African Journal of Biotechnology Vol. 11(42), pp. 10021-10025, 24 May, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.4309
ISSN 1684-5315 © 2012 Academic Journals

Full Length Research Paper

Antibacterial activities of extracts of leaf, fruit, seed and bark of *Phoenix dactylifera*

Sooad Al-daihan and Ramesa Shafi Bhat*

Biochemistry Department, College of Science, King Saud University, P. O. Box 22452, Riyadh 11495, Saudi Arabia.

Accepted 8 May, 2012

The antibacterial activities of different parts of local *Phoenix dactylifera* were investigated *in vitro*. Dried leaf, fruit, seed and tree bark were extracted with water, methanol and acetone. Antibacterial property of the extracts was evaluated against *Staphylococcus aureus, Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* using the disc diffusion method. Overall analysis of the antibacterial activity of various extracts revealed that the highest inhibitory activity was produced by the fruit extract (18.2 ± 0.55 mm) as compared to the leaf, bark and seed extracts. All the extracts from the different parts of the plant showed antibacterial activity against most tested microorganisms. On the whole, aqueous extracts have the least antibacterial activity as compared to methanol and acetone extracts. The antibacterial activity against the Gram-positive strains was the highest in the acetone fruit extract against *S. aureus* (18.2 ± 0.55 mm). The most active extract against Gram-negative bacteria was methanol extract from the leaves with a 13.5 ± 0.33 mm inhibition zone for *E. coli* followed by 12.5 ± 0.88 mm for *P. aeruginosa.* Phytochemical analysis revealed the presence of carbohydrates and alkaloids in all parts, and flavonoids, steroids, saponins and tannins were present in some parts.

Key words: Antibacterial activity, *Phoenix dactylifera*, disc diffusion assay, extracts, inhibition zone.

INTRODUCTION

Infectious diseases account for high proportion of health problems in the developing countries (Sashi et al., 2003). 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 resources (Mothana and Lindequist, 2005). Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava et al., 1996). 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, 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 industries (Uniyal et al., 2006). Although, hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated (Balandrin et al., 1985). 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 care (Sujatha, 2005). About 42% of 25 top selling drugs marketed worldwide are either directly obtained from natural sources or entities derived from plant products (Ramya et al., 2008).

The date palm (*Phoenix dactylifera*) is a monocotyledonous woody perennial fruit species belonging to the Arecaceae family (McClintock, 2007). The beneficial health and nutritional values of date palm for human and animal consumption have been claimed

*Corresponding author. E-mail: ramesa.aftab@gmail.com or rbhat@ksu.edu.sa. Tel: +96614785968. Ext: 1204.

for centuries (Barreveld, 2007). The fruit of the date palm contains tannin, which makes it an effective astringent. Dates have been used as a detersive and astringent in intestinal troubles, treatment for sore throats, colds, bronchial catarrh, fevers, gonorrhea, edema liver and abdominal troubles, and to counteract alcohol intoxication (Barh and Mazumdar, 2008). 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 disorders (Nadkarni, 1976). The seeds from the tree have been ground into a paste that is effective in treating ague (Morton 1987). The roots are used to treat toothache. The gum extracted from the trunk has been effectively used to treat diarrhea and urinary ailments (Morton, 1987). Dates are an excellent source of vitamin C and dietary fibers. They also supply vitamin A and B, different minerals and various amino acids. The antioxidants present in dates can also aid in lowering the risk of cancer and cardiovascular conditions, while ensuring a healthy immune system.

In the current study, we screened the antibacterial activity of different parts of *P. dactylifera* using aqueous, methanol and acetone extracts. Extracts of the leaf, fruit, seeds and bark were studied for their antibacterial effect against the Gram positive strains *Staphylococcus aureus* and *Streptococcus pyogenes* and the Gram negative strains *Escherichia coli* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Collection of plant material

Mosaifah cultivar of *P. dactylifera* (fresh leaves, fruits, bark and pits) were collected from Riyadh during July to August 2011. Collected material was washed thoroughly in running tap water, rinsed in distilled water and shade dried for one week in open air, crushed using mortar and pestle, reduced to powder using waring laboratory blender (MX-7011G) for 5 min at high speed and then stored in airtight closed bottles for two days before used for analysis.

