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Antibacterial activity of palm heart extracts collected from Iraqi Phoenix dactylifera L.

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Abstract

This study aimed to assess the antibacterial activity of palm heart (*Phoenix dactylifera L.*) extract *in vitro.* Fresh material of the palm heart was dried, crushed and then extracted with distilled water, methanol and acetone. Distilled water extract was divided into crude, residues (after filtration), protein and non-protein parts. All extracts were investigated to determine their inhibitory activity against *Staphylococcus aureus* (*S. aureus*), *Streptococcus pyogenes (S. pyogenes), Streptococcus mutans (S. mutans), Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), Proteus mirabilis (P. mirabilis), Enterobacter aerogenes (E. aerogenes), Morganella morganii (M. morganii)*, and *Pseudomonas aeruginosa (P. aeruginosa)* using agar well diffusion method, ceftriaxone (CTR) was used as a positive control. Phytochemical analyses were also performed to confirm the presence/absence of bioactive constituents. All extracts revealed antibacterial activity against some of the tested bacteria. When comparing the findings, aqueous (watery) extracts had the slightest antibacterial activity while the highest activity was related to acetone extraction. For Gram-positive bacteria, the highest activity of acetone extract was against *S. mutans* (10.7±0.13 mm) followed by *S. pyogenes* (10.6±0.17 mm), while Gram-negative most sensitive strain were *E. aerogenes* (15.3±0.20 mm) and *P. mirabilis* (14.4±0.48 mm) respectively. Phytochemical analyses have revealed the presence of steroids, alkaloids, pseudotannins, glycosides, carbohydrates, and aromatic amino acids. Finally, these findings indicate the activity of the heart of palm to fight germs, further studies may also be needed

Keywords: Heart of palm; Medicinal Plants; Extract; Acetone; Antibacterial

1. Introduction

Worldwide, one of the major health problems are bacterial infections, especially in developing countries [1, 2]. Synthetic antibacterial agents are used to fight these infections with some side effects [3]. Many factors including the prevalence and sometimes unsuitable using of antibiotics, widespread using of these agents in animal feeding as enhancers for growth, and increased transboundary passage of resistant bacteria are all responsible for developing antimicrobial-resistant bacteria [4]. The antimicrobial resistance problem in animals and humans will continue as the three factors stay [5]. However, alternative classes of drugs may also be necessary to develop to be as an alternative for the future to treat such infections [6]. Some plants are considered as a vital source

Nomenclature			
AC	Acetone alone	MHA	Muller-Hinton agar
ACE	Acetone extract	NA	Nutrient agar
ART	Alkaline reagent test	NB	Nutrient broth
CR	Crude extract	BA	Blood agar
CTR	Ceftriaxone	BHI	Brain-heart infusion
DW	Distilled water	NP	Nonprotein part
HoP	Heart of palm	PP	Protein precipitate
ME	Methanol alone	RE	Residue after filtration
MEE	Methanol extract	WR	Wagner's reagent

