Identification Phenolic and Biological Activities of Methanolic Extract of Date Palm Pollen (Phoenix dactylifera)

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Abstract

Pollens of the date palm (DPP) have been used for long time as a traditional Egyptian herbal medicine for improving male and female fertility. The chemical investigation of date pollen resulted from polar solvents. Six compounds which were identified as Caffeic Acid, Gallic Acid, Coumaric Acid, Chlorogenic Acid, Catchine and Quercetin. Also in this work, the impact of polar extract on biological activity was done against six species of pathogenic bacteria, it has a strong value at 22 mm inhibition zone with Staphylococcus Epidermidis. Antifungal activity was done against two species of pathogenic fungi, the extract and ketoconazole have the value with the two species. Finally, the extract was tested against three human cell lines, and the results showed that it has cytotoxic activity against all cell lines.

Keywords: Phoenix dactylifera, Antibacterial; Antifungal; Cytotoxic activity

Introduction

Date palm (Phoenix dactylifera L., Palmae) is native to the Middle East region over centuries ago [1]. In Folkloric practice, date represents an essential meal in some Arab area Miller et al., [2] and Al-Qarawi et al., [3]. The date palm (Phoenix dactylifera) is dioeciously, medium-sized tree with pinnate leaves containing about 150 leaflets having spines on the petiole. It consists of small yellowish flowers attached directly to the spikelet’s which develop into fruits [4]. There has been a tremendous interest in this plant as evidenced by the voluminous work in last few decades. Therefore, we aimed to compile an up to date of Phoenix dactylifera that covers its traditional and folk medicine uses, phytochemistry and pharmacology including various preclinical and clinical studies [5]. Phytochemically the whole pollen contains carbohydrates, alkaloids, steroids, flavonoids, vitamins and tannins. The phenolic profile of the plant revealed the presence of mainly cinnamic acids (ferulic, sinapic and coumaric acid and their derivatives, such as 5-caffeoylshikimic acid also called as dactyliferic acid), flavonoid glycosides (luteolin, methyl, luteolin, quercetin, and methyl quercetin), flavonoids (catechin, epicatechin) Four free phenolic acids (protocatechuic acid, vanillic acid, syringic acid, and ferulic acid) and nine bound phenolic acids (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferrulic acid, and o-coumaric acid) were tentatively identified [5]. Also another study evaluated the protective effects of Phoenix dactylifera pollen grains on liver and heart arteries in male rats fed with diet supplemented with different concentrations of date palm pollen grains (0.0%, 2.0% and 4.0%) [6]. The aim of our research is to identify the phenolic compounds found in methanolic extract and also study some of the biological effects of the extraction some types of bacteria, fungi, and three types of human cancers.

Materials and Methods

Collection of plant material

Date Palm pollen was collected in March (2012) from Sharkya Governate, Egypt. Some explants of palm pollen (Phoenix dactylifera) were collected and kept in a refrigerator at 4°C and were identified by botany department, Faculty of Science, Zagazig University.

Material for Antimicrobials

The bacterial and fungal strains were primarily obtained from the microbiology Lab, Botany Department, Faculty of Science, Zagazig University. Bacterial species tested were (Escherichia Coli, Klebsiella, Staphylococcus Epidermidis, Bacillus Cereus, Micrococcus Luteus, and Staphylococcus Aureus). And fungal species were (Candida Albicans and Aspergillus Niger) [7].

Materials for cytotoxic activity

Human tumor cell lines: [HELA (Cervical carcinoma cell line), MCF7 (breast carcinoma cell line) and CACO (intestinal carcinoma cell line)] [8].

Chromatography

Paper Chromatography

Sheets of Whatman paper No 1 or 3 MM were used for two-dimensional, comparative or preparative paper chromatography[9].

Column Chromatography

The separation of the phenolic and flavonoid components was performed by column fractionation of the extract or its fractions on one of the following stationary phases as stated in each case. A-Polyamide powder, polyamide 6-S for CC, Riedel-De Haen AG, seele-Hannover, Germany. B-Sephadex LH-20, (25-100 μm), Pharmacia fine chemicals.