Microorganisms

Reference bacteria strains were obtained from Botany Department of King Saud University, and they included *S. aureus, S. pyogenes*, *E. coli* and *P. aeruginosa.* The strains were maintained on agar slant at 4°C and activated at 37°C for 24 h on nutrient agar (Sigma-Aldrich, Germany containing 15 g/L agar, 1 g/L meat extract, 5 g/L peptone, 5 g/L sodium chloride and 2 g/L yeast extract) prior to any screening.

Phytochemical analysis

Phytochemical analysis of the crude powder of the leaves, fruits, bark and pits collected was determined as follows:

Molisch's test for carbohydrates

0.5 g of each powder was dissolved separately in 5 ml of distilled water and filtered. Few drops of Molisch's reagent were added to each solution, this was then followed by addition of 1 ml of concentrated H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers was taken as positive test (Sofowora, 1993).

Test for alkaloids

0.1 g of each powder was dissolved in 5 ml of methanol separately and the filtered. 2 ml of each filtrate from each sample were stirred with 5 ml of 1% aqueous HCl on water bath and then filtered. From the filtrate, 1 ml was taken individually into two test tubes. To the first portion, few drops of Dragendorff's reagent were added; occurrence of orange-red precipitate was taken as positive. To the second 1 ml, Mayer's reagent was added and appearance of buff-colored precipitate was taken as positive test for the presence of alkaloids (Sofowora, 1993).

Liebermann-Burchard test for steroids

0.2 g of crude powder of each sample was dissolved in 2 ml of acetic acid separately; the solutions were cooled well in ice followed by the addition of concentrated H_2SO_4 carefully. Color development from violet to blue or bluish-green indicated the presence of a steroidal ring (Sofowora, 1993).

Test for saponins

1 g of crude powder of each sample was boiled with 5 ml of distilled water separately and then filtered. To each filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 min. Frothing which persisted on warming was taken as an evidence for the presence of saponins (Sofowora, 1993).

Shinoda's test for flavonoids

About 0.5 g of each powder was dissolved in 5 ml of ethanol separately, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of concentrated HCl. A pink, orange or red to purple colouration indicates the presence of flavonoids (Trease and Evans, 2002).

Test for tannins

About 0.5 g of each portion of crude powder was stirred with about 10 ml of distilled water separately and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of each filtrate and occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins (Trease and Evans, 2002).

Extraction of plant material

Aqueous extraction

10 g of air-dried powder was added to 100 ml of distilled water and boiled on slow heat for 2 h. The mixture was filtered through eight layers of muslin cloth and centrifuged at 5000 g for 10 min. The supernatant was collected. This procedure was repeated; supernatants collected at 2-h intervals were pooled and concentrated to a final volume of one-fourth of the original volume of solvent used (which was 100 ml) (Harbone, 1973). It was then

Table 1. Antibacterial activity of various extracts of different parts of date palm (*Phoenix dactylifera*) against bacterial species tested by disc diffusion assay.

Plant extract	Zone of inhibition (mm)			
	Gram positive strain		Gram negative strain	
	S. aureus	*S. pyogenes*	*E. coli*	*P. aeruginosa*
Leaf				
AQ	10.5 ± 0.57	11.5 ± 0.58	12.5 ± 0.00	10.5 ± 0.79
ME	12 ± 0.00	12 ± 0.00	13.5 ± 0.33	12.5 ± 0.88
AC	13.2 ± 0.20	11 ± 0.5	12.2 ± 0.20	12.00 ± 00
Fruit				
AQ	12 ± 0.88	11 ± 0.00	11 ± 0.88	11 ± 0.57
ME	16 ± 0.20	13 ± 0.15	11 ± 0.00	12 ± 0.66
AC	18.2 ± 0.55	12± 00	10.5 ± 0.00	12 ± 0.55
Pit				
AQ	10 ± 0.00	8 ± 0.63	9 ± 0.22	9 ± 1.15
ME	11 ± 0.89	10 ± 0.19	11.5 ± 0.00	11.5 ± 0.66
AC	11.60 ± 0.88	9.00± 0.20	11 ± 0.50	11 ± 00
Bark				
AQ	9 ± 0.16	9 ± 0.86	10 ± 0.57	9± 0.88
ME	10 ± 0.33	10 ± 0.55	11 ± 0.66	10 ± 0.33
AC	11 ± 0.55	9.5± 0.80	10 ± 0.80	10 .5± 00
Kanamycin (30 µg/disc)	26.5 ± 0.33	28 ± 0.57	20 ± 0.33	25 ± 0.10

