Biological studies of isolated triterpenoids and phenolic compounds identified from *Wodyetia bifurcata* family Arecaceae

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Abstract

The chromatographic investigation of the aerial parts of *Wodyetia bifurcata* (family Arecaceae) revealed the presence of β-Amyrin, lupeol, apigenin, kaempferol, p-hydroxyl benzoic acid and gallic acid that isolated for first time from *Wodyetia bifurcata* and their structures are elucidated by different spectroscopic techniques as UV, $^{1}$H-NMR, $^{13}$C-NMR and El/MS spectra. The biological activities of different plant extracts are tested for anti-inflammatory, antioxidant and cytotoxicity.

Keywords: *Wodyetia bifurcata*, Arecaceae, Aerial part, Triterpenoids, phenolic compounds, Anti-inflammatory, Antioxidant, Cytotoxicity.

1. Introduction

*Wodyetia* is a genus of trees belonging to family Arecaceae. *Wodyetia bifurcata* L. (also called foxtail) is fast growing, large sized evergreen tree, up to 10 m tall, grows in El-Orman garden, Giza, Egypt. *Wodyetia bifurcata* L is endemic to Australia and it is found on north Queensland [18]. Triterpenoids are prevalent in the plant kingdom. Recent evidences support the beneficial effects of naturally occurring triterpenoids against several types of human diseases [27], anticancer [19], antioxidant [2], hepatoprotective [22], antifungal [28], antibacterial [16] and antileishmanial activity [9]. Flavonoids are a group of polyphenolic compounds, diverse in chemical structure and characteristics, found ubiquitously in plants. Until now, more than 9000 different flavonoid compounds were described in plants, where they play important biological roles by affecting several developmental processes [37]. There has been increasing interest in the research of flavonoids from dietary sources, due to growing evidence of the versatile health benefits of flavonoids including antioxidant activity [10], coronary heart disease [35], hepatoprotective [34], anti-inflammatory, [23], anticancer activities [38], antibacterial [21] while some flavonoids exhibit potential antiviral activities [5].

2. Results and discussions

The MS of the amyrins is characterized by an M+ of m/e 426 and a major fragmentation yielding a component of m/e 218 [15]. Data of $^{1}$H NMR for compound I showed eight methyl group corresponding to terpenoid of olean skeleton. The $^{13}$C NMR and El-MS of compound I were compared with that reported in that literature review [7, 8, 25]. The compound I is identified as β-Amyrin.