of antimicrobials. About 20% of wild plants have been tested to evaluate their biological or pharmacological properties, and a large number of new antimicrobials existing in the markets are derived from semi-synthetic or natural resources [7]. A large number of medicinally important plants are used in different countries to get drugs with potent and powerful effects [8]. Various parts of medicinal plants including fruit, flower, leaf, twigs exudates, root, and stem are used as raw drugs when extracted with diverse medicinal properties. While the folk healers collect smaller quantities of these raw drugs for local use, many others are collected in larger amounts and traded as raw material for herbal industries in the markets [9]. Hundreds of plants found in nature have been tested for antimicrobial activity but the vast majority have not been well enough evaluated [10]. In the field of pharmaceutical industry, many companies have interest in plant-derived medications because of the widespread trust regarding the safety of "Green Medicine" in comparison to expensive synthetic agents that absolutely have some bad side effects. According to the WHO (the world health organization) reports, about 80% of the world populace actually use herbal (plant-derived) preparations in the primary health care in some aspects [11]. Approximately 42% of 25 bestselling drugs globally are either obtained from natural sources directly or extracted from medicinal plant parts [12, 13]. *Phoenix dactylifera L.* (palm of date) is a monocotyledonous perennial, flowering and woody fruit species included in the Arecaceae family [12]. Date palm beneficial nutritional and health values for animal and human consumption have been supposed for centuries [14, 15]. The date fruit contains tannins that make it an efficacious astringent. In addition to using the dates as a detersive and astringent in troubles of the intestine, they have also been used in the treatment of a large number of conditions like sore throat, bronchial catarrh, colds, fever, gonorrhoea, edema, counteract alcohol intoxication and liver and abdominal troubles [16]. Many parts of the date palm also are used in folk medicine widely for treating numerous disorders like memory disorders, paralysis, fever, loss of consciousness, infection, and nervous disorders [12]. The roots are used in toothache treatment. Grounded seeds have been used as an adhesive that is effective in treating ague. The trunk's secretions (i.e. gum) have been used to treat urinary ailments and diarrhea effectively. Dates of *P. dactylifera L.* are rich in supplying the body with dietary fibers and vitamins A, B and C, in addition to various amino acids and minerals. Date antioxidants can also serve in decreasing the probability of getting cancer and cardiovascular system disorders, through establishing a strong immune system [17]. The heart of palm (HoP), represent a medulla or the inner part of palm trees. The HoP contains vitamins, phosphorus, potassium, iron, and calcium and has a significant role in supplying energy [18, 19]. Previously published works have reported antibacterial activity of fruit, leaf, pit, bark and seeds of date palm (*P. dactylifera L.*) against bacteria [12, 20]. In the present study, we aimed to investigate the activity of HoP of *P. dactylifera L.* against bacteria using aqueous (water), methanolic and acetone extracts. All the three extracts were evaluated for their antibacterial action against the Gram-positive strains *S. aureus*, *S. pyogenes* and *S. mutans* and the Gram-negative strains *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. aerogenes*, *M. morganii* and *P. aeruginosa*.

2. Materials and methods

2.1. Plant collection

The fresh HoP material of *P. dactylifera L.* was collected from Baquba city, Diyala, Iraq. Then it was washed with tap water, and dried in the shade at room temperature. About 1/4 of dried material were cut into small parts and then crushed using the blender for 10 minutes at high speed to be a fine powder for further use and finally stored in sealed bottles for 7 days before used for analyses.

2.2. Preparation of extracts of P. dactylifera L.

2.2.1. Cold crude aqueous extract

Four hundred grams of fresh HoP was added to 1200 milliliters of distilled water, and blended using the blender. The mixture was frozen at (-20 °C), thawed for 3 days with continuous shaking, filtered with a gauze piece and the ultrasonic system was used to break cell walls. Then, it was collected and concentrated to a suspended solution under reduced pressure using the Lypholyzer system [19].

2.2.2. Protein precipitate and nonprotein part of aqueous extract

Forty milliliters of cold acetone were slowly added to 60 ml of crude material of aqueous extract with continuous shaking for 60 minutes, the mixture was then refrigerated for 24 hours at 4 C°. To complete the precipitation process, cool ultracentrifugation 8385 x g for 20 minutes was used to get the precipitate fraction (fraction I), which represent the protein part of extract, and then it was collected and concentrated under reduced pressure using the Lypholyzer system after removing acetone completely, the remainder will be the non-protein fraction (fraction II) [21].

2.2.3. Methanol and acetone extracts

Ten grams of powder were macerated in 100 ml of each solvent (acetone and methanol) in a conical flask, each was thoroughly shaken for 24 hours. When the solvent was slowly evaporated at room temperature, the supernatant was collected [12].

2.3. Preliminary phytochemical screening

Chemical tests were carried out on the powder and its extracts using standard procedures for preliminary phytochemical screening, as follows:

2.3.1. Screening for steroids

Salkowski's test was used to investigate the presence of steroids in HoP extract. The test protocol attained by adding two drops of concentrated H_2SO_4 to 1ml of the tested solution (HoP) gradually on the test tube side. The development of red color confirms the existence of the steroids [22].