Values are mean inhibition zone (mm) ± S.D of three replicates. AQ, Aqueous; ME, methanol; AC, acetone.

autoclaved at 121°C and at 15 lbs pressure and stored at 4°C.

Methanol and acetone extraction

10 g of air-dried powder was added to 100 ml of methanol or 100 ml of acetone separately for methanol and acetone extract at the same time in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190 to 220 rpm for 24 h. The supernatant was collected and the solvent was slowly evaporated in wide mouthed evaporating bowls at room temperature for two to three days to make the final volume of one fourth of the original volume of solvent used (which was 100 ml) (Harbone, 1973) and stored at 4°C in airtight bottles.

Media preparation and antibacterial activity

The antibacterial assay was performed using paper disc diffusion method (Lai et al., 2010). Using a sterile cotton swab, the nutrient broth (Sigma-Aldrich, Germany; containing 1 g/L D(+)-glucose, 15 g/L peptone, 6 g/L sodium chloride, 3 g/L yeast extract) cultures were swabbed on the surface of sterile nutrient agar (Sigma-Aldrich, Germany; containing 15 g/L agar, 1 g/L meat extract, 5 g/L peptone, 5 g/L sodium chloride, 2 g/L yeast extract) plates and allowed to dry for 5 min. Sterile filter paper discs (5 mm in diameter) impregnated with different test extracts (100 µl disc) were then placed on the surface of inoculated agar plates. Kanamycin (30 µg/disc) was used as positive control. Respective solvents were used as the negative control. The plates were then incubated at 37°C for 24 h after which microbial growth was determined by measuring the diameter of the inhibition zone (mm) using a transparent scale. Each extract was analyzed in triplicate, the mean values are presented. Kanamycin disc (30 µg/disc) was used for comparing the bioassay.

RESULTS AND DISCUSSION

All the date palm extracts tested including leaf, fruit, seeds and bark showed antibacterial activity (Table 1). Results obtained in the present study show that plants extract possesses potential antibacterial activity against the tested organisms; however, methanol and acetone extracts was found to be more effective antimicrobial agents than the aqueous extracts (Table 1). The fruit and leaves extract showed better antibacterial activity than seed and bark extracts. In fact, high antibacterial activity against all test organisms was observed with acetone fruit extract against *S. aureus* (18.2 ± 0.55 mm). Leaf extract showed highest activity against *E. coli* in response to methanol extract (13.5 ± 0.33 mm). Seeds and bark extracts showed almost similar antibacterial activity

Table 2. Phytochemical analysis of different parts of date palm.

S/N	Constituent	Leaf	Fruit	Seed	Bark
1	Carbohydrates	+	+	+	+
2	Alkaloids	+	+	+	+
3	Steroids	+	+	+	-
4	Saponins	-	+	-	-
5	Flavonoids	-	+	-	+
6	Tannins	+	+	-	+

+, Present; -, absent.

against all the tested bacteria. All the extracts were found to be less effective than the standard antibiotic kanamycin.

The results of the phytochemical screening of crude powder of leaf, fruit, seeds and bark of date palm are presented in Table 2. Phytochemical analysis revealed the presence of carbohydrates and alkaloids in all the parts of the plant. The other secondary metabolites like flavonoids, steroids, saponins and tannins were present in some of the parts (Table 2).

The present study shows that date palm leaf and fruit extracts were effective inhibitors of bacteria growth than pits and bark extracts. The methanol and acetone extracts were more effective against all test bacteria than the aqueous extracts. This may be due to the ability of methanol and acetone to extract a wide range of chemical constituents of plant material, while water extracted less number of ingredients (Cowan, 1999). These results confirm the report of previous studies that methanol is a better solvent for more consistent extraction of antimicrobial substances from medical plants as compared to other solvents such as water and hexane (Ahmad et al., 1998; Eloff, 1998; Lin et al., 1999, Karaman et al., 2003).