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Physical Test

UV-Spectroscopic analysis

Investigated material and UV measurements were then carried out [10].

Ultra-violet Spectrophotometric analysis

Chemically pure materials dissolved in analytically pure methanol were subjected to UV spectrophotometric investigation in 4 ml capacity quartz cells Zeiss spectrometer PMQ-II. In case of flavonoids, AlCl₃, AlCl₃/HCl, fused NaOAc/H₂BO₃ and NaOMe reagents were separately added to methanolic solution of the investigated material and UV measurements were then carried out.

NMR Spectroscopic analysis

1H chemical shifts(δ) were measured in ppm, relative to dmsod-δ6 and converted to TMS scale.

Nuclear magnetic resonance spectroscopic analysis

JeolEc 500 MHz NMR Spectrometer at 500 MHz, (Institute Fur Chemie, Humboldt Universität zu Berlin, Germany). 1H chemical shifts were measured in ppm, relative to TMS and 13C NMR chemical shifts to DMSO-d6 and converted to TMS scale by adding 39.5. Typical conditions: spectral width=8 KHz for 1H and 30 KHz for 13C, 64 K data points and a flip angle of 45°C.

Mass spectroscopic analysis

The isolation pure compounds were subjected (FAB–MS).

Mass spectrometric analysis

The isolated pure compounds were subjected, in most cases to Fast Atom Bombardment (positive and negative) mass spectroscopic analysis (FAB–MS) on MM 7070 E spectrometer (VG analytical). Some other compounds were subjected to electron spray ionization mass spectroscopic analysis (ESI–MS) a Varian Mat1 12-ET Spectrometer. All measurements were carried out at Institute Fur Chemie, Humboldt Universität zu Berlin, Germany.

Methods

Preparation of extracts

The yellowish powder pollen was extracted exhaustively with petroleum ether, diethyl ether and methanol (according to polarity). For each extraction the powder was left 24 hrs, in a Soxhlet apparatus. After that the methanolic extract was concentrated under vacuum for several times led to the separation of six pure phenolic compounds. The structure of these compounds was confirmed by comparison of their physical and spectral data with those of reported compounds [10].

Methods of antibacterial activity

The antibacterial activity was studied by using cup plate agar diffusion method by measuring diameter of zone of inhibition in mm. The activity was reported by measuring the diameter for zone of inhibition in mm. Ciprofloxacin was used as standard drug for antibacterial activities. Nutrient agar was employed as culture medium and DMSO were used as solvent.

Methods of antifungal activity

Antifungal activity was screened for the newly separated samples. The cup plate method was employed to study the preliminary antifungal activity of Candida Albicans and Aspergillus Niger. The extract was dissolved in 5 ml of Dimethyl Sulfoxide (1000 μg/ml) Volume and 1mg/ml. Ciprofloxacin was used as standard drug (50 and 100 μg/ml) and dimethyl sulfoxide as a control. The observed zone of inhibition was measured in mm.

Methods of antitumor activity:

Potential cytotoxicity of the extract was tested using the method of Shkhan et al., [11]. Cells were plated in 96-multwell plate (104 cells/well) for 24 hrs. Before treatment with the extract allow attachment of cell to the wall of the plate. Different concentrations of the extract under test (50, 100, 125, 250 and 500 μg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the extract for 48 hrs at 37°C and atmosphere of 5% CO₂. After 48 hrs; cells were fixed, washed and stained with Sulforhodamine B strain. Excess strain was washed with acetic acid and attached strain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and extract concentration is plotted to get the survival curve of each tumor cell line after the specified compound [7].

