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Antimicrobial Activity of Different Parts of *Phoenix dactylifera*

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ABSTRACT

In many parts of the world there is a rich tradition in the use of herbal medicine for the treatment of many infectious diseases. Because of the side effects and the resistance that pathogenic microorganisms build against the antibiotics, much recent attention has been paid to extract the biologically active compounds from plant species used in herbal medicine. In this study different parts of Phoenix dactylifera from Kerman-Bam region were collected. Plant samples were dried in shade and extracted with methanol, chloroform and aqueous by maceration method for 10 days at room temperature. Microorganisms (five gram negative and three gram positive bacteria) were cultured on brain heart infusion agar and antibacterial activity tested by agar well diffusion assay. As a precaution for not missing any trace amounts of antimicrobials, a concentration of 40 mg/ml of each extract was prepared in dimethyl sulfoxide: methanol (1:1 v/v) solvent and administered in each well. Cultured plates were incubated at 35°C. After 48 hours the bioactivity was determined by the measurement of the diameter of inhibition zones (DIZ). Finally MIC and MBC were determined. Some extracts showed antibacterial activity against some bacteria and methalonic extract of palm seed had the most effects. In gram positive bacteria, *Staphylococcus aureus* (PTCC 1112) and in the gram negative bacteria *Escherichia coli* (PTCC 1330) were the most sensitive bacteria. MIC and MBC value were 1.25 and 2.5 mg/ml, respectively. According to the results from this study, it is suggested that different parts of Phoenix dactylifera may be used in the treatment of the infections including gram positive bacteria. Efforts should go on to screen more local flora in different regions, as many investigations have shown that environment is very effective in biological properties in plants.

1. Introduction

There is usually a battle between human and microorganisms causing infections and various diseases. Different factors may cause antibiotic resistance, for example, bacteria can acquire resistance via mutation or through acquisition of resistance factors from other organisms (Tenover, 2006). Acquired resistance factors through transformation, conjugation and transduction may lead to activation of a bacterium to produce enzymes in order to make antibiotics inefficacious. Resistant microbes are capable of spreading and emerging a massive infection. At the present time, regarding spread of antibiotic resistance and background of using plants extract to cure different diseases, researchers try to find those plants with antibiotic effects (Tenover, 2006). Date palm has

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several medicinal effects such as antioxidant and anti-mutagen, and it is rich in minerals in which the potassium is the most. High levels of selenium existed in date preventing from cancer and reinforce function of the immunity system. Moreover, fluoride in date preventing teeth decays. Date fruit contains fourteen types of unsaturated fatty acids such as palmitic, oleic and linoleic acid. Amount of Oleic acid in date is between 41% -58.8%, which means that date is the main source of Oleic acid. Date fruit also contains 6 main vitamins A, C, B1, B2, nicotinic acid and folic acid (Tenover, 2006). Each parts of date have its own special effects and applications e. g. date kernel is capable of adding to the medium of *Streptococcus thermophilus* as a source of nitrogen. Adding different amounts of hydrolyzate of date kernel as the only source of nitrogen caused an increase in *Streptococcus thermophilus* growth despite the inadequacy of nitrogen in the kernel (Tenover, 2006). The palm pollen has four biological compounds. The pollen smoothening the blood circulation through diluting and improves physical and mental ability in senility. The pollen also is rich in fat that releases much energy, prevents from senility diseases and it seems to be useful for elderly joint erosion (Parker and Swanson, 2002). Antimicrobial effects of methanol, aqueous and chloroform extracts of root, leaves, pollen, date kernel and Sago against several Gram-positive and Gram-negative bacteria and *Candida albicans* yeast.

2. Material and methods

2.1. Plant Samples

In early spring, pollination time of palm trees, different parts of Mazafati cultivar of date including root, leaves, date kernel, Sago, pollen and spathe collected in Poshtrood district of Narmashir town in Bam city and then transferred to Research Laboratory of Microbiology in Islamic Azad University of Kerman. The samples were rinsed well with water and then dried at room temperature and shade (Momo et al., 2011).

2.2. Microorganisms tested

Microorganisms studied in this project were provided from Iranian Research Organization for Science and Technology (IROST) which were: *Staphylococcus aureus* (PTCC 1112,1431,1764), *Escherichia coli* (PTCC 1399,1330,1270), *Bacillus cereus* (PTCC 1015), *Shigella dysenteriae* (PTCC 1188), *Salmonella typhi* (PTCC 1609), *Klebsiella pneumonia* (1290), *Serratia marcescens, Candida albicans* (5027).

20 g of each plant sample chopped completely and added to Erlenmeyer flask containing 250 ml of solvent. The solvents that have been used in this study were methanol, chloroform and water. Extraction has been performed by maceration method. The Erlenmeyer flasks that contain the sample and solvent placed in room temperature for seven days and stirred every day. After seven days, the extracts filtered by Whatman Paper No. 1 and they dried and concentrated in vacuum through distillation system. As a result, different concentrations in solvent (DMSO: Methanol 1:1 V / V) achieved and the first concentration used was 40 mg/ml. Then, successive dilutions prepared to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), respectively (Momo et al., 2011). Successive dilutions (20, 10, 5, 5/2 and 25/1 mg/ml) in the solvent (DMSO: Methanol 1:1 V/V) prepared to determine MIC and MBC, respectively (Al-Shahib and Marshall, 2003).

3. Result

As resulted from Table 1, the best and the most effective methanol extracts from *Phoenix dactylifera* were date kernel, spathe, pollen, Sago and root. Date kernel extract has the highest zone of growth inhibition and leaf extract was the only that has no antimicrobial effects. Results of Table 2 which are related to the chloroform extracts, showed that the extracts obtained by chloroform has no antimicrobial effects and in rare cases, e.g. spathe has been shown a weak antibacterial effects. Results of methalonic extracts are very similar to the aqueous ones (Tables 3 and 4).

