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FULL LENGTH ARTICLE

Health-benefits of date fruits produced in Saudi Arabia based on in vitro antioxidant, anti-inflammatory and human tumor cell proliferation inhibitory assays

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Abstract Date fruits are reported to exhibit health-beneficial effects in addition to its nutritional value. Fruits collected from commercial date palm trees were sequentially extracted with water and methanol. All varieties of date fruits contained sugars, phenolics, triterpenoids, triglycerides, fatty acids and steroids, where sugars were the predominant components. Water and methanolic extracts of date fruits were assayed for antioxidant, antiinflammatory and human tumor cell proliferation inhibitory activities. In MTT antioxidant assay, methanolic extracts at 250 μg/mL exhibited moderate activity with absorbance values between 0.14 and 0.41. Water and methanolic extracts at 100 μg/mL inhibited lipid peroxidation (LPO) by 50–67% and 58–82%, respectively. In anti-inflammatory assay using cyclooxygenase enzymes (COX-1 and -2), water and methanolic extracts at 100 μg/mL showed COX-1 enzyme inhibition by 26–36% and 33–41%, and COX-2 by 45–48% and 48–52%, respectively. At 100 μg/mL concentrations, methanolic extracts of all date fruits showed marginal cell proliferation inhibitory activity against human gastric, prostate, colon, breast and lung tumor cell lines. The bioassay results suggest that varietal difference is not a

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significant factor among the 29 date fruits studied when compared for health-beneficial effects resulting from non-nutritional components present in it.

1. Introduction

Date palm *Phoenix dactylifera* L. (Palmaceae) is grown under arid conditions primarily in the Middle East, North Africa and United States. Its fruit is an important food and dietary component in many countries. Dates are implicated to possess medicinal properties in addition to its nutritional value (Vayalil, 2012). Several studies have reported date fruit with a wide range of bioactivities, such as antioxidant activity due to the presence of phenolics, carotenoids and anthocyanins in it (Mohammed and Al-Okbi, 2004; Saleh et al., 2011; Vayalil, 2002), antimutagenic (Vayalil, 2002), anti-inflammatory (Mohammed and Al-Okbi, 2004), antihyperlipidemic (Tang et al., 2013), antibacterial (Sallal and Ashkenani, 1989) and antifungal (Sallal et al., 1996) activities.

Over 450 date palm varieties or cultivars are grown in the Kingdom of Saudi Arabia and yield more than 1 million metric tons of date fruits accounting for about 14% of the total world production (FAOSTAT, 2014). We had earlier reported the functional food components in Ajwa date fruit, the most expensive date fruit in the market (Zhang et al., 2013). In this study, we determined the health-benefits of 29 significant varieties of date fruits, Barni Al Madinah, Hulwa, Khashram, Khodry, Khalas, Deglet Noor, Dekhaini, Rabeaa, Rushodia, Ruthana, Ruthana Al Sharag, Sabaka, Sukkari Al Qassim, Sullaj, Shalabi, Shaishee, Safawi, Sefri, Segae, Ajwa, Anbara, Luban, Mabroom, Majhool, Mutwah, Meneifi, Nabtat Ali, Naboot Seif and Hilali using in vitro bioassays. The antioxidant activity was evaluated using MTT [3-(4, 5-dimethylthia zole-2-yl)-2,5-diphenyltetrazolium bromide] (Liu and Nair, 2010) and lipid peroxidation (LPO) assays (Bowen-Forbes et al., 2009; Liu and Nair, 2012; Zhang et al., 2013). Using cyclooxygenase enzymes (COX-1 and -2), we determined the anti-inflammatory activity of the date varieties studied (Bowen-Forbes et al., 2009; Liu and Nair, 2012; Zhang et al., 2013). In addition, human tumor cell lines, AGS (gastric), DU-145 and LNCaP (prostate), HCT-116 (colon), MCF-7 (breast) and NCI-H460 (lung) were employed to evaluate the cell proliferation inhibitory activity of water and methanolic extracts of all date fruits (Bowen-Forbes et al., 2009; Liu and Nair, 2012).

2. Material and methods

2.1. General experimental procedures

All solvents used were of ACS reagent grade (Sigma–Aldrich Chemical Company, St. Louis, MO, United States). 250 μm silica gel plates (Analtech, Inc., Newark, DE, United States) were used for thin-layer chromatography (TLC). TLC plates were viewed under UV (ultraviolet) light at 254 and 366 nm in Spectroline CX-20 ultraviolet fluorescence analysis cabinet (Spectroline Corporation, Westbury, NY, United States), and sprayed with 10% sulfuric acid solution. MTT [3-(4, 5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide], *tert*-butylhydroquinone (TBHQ), butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), Adriamycin, aspirin, naproxen and ibuprofen were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, United States). Similarly, the nonsteroidal anti-inflammatory drug (NSAIDs) Celebrex® was physician's professional sample provided by Dr. Subhash Gupta, Sparrow Pain Center, Sparrow Hospital, Lansing, Michigan. COX-1 and -2 enzymes were prepared in our laboratory from ram seminal vesicles (Oxford Biomedical Research, Inc., Rochester Hills, MI, United States) and insect cells cloned with human PGHS-2 enzyme, respectively. Arachidonic acid was purchased from Oxford Biomedical Research, Inc. (Rochester Hills, MI, United States). 1-Stearoyl-2-lino leoyl-sn-glycero-3-phosphocholine (SLPC) was purchased from Avanti Polar Lipids (Alabaster, AL, United States). The fluorescent probe, 3-(p-(6-phenyl)-1,3,5-hexatrienyl) phenylpropionic acid was purchased from Molecular Probes (Eugene, OR, United States). Fetal bovine serum (FBS) and Roswell Park Memorial Institute 1640 (RPMI-1640) medium were purchased from Gibco BRL (Grand Island, NY, United States). Human tumor cell lines DU-145 and LNCaP (prostate), MCF-7 (breast) and NCI-H460 (lung) were purchased from the National Cancer Institute (NCI, Bethesda, MD, United States). AGS (gastric) and HCT-116 (colon) were purchased from American Type Culture Collection (ATCC, Rockville, MD, United States). All cell lines, enzymes and reagents were stored in the Bioactive Natural Products and Phytoceuticals Laboratory at Michigan State University (East Lansing, MI, United States). Centrifugation was carried out on a Sorvall RC-5C refrigerated high-speed centrifuge (DuPont, Newtown, CT, United States). MTT antioxidant assay plates were read on Bio-Tek Elx800 universal microplate reader (Bio-Tek Instruments, Inc., Winooski, VT, United States). The fluorescent reading in the LPO assay was carried out on a Turner model 450 Fluorometer (Barnstead/Thermolyne Corporation, Dubuque, IA, United States). COX assays were performed in Instech micro oxygen chamber using an oxygen electrode (Instech Laboratories, Plymouth Meeting, PA, United States) attached to a YSI model 5300 biological oxygen monitor (Yellow Springs Instrument, Inc., Yellow Springs, OH, United States).

2.2. Date fruits

The 29 varieties of date fruit samples were collected from commercial farms in Saudi Arabia. The varietal identity of the fruit collected was determined based on the information provided by the farmer and by using the database kept in the Ministry of Agriculture, Saudi Arabia (Table 1) (Anonymous, 2006). Date fruits were packaged and shipped to Michigan State University and kept in −20 °C till analyses.

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