Results

Structure elucidation of phenolic compounds in methanol extract

Two dimensional paper chromatography of the extract was applied on What man paperNo 1, irrigated in the solvent system6%(acetic acid-water) (HOAc-6%), followed by butanol: acetic: water (4:1:5) revealed the presence of mainly six phenolic compounds, corresponding spots gave positive response towardsFeCl₃ spray reagent, some of which appeared under UV light as dark purple spots which turned orange or lemon yellow or reddish orange when fumed with ammonia vapor.

Investigation of the phenolic compounds was done by fractionation of the extract over polyamide column chromatography and elution with methanol/bidistilledwater. Thenit was subjected to re-chromatography for several times led to the separation of six pure phenolic compounds. The structure of these compounds was compared by comparison of their physical and spectral data with those of reported compounds.

Caffeic acid: - Rf values (x 100): 25 (H2O), 45 (HOAC-6%), 81 (BAW). UV Spectral Data: MeOH 218, 245, 298, 325. 1H- NMR Spectral Data (DMSO-d6): δ (ppm):6.2 (d, J=16Hz, β-H), 6.76(J=7.5 Hz, 5-H), 6.88 (dd, J=7.5 Hz and J=2.5 Hz, 6-H), 6.98 (d, J=7.5 Hz and J=2.5 Hz, 6-H), 6.98 (d, J=2.5Hz, 2-H), 7.48 (d, J=16Hz, α-H).

Gallic acid: Rf values (x100): 44 (H₂O), 55(HOAc-6%), 72 (BAW), UV (MeOH): Amax ___272 nm. 1H- NMR (DMSO-d6): δ 6.98 (s, 2H, H-2 and H-6), 13C-NMR (DMSO-d6): δ 120.6 (C-1), 108.8 (C-2 and C-6), 145.5(C-3 and C-5), 138.1(C-4), 167.7(C=O).Ms (m/z): 170.9 [M+ 1, 5.1%], 169.0 [M’ - H, 100%, ion Aj], 167.9 [M’-H, 63.9%], 153 [M’ - OH, 11.2%], 145 [ion A-C, 3.5%], 139 [ion A–HCHO, 11.3%], 126 [M’-CO₂, 6.5%] and 123 [ion A–HCOOH, 27.1%]. Anal. Calcd for C₇H₆O₅ (170.02): C, 65.90; H, 4.79; O, 29.31.

Catechin: Rf values (x 100): 0.33 (H2O), 0.54 (HOAC-6%), 0.60 (BAW). UV Spectral data: λmax (nm)=278 (H-NMR Spectral Data (DMSO-d6)) (ppm): 4.51 (d, J=7.3, H-2), 3.84 (m, H-3), 5.90 (d, J=2.2, H-6), 5.72 (d, J=2.2, H-8), 6.74 (d, J=1.9, H-2'), 6.7 (d, J=8, H-5'), 6.61 (dd, j=8, 1.9 , H-6'). 13C-NMR Spectral Data (DMSO-d6): δ (ppm) 81.0 (C-2), 66.4 (C-3), 27.7(C-4), 156.1 (C-5), 95.3 (C-6), 156.4 (C-7), 94.0 (C-8), 155.3 (C-9), 99.2 (C-10), 130.7 (C- 1'), 114.5 (C-2'), 144.8 (C-3'), 144.8 (C-4'), 115.1 (C-5'), 118.4 (C-6').

p-Coumaric acid: Rf values (x100): 43 (H2O), 45 (HOAC-6%), 90 (BAW) UV (MeOH): λmax=283, 310 nm, UV (MeOH + NaOMe): λmax=228, 333 nm, H-NMR Spectral Data (DMSO-d6): δ 6.6 (d, J=15Hz, α), 6.72(d, J=8Hz, H-3 and H-5), 7.32 (d, J=8 Hz, H-2 and H-6), 7.52(d, J=15Hz, H-β). Anal. Calcd for C9H8O3 (164.16): C, 65.85; H, 4.91; O, 29.24. Found: C, 65.80; H, 4.80; O, 29.40.

