¹³. The plates were then incubated at 37°C for 24 hours before zones of inhibition were measured using a caliper¹⁴. Each extract was analyzed in triplicate; the mean values are presented.

RESULTS: All the date palm species tested showed antibacterial activity (**Table 1**). The Endocarp extract showed a low activity against *Klebsiella pneumonia* and high activity against the other bacterial isolates except for *Staphylococcus aureus* which was of low susceptibility to this extract. The date tissue and endocarp didn't show any activity against *Klebsiella pneumoniae* with a low or no activity against *Pseudomonas aeruginosa*. Date tissue extract alone didn't exhibit any activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. All types of extract were active against *Escherichia coli*.

TABLE 1: ANTIBACTERIAL ACTIVITY OF PALM DATE ENDOCARP, DATE TISSUE AND ENDOCARP AND DATE TISSUE TOGETHER AGAINST GRAM NEGATIVE AND GRAM POSITIVE BACTERIA IN AGAR WELL DIFFUSION

Tested bacteria	Gram (-) bacteria				Gram (+) bacteria
	Ec	Kp	Ps	Se	Sa
Endocarp					
KSA	10	4	14	14	8
Jor	14	9	11	15	5
Alg	11	16	2	15	5
Date tissue and endocarp					
KSA	6	N	5	8	4
Jor	12	N	N	N	18
Alg	9	N	4	11	12
Date tissue					
KSA	14	N	N	18	17
Jor	12	N	N	N	16
Alg	12	N	N	18	18
DMSO (-ve control)	-	-	-	-	-
Amoxicillin (+ve control)	+++	+++	+++	++	+++

Sa – *Staphylococcus aureus*, Ps – *Pseudomonas aeruginosa*, E. coli – *Escherichia coli*, Se- *Salmonella enteritidis*, Kp- *Klebsiella pneumoniae*; DMSO is a negative control and Amoxicillin is a positive control; N: non detected. Key to interpretation (-) = no inhibition zone = inactive; 1–5 mm (+) = less active; 5–10 mm (++) = moderately active; 10–15 mm (+++) = highly active. KSA: Kingdom of Saudi Arabia; Jor: Jordan; Alg: Algeria

DISCUSSION: The results obtained in the present study showed that there were some differences in the antibacterial activity between the endocarp, date tissue and endocarp and date tissue together. Plant extract possesses potential antibacterial activity against the tested organisms. Many of the fruit extracts were found to be effective against the tested clinical isolates and were comparable to the standard antibiotic Amoxicillin.

As long as we know this study is the first to compare the antibacterial activity of different components of the date palm fruit. Date palm is a well-known fruit used all over the world for its high nutritional value as well as the use of date palm in folk medicine to treat a variety of diseases including the infectious diseases along with too many other diseases like memory disturbances, fever, inflammation, paralysis, loss of consciousness and nervous disorders².

In this study, all the tested extracts were found to be less effective than the standard antibiotic Amoxicillin. The results in this study showed that date palm fruit tissue, Endocarp and date palm tissue and endocarp together were effective inhibitors of pathogenic bacterial growth with some differences in the susceptibility of various bacterial species to each of the palm species extract.

Among the different extracts of date palm, Endocarp extract showed a moderate to high activity against all Gram negative tested bacteria and a low activity against Gram positive bacteria. This may be due to the presence of tannins in the endocarp. Tannins are known for their astringent property and antimicrobial activity¹⁵.

Phytochemically the date palm contains carbohydrates, phenolic compounds, alkaloids, steroids, flavonoids, vitamins and tannins¹⁶. The phenolic profile of the plant revealed presence of mainly cinnamic acids, flavonoid glycosides, flavanols, four free phenolic acids and nine bound phenolic acids¹⁷.

In our study all date fruit extracts showed a high antibacterial activity against *E. coli* which is different from the results of¹⁸ Ayachi *et al* (2009) who found that date fruits extracts showed a lower antibacterial activity against *E. coli*. The difference may be due to the differences in the nature of bacterial isolates used in the two studies since we have used clinical isolates.

In our results, crude extract of endocarp inhibits the growth of all Gram negative and Gram positive bacteria with some variations in their activity.

The crude extract of the other two components of the date palm fruit exhibited high activity against *S. aureus* and had a moderate to no activity against Gram negative bacteria which is in the contrast to the report from the study by Saddiq and Bawazir (2010)¹⁹ as they reported good antibacterial activity of the aqueous extract of date palm against Gram negative bacteria (*K. pneumonia* and *E. coli*).

The three tested palm dates showed some variations in the activity against the pathogenic bacteria used in this study. The activity of the KSA date tissue and endocarp against *S. aureus* was low while it was moderate to high for the Algerian and Jordanian dates respectively. The Algerian endocarp extract activity was high against *K. pneumonia* while the Jordanian and KSA endocarp extract exhibited moderate to low activity respectively. The difference of activity between the three palm date types may be due to the high diversity in chemical composition depending on the genotype, environment, season and cultural practices of these palm dates¹.

CONCLUSION: Based on our results, it can be concluded that endocarp, date tissue and endocarp with the whole date tissue possess have a significant antibacterial activity. The results also suggest that fruit can serve as potential source of bioactive healthy compounds in the diet, recommending that their consumption could be useful in the prevention of diseases.

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