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Phenolics and Lipid Antioxidants from *Washingtonia robusta*

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Our phytochemical investigations of *Washingtonia robusta* Wendl. leaves detected the previously isolated flavonoids and, in addition, luteolin and its 7-*O*-glucoside, tricin 7-*O*-glucoside, isovitexin 3′-*O*-methyl ether, isoorientin 3′-O-methyl ether and *p*-hydroxybenzoic acid. The unsaponifiable material of the lipoidal fraction exhibited twenty hydrocarbons, four sterols, one triterpene and three fat-soluble vitamins, while the saponifiable portion contained thirteen saturated and unsaturated fatty acids. The different successive extracts were tested for their antioxidant activities. The aqueous methanolic extract showed 61% (AA) relative per cent inhibition, followed by the ether extract 52% while pet. ether, chloroform and methanol extracts exhibited the lowest inhibition at 13%, 10% and 8%, respectively. All compounds were separated and identified by chemical and physical spectroscopic analysis including acid hydrolysis, HPLC, GLC, ^{1}H NMR, ^{13}C NMR, COZY experiments, CMS and UV spectra.

Key Words: ***Washingtonia robusta*, Palmae, Coryphoideae, Flavone glucosides, Methylated C-glycosides, Antioxidant activity.**

INTRODUCTION

Washingtonia robusta Wendl. represents one of about 3,000 species of the Palmae family, subfamily *Coryphoideae*[1]. *W. robusta* is native to northwest Mexico and has the common name Mexican fan palm and has been cultivated in Egypt and elsewhere[2]. The plant fruit is eaten for its sweetness and the leaves are used for making baskets[3]. Previous phytochemical studies of *W. robusta* attended free amino acids[4], polysaccharides[5], negatively charged flavonoids and flavone C-glycosides as chemosystematic markers[6], tricin aglycone[7]. There are no previous publications concerning the biological activity of *W. robusta*; however

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the related species *Phoenix dactylifera* L. showed urine regulation properties[8], and useful as an aphrodisiac, purgative, expectorant, tonic and for fever and blood problems[9]. The extract of *P. dactylifera* also exhibited antibacterial and antifungal[10] and gonadotrophic properties[11]. We here describe the isolation and identification of additional chemical constituents from *W. robusta* including methylated C-glycosides, sterols, a triterpene, saturated and unsaturated fatty acids and fat-soluble vitamins. Various extracts of *W. robusta* were examined for their antioxidant activity.

EXPERIMENTAL

Preparation of Plant Extracts

Leaves of *W. robusta* were collected from the Orman Garden, Giza, Egypt, during March–May 2001. The collected samples were authenticated by Dr. Tereez Iabib. A voucher specimen has been deposited in the National Research Centre Herbarium.

The dried powdered leaves of *W. robusta* (500 g) were continuously defatted with pet. ether (40–60°C) in a Soxhlet apparatus. The defatted powder was dried, then extracted with 50% methanol in a Soxhlet until exhaustion. The dried methanolic extract was suspended in water and partitioned successively with chloroform, ethyl acetate and *n*-butanol. The ethyl acetate extract was chromatographed over a silica gel column eluted with chloroform followed by stepwise addition of methanol affording *p*-hydroxybenzoic acid and luteolin aglycone. The *n*-butanol extract was chromatographed over a silica gel column eluted with chloroform followed by stepwise addition of methanol affording the rest of the flavonoids. All separated compounds were finally purified over Sephadex LH-20 using 1 : 1 methanol : water.

NMR spectra (^1H and ^{13}C) were recorded on a Varian Inova-500, and/or Unity Plus 300 spectrometer using DMSO-d_6 as solvent and TMS as standard ($\delta = 0$) and chemical shift in ppm δ values. Mass spectra (EI and CI) were recorded on Finnigan MAT-TSQ spectrometer while UV-Vis spectrophotometers of the types Bechman DU7 and Shimodza UV 240 were used for recording UV spectra. HPLC apparatus of the type Philips PU 4110 UV-Vis detector, Philips PU 4100 pump with P-3120 computer monitor was used for successful isolations. GC chromatography was done using Hewlett-Packard (HP 6890 Series GC system). Saponification was attained through refluxing the diethyl ether with 10% KOH. The fatty acids were methylated by reflux with H_2SO_4 for 2 h.