Table 1. Evaluation of the antimicrobial effects of methanol extracts from different parts of *Phoenix daylifera* against microorganisms in 40mg/ml concentration.

Microorganism	**PTCC No.**	**Root**	**Leaf**	**Pollen**	**Spathe**	**Seed**	**Sago**
Escherichia coli	1399	_	_	_	_	_	_
Escherichia coli	1330	12	_	13	14	20	12
Escherichia coli	1270	_	_	_	_	_	_
Staphylococcus aureus	1112	12	_	14	16	18	12
Staphylococcus aureus	1431	13	_	13	15	17	13
Staphylococcus aureus	1764	13	_	14	13	15	13
Bacillus cereus	1015	13	_	15	17	21	15
Shigella dysenteriae	1188	_	_	_	_	_	_
Salmonella typhi	1609	_	_	_	_	10	_
Klebsiella pneumonia	1290	_	_	_	_	_	_
Serratia marcescens		_	_	_	_	_	_
Candida albicans	5027	_	_	_	_	_	_

Inhibition zone diameter in millimeters on t he numbers show.

Table 2 . Evaluation of the antimicrobial effects of choloroformic extracts from different parts of Phoenix daylifera against microorganisms in a concentration of 40mg/ml.

Microorganisms	**PTCC No.**	**Root**	**Leaf**	**Pollen**	**Spathe**	**Seed**	**Sago**
Escherichia coli	1399	_	_	_	_	_	_
Escherichia coli	1330	_	_	13	15	_	_
Escherichia coli	1270	_	_	_	_	_	_
Staphylococcus aureus	1112	_	12	8	11	_	_
Staphylococcus aureus	1431	_	_	_	_	_	_
Staphylococcus aureus	1764	-	_	_	_	_	_
Bacillus cereus	1015	_	14	_	12	_	_
Shigella dysenteriae	1188	_	_	_	_	_	_
Salmonella typhi	1609	_	_	_	_	_	_
Klebsiella pneumoniae	1290	_	_	_	_	_	_
Serratia marcescens		_	_	_	_	_	_
Candida albicans	5027	_	_	_	_	_	_

Inhibition zone diameter in millimeters on the numbers show.

Table 3. Evaluation of the antimicrobial effects of aqueous extracts from different parts of Phoenix daylifera against microorganisms in 40 mg/ml concentration.

Microorganisms	PTCC No.	Root	Leaf	Pollen	Spathe	Seed	Sago
Escherichia coli	1399	_	_	_	_	_	_
Escherichia coli	1330	12	17	_	-	_	_
Escherichia coli	1270	_	12	_	12	12	10
Staphylococcus aureus	1112	14	14	12	14	17	_
Staphylococcus aureus	1431	_	15	_	_	_	_
Staphylococcus aureus	1764	-	14	_	_	_	_
Bacillus cereus	1015	12	15	12	10	10	13
Shigella dysenteriae	1188	_	_	_	_	_	_
Salmonella typhi	1609	_	_	_	_	_	_
Klebsiella pneumoniae	1290	_	_	_	_	_	_
Serratia marcescens		_	_	_	_	_	_
Candida albicans	5027	_	_	_	_	_	_

Inhibition zone diameter in millimeters on the numbers show.

Table 4. Determination of MIC and MBC value (mg/ml)

Sample / Bacteria	*E. coli* (1330)	*S. aureus* (1112)	*S. aureus* (1431)	*S. aureus* (1764)	*B. cereus* (1015)
Root	MIC:1.25	MIC:1.25	MIC:20	MIC:10	MIC:1.25
	MBC:5	MBC:2.5	MBC:40	MBC:20	MBC:20
Pollen	MIC:1.25	MIC:1.25	MIC:2.5	MIC:1.25	MIC:1.25
	MBC:2.5	MBC:1.25	MBC:5	MBC:10	MBC:1.25
Spathe	MIC:2.5	MIC:1.25	MIC:2.5	MIC:10	MIC:2.5
	MBC:2.5	MBC:10	MBC:10	MBC:20	MBC:20
Seed	MIC:1.25	MIC:1.25	MIC:1.25	MIC:2.5	MIC:1.25
	MBC:2.5	MBC:2.5	MBC:2.5	MBC:5	MBC:2.5
Sago	MIC:1.25	MIC:1.25	MIC:1.25	MIC:5	MIC:1.25
	MBC:2.5	MBC2.5	MBC:2.5	MBC:10	MBC:2.5

4. Discussion

It has been shown that date kernel extract is the most effective on the bacteria and sensitive Gram-negative bacterium is *Escherichi coli* (PTCC 1330) and the most sensitive Gram-positive bacterium is *Bacillus cereus* (PTCC 1015). Also, the most resistant Gram-negative bacteria are *Shigella* dysenteriae PTCC 1188, *Salmonella Typhi* PTCC 1609, *Klebsiella pneumoniae*, and the most resistant Gram-positive bacteria are *Staphylococcus aureus* PTCC 1290, PTCC 1764 and PTCC 1431. *Candida albicans* fungus was the most resistant among all other extracts of the palm plant. Therefore, it was shown that these extracts do not have antifungal effects. Table 4 also shows the MIC and MBC values of active extracts. Due to very low and effective concentrations of the extracts, date kernel and Sago extracts are appropriate subjects for more extensive antimicrobial researches. We have shown that microbial ingredient of the different parts of *Phoenix dactylifera* is polar that entered into water

and methanol solvents. According to the results from this study, it is suggested that different parts of *Phoenix dactylifera* may be used in the treatment of the infections including gram positive bacteria.

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