2.3.2. Tests for flavonoids

According to the ferric chloride test procedure, described by Louis et al., three drops of neutral ferric chloride ($FeCl_3$) solution was added to 1 ml of test solution (HoP). A blackish red color indicates the presence of flavonoids. The flavonoid screening could be confirmed by alkaline reagent test (ART) as per Reynolds and Safowora [23, 24].

2.3.3. Tests for alkaloids

Wagner's test was used to check the presence of alkaloids. Two drops of reagent (Wangers' reagent) should be added to 1 ml of aqueous extract, then yellow to brown precipitates indicate the presence of the alkaloids [25].

2.3.4. Screening for amino acids and proteins

Biuret and xanthoproteic tests were used in qualitative testing of proteins and amino acids. In biuret test protocol, two to three drops of 1% $CuSO_4$ (copper sulfate solution) were added to 1 ml of 40% NaOH solution till a blue colour has appeared, then 1 ml of extract was added. The presence of protein (and amino acids) is demonstrated when a pink to purple colour raised. The xanthoproteic test was performed by adding 1 ml of concentrated nitric acid (HNO_3) to 1 ml of HoP extract till white precipitates are formed, then the test tube content was boiled for 5 minutes and cooled, after that 20% of NaOH (sodium hydroxide) was added. The xanthoproteic test positive result was achieved by a pale orange color which indicated the presence of aromatic amino acids [26].

2. 3.5. Screening for carbohydrates

The carbohydrate contents were screened by both Fehling's and Benedict's tests. The protocol of Fehling's test was carried out by adding 5 ml of Fehling's A reagent to 5 ml of Fehling's B reagent along with well mixing, then 2 ml of Fehμing's mixture was added to the same volume of crude extract sample and heated to boiling. Brick red deposits appear at the bottom of the test tube to confirm the presence of reducing sugars. In Benedict's test when 2 ml of Benedict's reagent mixed with crude extract sample and boiled, the reddish-brown precipitates denoted the presence of carbohydrates [26, 27].

2.3.6. Tests for glycosides

The Liebermann's test along with Salkowski's test was used to test the presence of glycosides in HoP crude extract. According to Liebermann's protocol, the HoP crude extract is mixed with 2 ml of chloroform ($CHCl_3$), then 2 ml of acetic acid was added and the mixture allowed to be cooled on ice. When the mixture cooled, concentrated H_2SO_4 was added. A color change from purple or blue to green indicates the presence of a steroidal nucleus, i.e., glyconeportion of glycoside. In Salkowski's test, the crude extract sample was added to 2 ml of chloroform, then 2 ml of concentrated H_2SO_4 was added carefully and shaken gently. The reddish-brown color appears to indicate the presence of a steroidal ring [24, 26].

2.3.7. Tests for phenolics and tannins

The ferric chloride test was used to check the presence of phenolics and tannins by adding few drops of (0.1% ferric chloride solution) to 1 ml of extract. A greenish-black or dark-blue color solution demonstrates the presence of phenolic compounds or tannins, while the brown color indicates the presence of pseudo-tannins. The dichromate test was also used to support the ferric chloride test result. The test protocol was carried out by adding 2 ml of 20% $K_2Cr_2O_7$ (aqueous potassium dichromate solution) to 1 ml of the extract tested, a yellow coloured deposit indicate the presence of tannins and phenolics [28].

2.4. Bacteriological assays

2.4.1. Microorganisms

Well-identified bacterial strains by an expert microbiologist in Al-Miqdadiyah General Hospital were used; they included *S. aureus*, *S. pyogenes*, *S. mutans*, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. aerogenes*, *P. aeruginosa*, and *M. morganii*. The *S. mutans* was preserved on brain heart infusion (BHI) by inoculation of 0.01 ml of bacterial suspension in 10 ml bottle containing (BHI + 20% glycerin), incubation at 37 °C overnight, and then it was stored at -20 °C for long term storage. Other microorganisms were stored on nutrient agar slants at 4 °C. All microorganisms were reactivated by streaking on nutrient agar, and blood agar (streptococci) in both aerobic and anaerobic conditions (using the candle jar) at 37 °C for 24 hours prior to screening.