The $^{1}$H NMR data of compound II showed seven tertiary methyl singlets and one secondary hydroxyl group. It also showed olefinic protons at δ 4.41 and 4.67. The compound showed 30 signals for the terpenoid of lupine skeleton which is represented by seven methyl groups. The carbon bonded to the hydroxyl group C-3 appeared at δ 79.1, while the alkenic carbons appeared at δ 151.1 and 109.5. El-MS spectrum of compound II showed the molecular ion at m/z 426 (10%) [M+] corresponding to the formula C30H50O, together with fragments at m/z 411 (6%) [M+15] and 408 (5%) [M+18] which were due to the loss of methyl group and a molecule of water from the molecular ion peak other fragments occurring at m/z 385 (4%) (M+2), m/z 220 (8%) (M-CH3), m/z 218 (46%) (M-C2H5O), m/z 207(51%) (M-C3H7) and m/z 189 (70%) (M-C4H9) were characteristic for lupane series [15]. Data of $^{1}$H NMR, $^{13}$C NMR and El-MS were compared with that reported in that literature review [1, 3, 13, 36]. The compound II is identified as lupeol.
UV spectral data of compound III showed two major absorption bands; band (I) at 331 nm and band II at 272 nm, which is typical of a flavone nucleus with no hydroxyl group at position 3 \[20\]. The addition of sodium methoxide resulted in a bathochromic shift (+\(\Delta 69\) nm) in band I with an increase in intensity, which proved that position 4\(\prime\) has a free OH group \[20\]. The bathochromic shift in band II on addition of NaOAc (+\(\Delta 9\) nm) compared with the same band in MeOH suggested the presence of a free hydroxyl group at carbon number 7. On addition of H\(_3\)BO\(_3\) to NaOAc the hypochromic shift in band I (+\(\Delta 45\) nm) suggested the absence of any ortho-dihydroxyl groups. The \(^1\)H-NMR spectrum of compound III revealed the presence of two aromatic protons of the A-ring revealed as two doublet at \(\delta 6.2\) and \(\delta 6.0\) each proton has \(J=1.5\) Hz due to meta coupling assigned to H-8 and H-6, respectively. H-3 appeared at \(\delta 6.4\) as a single signal as no neighbor proton appears. The aromatic protons of the B-ring as two doublets at \(\delta 7.6\) and \(\delta 7.0\) each doublet with \(J=5.7\) Hz due to ortho coupling assigned to H-2', 6' (H-2', 6' are superimposed) and H-3', 5' (H-3', 5' are superimposed) respectively. Note that the H-3', 5' doublet occurs upfield from H-2', 6' due to shielding effect of the hydroxyl group and the deshielding influence of C-ring on H-2', 6'. The EI-MS spectrum of compound III showed the presence of phenolic group with melting point 215-216 ºC. The \(^1\)H-NMR spectrum of compound IV showed signals at \(\delta 6.2\) each proton has \(J=1.2\) Hz due to ortho coupling assigned to H-2', 6' (H-2', 6' are superimposed) and H-3', 5', (H-3', 5' are superimposed) respectively. Note that the H-3', 5' doublet occurs upfield from H-2', 6' due to shielding effect of the hydroxyl group and the deshielding influence of C-ring on H-2', 6'. The EI-MS spectrum of compound IV showed the molecular ion peak as the base peak (M\(^+\)) at m/z 270 (95%) corresponding to molecular formula C\(_{15}\)H\(_9\)O\(_5\). Peaks at m/z 152 (43%) and m/z 118 (48%) it suggests the fragments of H-2', 6' (H-2', 6' are superimposed) and H-3', 5' (H-3', 5' are superimposed) respectively. The compound VI is identified as Colourless crystals; it gave violet color with ferric chloride solution indicating the presence of phenolic group with melting point 215-216 ºC. The \(^1\)H-NMR of compound V showed signals at \(\delta 7.58\) (2H, d, \(J=2.1\) Hz, H-2, 6), 6.92 (2H, d, \(J=1.2\) Hz, H-3, 5). Data of \(^1\)H NMR, UV spectra and EI-MS were compared with that reported in that literature review \[6, 24, 31\]. The compound VI is identified as kaempferol.

### 3. Experimental

#### 3.1 Plant Material

The aerial parts of *Wodeytia bifurcata* were collected from the Egyptian Orman garden, Giza, Egypt at June 2011. The plant was identified by Agricultural Engineer Terese Labib, El Orman Botanical Garden.

#### 3.2 Extraction and isolation

The air dried powdered leaves and stems of *Wodeytia bifurcata* (1 kg) were macerated in 3 L 80% aqueous methanol with occasional stirring at room temperature for three days. The methanolic extract was concentrated and dried under vacuum. The dried residue (85 g) was suspended with H\(_2\)O and extracted with butanol. The butanol extract was separated and evaporated to dryness under reduced pressure to yield a semisolid residue (Fraction I, 28 g). The aqueous liquor was evaporated till dryness under vacuum to yield a residue (Fraction II, 35 g).

The fraction I subjected to column silica gel achieved with gradient elution using chloroform: methanol (100% CHCl\(_3\) to 100% MeOH) to yield (fraction I-a, 85:15 chloroform: methanol) and (fraction I-b, 80:20 chloroform: methanol) which are subjected to further purification by silica gel to yield compound I and compound II subjected to more purification.

\(~68~\)
with preparative-TLC silica gel G F254 eluate with CHCl3: MeOH: H2O (13:7:2). The fraction II subjected to column silica gel achieved with gradient elution using chloroform: methanol (100% CHCl3 to 100% MeOH) to yield (fraction II-c, 70:30 chloroform: methanol) and (fraction II-d, 40:60 chloroform: methanol) which are major fraction which is subjected to further purification by silica gel column to yield fractions which were further purified by rechromatography on sephadex LH-20 column using MeOH, MeOH/H2O and H2O as eluents to yield compound III, compound IV, compound V and compound VI.