Among the different extracts of date palm, fruits extract showed maximum activity. This may be due to the presences of carbohydrates, alkaloids, steroids, saponins, flavonoids and tannins in the fruit parts (Table 2). Tannins are known for their astringent property and antimicrobial activity (Cowan 1999). Phytochemically, the date palm contains carbohydrates, phenolic compounds, alkaloids, steroids, flavonoids, vitamins and tannins (Biglari et al., 2008). The phenolic profile of the plant revealed presence of mainly cinnamic acids, flavonoid glycosides, flavanols, four free phenolic acids and nine bound phenolic acids (Dowson, 1982; Mosa et al., 1986; Ziouti et al., 1996; Eong et al., 2006; Biglari et al., 2008). Phenol is well known as a chemical antiseptic. The studies of Cheesbrough (1984) and El-Shanawny (1996) indicated that phenolic compounds cause inhibition of a wide range of microorganisms. Compounds like alkaloids, flavonoids and tannins have been reported to inhibit bacteria growth and are capable of protecting certain plants against bacterial infection (Clark, 1981; Mather and Gonzalez, 1982). In the present study, date fruit extracts showed a lower antibacterial activity against *E. coli* which is supported by the results of Ayachi et al. (2009) who found that dates fruits extracts showed a lower antibacterial activity against *E. coli* with respective means (7.5, 8 and 9.5 mm of inhibiting diameter for the three variety of dates). In our results, crude extract of pits checked the growth of all Gram negative bacteria which is in agreement with the report from the previous study by Saddiq and Bawazir (2010) as they reported antibacterial activity from aqueous extract of date palm pit against Gram negative bacteria (*K. pneumonia* and *E. coli).*

Conclusion

Based on our results, it can be concluded that leaf, fruit, seeds and bark of date palm possess 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. Further research is needed for the isolation and identification of active principles present in the extracts which could possibly be exploited for pharmaceutical use.

ACKNOWLEDGEMENT

We extend our appreciation to Deanship of Scientific Research, King Saud University for funding the work through the research group project no RGP-VPP-063.

REFERENCES

Ahmad I, Mehmood Z, Mohammad F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol. 62: 183–193.

Ayachi A, Alloui N, Bennoune O, Yakhlef G, Daas Amiour S, Bouzid W, Djemai Zoughlache S, Boudjellal K, Abdessemed H (2009). Antibacterial activity of Some Fruits; Berries and Medicinal Herb Extracts Against Poultry Strains of Salmonella American-Eurasian J. Agric. Environ. Sci. 6(1): 12-15.

Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH (1985). Natural plant chemicals: Sources of industrial and medicinal materials. Sci. 228: 1154-1160.

Barh D, Mazumdar BC (2008) .Comparative nutritive values of palm saps before and after their partial fermentation and effective use of wild date *(Phoenix sylvestris* Roxb *)* sap in treatment of anemia. Res. J. Med. Med. Sci. 3: 173-176

Barreveld WH (2007). Date Palm Products. FAO Agricultural Services Bulletin No. 101. (http://www.fao.org/docrep/t0681E/t0681e00. htm#con).

Biglari F, Abbas FM, AlKarkhi, Azhar ME (2008). Antioxidant activity and phenolic content of various d ate palm (*Phoenix dactylifera*) fruits from Iran. Food Chem., 107: 1636–1641.

Cheesbrough M (1984). Tropical Health Technology, Cambridge university press U.K. 2:2-392.

Clark WS (1981). Antimicrobial Activities of pherolic consistuents of *Mangolia gradiflora*, L. J. Pharm. Sci. 10: 951.

Cowan MM (1999).Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12: 564-82.

Dowson VHW (1982). Date production and protection. FAO plant production and protection. Food and Agriculture Organization of the United Nations, p. 35.