2.4.2. Media preparation

The culture media used in this experiment included nutrient agar medium (NA, 28g/ 1000 ml D.W.), Muller-Hinton agar medium (MHA, 38g/ 1000 ml D.W.), blood agar medium (BA, 40 g/ 1000 ml D.W. + 5% blood), and nutrient broth (NB, 13g/ 1000 ml D.W.) from *Oxoid*™ (*RG24 8PW*, United Kingdom). These media were prepared according to the manufacturers' instructions and used in the reactivation of microorganisms, and antibacterial susceptibility testing.

2.4.3. Antibacterial activity

The Agar well diffusion method was used, since 5 mm diameter holes were made in the MHA media aseptically (*Streptococcus* spp. were tested on MHA supplemented with 5% sheep blood) to assess antibacterial activity [29]. Using a sterile swab, bacterial lawn for each bacterial species was made on 6 Petri-plates containing MHA and allowed away for 5 minutes to dry. After drying, the tested extracts were embedded as (50 µl) into previously made holes in the media; the respective solvents (D.W., acetone AC, and methanol ME) were used in the same volumes as negative controls while the positive control was ceftriaxone CTR (30 µg/ disc). All plates were incubated at 35±2 °C for 24 hours, and then inhibition zones diameter were measured using a transparent scale. Aqueous

extract parts were tested as well as acetone and methanol extract in triplicate (i.e., for each strain, 3 Petri-plates for aqueous extract parts testing and 3 plates for methanolic and acetone extracts).

3. Results and discussion

3.1. Antibacterial activity of aqueous extract

Aqueous extract was divided into crude extract (CR), protein precipitate (PP), non-protein part (NP) and residues (RE, i.e. the remained material after filtration). All parts were examined to determine their activity against tested strains in the presence of negative and positive controls (the water and ceftriaxone respectively). Each part of the extract showed an activity against some of the tested bacteria (Table 1).

3.2. Antibacterial activity of methanolic and acetone extract

Tested strains were also subjected to assess their susceptibility to methanol and acetone extracts, respective solvents were used as negative control and CTR as positive control (Table 2).

Table 1: Antibacterial effect of different parts of aqueous extracts against tested microorganisms using disc diffusion method

Extract	Inhibition zones (mm/ 50 µL)								
	Gram +ve strains				Gram −ve strains				
	S. aureus	*S. pyogenes*	*S. mutans*	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*P. aeroginosa*	*E. aerogenes*	*M. morganii*
DW	–	–	–	–	–	–	–	–	–
CR	7.5±0.21	6.5±0.13	8.7±0.25	8.8±0.47	7.5±0.21	–	11.6±0.10	6.4±0.14	6.6±0.52
RE	–	–	–	–	–	7.4±0.22	–	–	–
NP	–	–	–	–	7.7±0.16	15.3±0.40	8.4±0.18	–	–
PP	7.5±0.26	–	7.4±0.12	6.3±0.38	7.5±0.13	8.07±0.38	–	–	–
CTR	17.4±0.25	21.7±0.42	18.5±0.10	31.0±0.62	28.0±0.82	40.3±0.63	30.3±0.18	29.8±0.35	19.7±0.68

Abbreviations: DW (distilled water), CR (crude), RE (residues, i.e. the part of remained material after filtration), NP (non-protein part), PP (protein part), CTR (ceftriaxone), +ve (positive),−ve (negative).

Table 2: Antibacterial strength of methanolic and acetone extracts against tested species using the disc diffusion method

Extract	Inhibition zone (mm/ 50 µL)								
	Gram +ve strains				Gram −ve strains				
	S. aureus	*S. pyogenes*	*S. mutans*	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*P. aeroginosa*	*E. aerogenes*	*M. morganii*
AC	–	–	–	–	–	–	–	–	–
ACE	7.6±0.21	10.6±0.17	10.7±0.13	8.5±0.11	11.5±0.53	14.4±0.48	10.4±0.36	15.3±0.20	8.6±0.24
ME	–	–	–	–	–	–	–	–	–
MEE	10.6±0.31	9.7±0.21	7.4±0.17	–	8.6±0.23	11.5±0.40	9.2±0.38	15.8±0.14	8.5±0.32
CTR	17.6±0.23	21.4±0.31	19.6±0.07	30.0±0.53	29.1±0.45	38.8±0.48	30.3±0.18	30.6±0.36	20.5±0.56

Abbreviations: AC (acetone, as alone), ACE (acetone extract), ME (methanol, as alone), MEE (methanolic extract) and CTR (ceftriaxone).