Quercetin: Rf values (x100): 00 (H2O), 07 (HOAC-6%), 90 (BAW). UV (MeOH): λmax=255, 268, 370nm.(MeOH + NaOAc): λmax=254, 276, 375 nm.(NaOAc + H3BO3): λmax=272, 388nm.(MeOH + AlCl3): λmax=270, 360, 440 nm. (AlCl3 + HCl): λmax=258, 400 nm.H-NMR Spectral Data (DMSO-d6): δ 6.19 (d, J=2,5, H-6), 6.4 (d, J=2,5, H-8), 7.64 (d, J=2,5, H-2'), 7.53 (dd, J=2.5 and 8.5, H-6'). 13C-NMR Spectral Data (DMSO-d6): δ 147.0 (C-2), 135.8 (C-3), 176.2 (C-4), 160.5 (C-5), 99.2 (C-6), 164.0 (C-7), 93.7 (C-8), 156.4 (C-9), 103.5 (C-10), 122.2 (C-1'), 115.3 (C-2'), 145.1 (C-3'), 148.0 (C-4'), 115.6 (C-5') and 120.2 (C-6'). Ms (m/z): 300.8 [M - - H, 100%, ion A], 299.8 [M --H2, 28.3%], 270.9 [ion A-C7H6O3, 10.1%, ion B] and 117.3 [ion B–HCOOH, 5.3%]. Anal. Calcd for C15H10O7 (302.04): C, 59.61; H, 3.33; O, 37.06. Found: C, 59.30; H, 3.40; O, 37.30.

Chlorogenic acid: Rf values (x100): 67 (H2O), 65 (HOAC-6%), 59 (BAW) UV (MeOH): λmax=229, 275, 400, 1H- NMR Spectral Data (DMSO-d6): δ Caffeic acid moiety: 7.42 (d, J=16Hz, β-H), 7.05 7.05 (d, J=2Hz, 6-H), 6.96 (d, J=7.5 Hz and J=2 Hz, 2-H), 6.79 (d, J=7.5Hz, 3-H), 6.19 (d, J=16Hz, α-H). Quinic acid moiety: 5(m, 1'-H), 1.88 (m, 2'-H and 6'-H), 3.85 (m, 3'-H and 5'-H), 3.5 (m, 4'-H). 13C-NMR Spectral Data (DMSO-d6) δ (ppm) Caffeic acid moiety: 126.1 (C-1), 115.2 (C-2), 144.9 (C-3), 148.5 (C-4), 116.29 (C-5), 121, 5 (C-6), 146.2 (C-7), 115.2 (C-2), 115,2(C-8), 116.5 (C-9) Quinic acid moiety: 76.6 (C-1'), 68.6 (C-3'), 71.8(C-4'), 71 (C-5'), 180 (C-7'), signals of C-2'.
Results of Antibacterial Activity

As we can see from the data in Figure 1; the methanolic extract had different antibacterial activities of palm pollen. Antibacterial activity was done against six species of pathogenic bacteria (Escherichia Coli, Klebsiella, Staphylococcus Epidermidis, Bacillus Cereus, Micrococcus Luteus and Staphylococcus Aureus), but it has a strong value at 22 mm inhibition zone with Staphylococcus Epidermidis which equal to antibiotic value with the same species.

Results of Antifungal Activity

Antifungal activity was done against two species of pathogenic fungi (Candida Albicans, Aspergillus Niger). Also we can see from the data in Figure 2; that the methanolic extract and ketoconazole have the value with the two species.

Results of Anti-tumor activity

The methanolic extract of date palm pollen (Phoenix dactylifera) was tested against three human cell lines [HELA-1 (Cervical carcinoma cell line), MCF7 (breast carcinoma cell line) and CACO (intestinal carcinoma cell line)], and IC50 value of the different cell lines of the methanolic extract of date palm pollen were reported in Figure 3.

Conclusion

The methanolic extract of (DPP) include on active chemical compounds (six phenolic compounds) which were more active towards six species of pathogenic bacteria, two species of fungi and last two cell lines (MCF-7 and CACO).

References