Extraction of Lipoidal Material

The dried leaves of *W. robusta* (400 g) were extracted with diethyl ether (2X1L) yielding 2.8 g lipoidal material. From the lipoidal content, the unsaponifiable portion yield was 56% (1.57 g); of the 1.57 g, cholesterol represented 7.16%, campesterol 2.69%, stigmasterol 2.37%, β-sisterol 6.83% and β-amyrin 1.82%. The soluble vitamins from the 1.57 g were β-carotene (272 mg), lutein (380 mg) and α-tocopherol (172 mg). The saponifiable material represents 38% of the lipoidal materials (1.1 g) containing montanic acid (385 mg) and oleic acid (94

mg). HP-1 methyl siloxane capillary column, length 30 cm, diameter 530 μm, thickness 2.56 μm, at 250°C, flow rate 30 m/min with flame ionization detector, FID, initial temp. 60°C increasing rate 10°C/min, final temp. 280°C was used for identification of USM. HP-INNO column wax polyethylene glycol, capillary column, length 30 cm, diameter 530 μm, thickness 1 μm, oven rate 2°C/min, initial temp. 60°C, final 280°C, flow 16.1 m/min, FID detector flow rate 300 m/min was used for the identification of fatty acid methyl esters. HPLC grade methanol/acetonitrile 20 : 80 and/or 10 : 90 as a mobile phase was used for separation of fat-soluble vitamins using Vydac 4.6 × 250 mm column, flow rate: 1 mL/min and UV-Vis detector.

Antioxidant Activity

β-Carotene solution (1 mL), 0.2 mg/mL in chloroform was added to a round bottom flask (100 mL) containing 0.02 mL of linolic acid and 0.2 mL of Tween-20. The mixture was then dosed with 0.2 mL of 80% methanol or 50 mg/L of dL-α-tocopherol as standard of the plant extract being examined. After evaporation to dryness under vacuum at room temperature, 50 mL of oxygenated distilled water was added and the mixture was shaken to form a liposome solution. The samples were then subjected to thermal autooxidation at 50°C for 2 h. The absorbance of the solution at 470 nm was measured immediately after preparation at 0 time and at the end of the experiment (120 min). All samples were assayed in triplicate. The antioxidant activity was calculated as per cent inhibition relative to control.

RESULTS AND DISCUSSION

In addition to the previously isolated flavonoids (orientin 7-glucoside-KSO_3, luteolin 7-rutinoside, orientin, vitexin, isovitexin, vitexin 7-glucoside-KSO_3)[6], the extraction of the leaves of *W. robusta* afforded six other known flavonoids, namely, luteolin and its 7-*O*-β-D-glucoside, tricin 7-*O*-β-D-glucoside, *p*-hydroxybenzoic acid, isoorientin 3′-*O*-methyl ether and isovitexin 3′-*O*-methyl ether. The isolated compounds were all identified on the basis of their UV, ^{1}H NMR, ^{13}C NMR and CIMS spectra in comparison with literature data. The ether extract afforded lipoidal material, which upon saponification followed by GC/MS, yielded twenty hydrocarbons among which decosane predominates. The unsaponifiable material also contained campesterol, stigmasterol, β-sitosterol and cholesterol as major components and β-amyrin as the only triterpene. HPLC of the unsaponifiable material of the diethyl ether extract yielded the fat-soluble vitamins β-carotene, lutein and α-tocopherol[12]. Thirteen fatty acids were also detected by GC/MS of the methylated saponifiable material including oleic and montanic acids as the major components of unsaturated and saturated fatty acid fraction[13, 14]. The antioxidant activity of the successive extracts (pet. ether, ether, chloroform, methanol and aqueous methanol) was determined[15]. The highest antioxidant activity of the ether extract is attributed to the presence of α-tocopherol and β-carotene (by their quenching of singlet oxygen, superoxide and peroxyl free radicals)[16, 17], campesterol, stigmasterol, *p*-sitosterol and lutein[18–20]. The methanol extract antioxidant activity is related to the presence of flavonoids[21, 22]. A linear relationship between antioxidant

properties and total phenolic contents of medicinal herbs is reported[23] while the major and powerful antioxidant compounds in some plants are attributed to phenolic acids and flavonoid aglycones[24, 25]. *W. robusta* contains compounds belonging to most of the above classes of plant compounds exhibiting antioxidant activity.

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