3.3. Phytochemical analyses

The results of qualitative phytochemical screening demonstrated the presence of steroids, glycosides, carbohydrates, alkaloids, pseudotannins, and aromatic amino acids (Table 3).

Table 3: The phytochemical screen to investigate the presence/absence of bioactive compounds

Bioactive constituent	Test/s used for investigation	Result
Carbohydrate	Fehling's method	+
	Benedict's method	+
Flavonoids	Ferric chloride method	─
	Alkaline reagent method	─
Alkaloids	Wagner's method	+
Steroids	Salkowski's method	+
Pseudo tannins	Ferric chloride method	+
Tannin and phenolic compounds	Dichromate method	─
	Ferric chloride method	─
Glycosides	Liebermann's method	+
	Salkowski's method	+
Amino acids and proteins	Biuret method	─
	Xanthoproteic method	+

(+) positive = present, (─) negative = absent

In Tables 1 and 2, the results showed a positive activity of all the three tested extracts of HoP of *P. dactylifera L.* against used bacteria. Our findings confirm that the extraction of HoP with water, methanol, and acetone has potential activity against tested bacteria; however, the efficacy of extraction was at the highest level in acetone (Table 1) and at the lowest level with the aqueous extraction (Table 2). Among all tested bacteria, the findings revealed that the Gram negative strain of *E. erogenous* (15.34±0.20 mm) has the highest sensitivity to acetone extract, while the more susceptible Gram-positive specie was *S. mutans* (10.72±0.13 mm). All extracts had less activity than the standard antibiotic ceftriaxone (CTR). The phytochemical analyses conducted on the crude HoP extract showed the presence of steroids, alkaloids, glycosides, pseudotannins, carbohydrates, and aromatic amino acids (Table 3). As methanolic and acetone extracts revealed better results than aqueous ones, this may be due to acetone and methanol ability to withdraw a large number of chemical components from plant material more than the water ability in extraction of such ingredients [30]. In addition, our findings support the previously published reports which suggested that methanol is a good solvent for more stable extraction of antimicrobials derived from medicinal plants when compared to other solvents like hexane and water [31–34].

The HoP potential activity against bacteria may be due to the presence of phytochemicals including steroids, alkaloids, glycosides, pseudotannins, carbohydrates, and aromatic amino acids (Table 3). Steroids are very important compounds, especially due to their association to compounds such as sex hormones; and they have also been reported to have antibacterial properties [35, 36]. Alkaloids have been utilized in pharmaceutical preparation for centuries; their cytotoxicity is one of their common properties. Several researchers have reported the activity of alkaloids as antispasmodic, analgesic and antibacterials [37]. Compounds like tannins, alkaloids, and flavonoids have been described to fight the growth of bacteria and are able to protect against bacterial infections in certain plants [17, 38]. Many published studies report glycosides efficacy in lowering blood pressure (BP) [29]. The outcomes in this study therefore propose that the identified phytochemicals may be the bioactive elements, and these plants are demonstrating to be a valuable reservoir of bioactive constituents of substantial medicinal merit increasingly.

In the current study, findings regarding a lowest inhibition activity against *E. coli* agreed with the findings of the Saudi researchers Al-daihan and Bhat (2012) who reported that the fruit of date extracts revealed the lowest antibacterial activity to inhibit *E. coli* with 7.5, 8, and 9.5 mm zones of inhibition respectively (12). In conclusion, HoP extractions has significant effectiveness against the

bacterial species studied in this work and it could represent a potential source to produce new antibacterial agents. Further studies are recommended to discuss the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), studying the efficacy *in vivo*, and other studies to get a pure spare of antibacterials.

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