Eloff JN (1998). Which extract should be used for the screening and isolation ofantimicrobial components from plants? J. Ethnopharmacolol. 60: 1–8.

El- Shanawny MAA (1996). Medicinal plants used in Saudi traditional Medicine, King Abdul- Aziz City for Science and Technology, Riyadh. Pp. 277.

Eong YJ, Hong FA, Tomas-Barberan, Adel A, Kader S, Alyson E (2006). The flavonoid glycosides and procyanidin composition of Deglet Noor dates (*Phoenix dactylifera*). J. Agric. Food Chem. 54: 2405-2411.

Harbone JB. (1973) Phytochemical Methods. London: Chapman and Hill. Pp. 49-188.

Karaman I, Şahin F, Güllüce M, Öğütçü H, Şengül M, Adigüzel A *(*2003*)* .Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J. Ethnopharmacol. 85*:* 231–235*.*

Lai HY, Lim YY, Kim KH (2010). *Blechnum Orientale* Linn - a fern with potential as antioxidant, anticancer and antibacterial agent. BMC Comple. Alt. Med. 10: 15.

Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE , Jager AK, van Staden J (1999). Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. J. Ethnopharmacol. 68: 267–274.

Mather S, Gonzalezi L (1982). Identification of Terpenoids from leaves of *Piptocarpha perctoca* and their biological activitie. J. Nat. Prod. 45: 495-496.

McClintock E (2007) Arecaceae palm family. The Jepson Manual: http:ucjeps.berkeley.edu/cgi-bin/get_JM_treatment.pl?Phoenix+dactylifera

Morton J (1987). Date. In: Fruits of warm climates. Julia F. Morton, Miami, FL, pp. 5–11.

Mothana RA, Lindequist U (2005) Antimicrobial activity of some medicinal plants of the island Soqotra. J. Ethnopharmacol. 96(1-2): 177-181.

Mosa JS, Hifnawy MS, Mekkawi AG (1986). Phytochemical and biological investigations on date palm seeds (*Phoenix dactylifera* L.) produced in Saudi Arabia Arab. Gulf J. Sci. Res. 4: 495-507.

Nadkarni KM (1976) (Ed). Indian Materia Medica Vol 1. Mumbai: Bombay popular prakashan Pvt. Ltd.

Ramya S, Govindaraji V, Kannan NK, Jayakumararaj R (2008). In Vitro Evaluation of Antibacterial Activity Using Crude Extracts of *Catharanthus roseus* L. (G.) Don. Ethnobotanical Leaflets 12: 1013-1018.

Sashi KJ, Ramya M, Janardhan K (2003). Antimicrobial activity of ethnomedicinal plants of Nilgiri Biosphere reserve and Western Ghats. Asian J. Microbiol. Biotechnol. Environ. Sci. 5: 183- 185

Saddiq AA and Bawazir AE (2010). Antimicrobial activity of date palm (*Phoenix dactylifera)* pits extracts and its role in reducing the side effect of methyl prednisolone on some neurotransmitter content in the brain, hormone testosterone in adulthood. Acta Hort. (ISHS) 882: 665-690.

Sofowora A (1993).Screening Plants for Bioactive Agents. In: Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria, pp. 134-156.

Srivastava J, Lambert J, Vietmeyer N (1996). Medicinal plants: An expanding role in development. World Bank Technical Paper. No. 320.

Sujatha S (2005).Complementary and alternative therapies in palliative care: a transition from modern medicine to traditional medicine in India. J. Cancer Pain and Symptom Palliation 1: 25-9.

Trease GE, Evans WC (2002). Pharmacognosy. 15th Ed. Saunders Publishers, London. pp. 42-44, 221-229, 246-249, 304-306, 331-332, 391-393.

Uniyal SK. Singh KN, Jamwal P, Lal B (2006). Traditional use of medicinal plants among the tribal. communities of Chhota Bhangal, Western Himalayan J. Ethnobiol. Ethnomed., 2: 1-14.

Ziouti AC, Modafar EL, Fleuriet AS, Boustani EL, Macheix JJ (1996). Phenolic compounds in date palm cultivars sensitive and resistant to *Fus arium oxysporum*. Biologia. Plantarum. 38: 451-457.