Compounds (I)

1H NMR (CDCl3, 300 MHz) at δ; 0.68, 0.78, 0.80, 0.86, 0.90, 0.93, 1.05, 1.18 (each 3H, s, C-25, C-23, C-30, C-29, C-24, C-26, C-27, C-28), 3.1 (dd, J = 7.2, 3.1 Hz, H-3) 5.7 (d, J = 7.2 Hz, H-12).

13C NMR (CDCl3, 300 MHz): 29.12 (C-1), 30.48 (C-2), 34.33 (C-7), 35.32 (C-16), 37.66 (C-10), 38.42 (C-1), 39.88 (C-4), 40.22 (C-13), 41.82 (C-22), 42.12 (C-8), 43.42 (C-14), 44.22 (C-17), 49.56 (C-19), 49.84 (C-18), 50.12 (C-9), 52.09 (C-5), 110.89 (C-29), 115.66 (C-13), 145.66 (C-12), 148.42 (C-21), 150.63 (C-20).

EI-MS: showed the molecular ion at m/z 426 (14%) [M+] corresponding to the formula C20H30O, together with fragments m/z 411 (5%), 275 (3%), m/z 257 (2%), 218 (95%), 203 (46%), 189 (33%), 175 (17%), 161 (3%).

Compounds (II)

1H NMR (CD3OD, 300 MHz) showed signals at δ; 0.77, 0.86, 0.88, 0.90, 0.92, 0.96, 1.11 (each 3H, s), 3.64 (1H, dd, J = 5.2 Hz, H-3) 4.41 (1H, s, H-29a), 4.67 (1H, s, H-29b) showed seven tertiary methyl singlets and one secondary hydroxyl group. It also showed olefinic protons at δ 4.41 and 4.67. 13C NMR (CDCl3,300MHz): at δ; 14.36 (C-27), 15.73 (C-24), 16.77 (C-26), 17.62 (C-25), 18.42 (C-28), 19.12 (C-6), 20.53 (C-30), 21.33 (C-11), 25.55 (C-2), 26.63 (C-12), 27.21 (C-15), 28.43 (C-23), 30.48 (C-21), 34.33 (C-7), 35.32 (C-16), 37.66 (C-10), 38.42 (C-1), 39.88 (C-4), 40.22 (C-13), 41.82 (C-22), 42.12 (C-8), 43.42 (C-14), 44.22 (C-17), 49.56 (C-19), 49.84 (C-18), 50.12 (C-9), 54.29 (C-5), 77.40 (C-3), 110.89 (C-29), 150.63 (C-20).

Compounds (III)

UV spectral data at λmax (nm): MeOH; 272, 331, NaOMe; 268, 332, 400, AlCl3; 279, 306, 351, 387, AlCl3/HCl; 279, 304, 346, 386, NaOAc; 281, 306, 387, NaOAc/CH3BO3; 268, 342. 1H NMR (DMSO-d6, 270 MHz) at δ; 7.6 (2H, d, J = 5.7 Hz, H-2’, H-6’), 7.0 (2H, d, J = 5.7 Hz, H-3’, H-5’), 6.4 (1H, s, H-3), 6.2 (1H, d, J = 1.5 Hz, H-8), 6.0 (1H, d, J = 1.5 Hz, H-6). The EI-MS spectrum showed the molecular ion peak as the base peak (M+) at m/z 270 (95%) corresponding to molecular formula C15H10O5 together with fragments at m/z 152 (43%), 118 (48%), 121 (20%) and 149 (26%).

Compounds (IV)

UV spectral data at λmax (nm): MeOH; 265, 294, 322, 367, NaOMe; 277, 294, 315, 420, 420, AlCl3; 268, 303, 350, 424, AlCl3/HCl; 269, 303, 348, 424, NaOAc; 276, 302, 387, NaOAc/CH3BO3; 266, 248, 320, 372. 1H-NMR (DMSO-d6, 270 MHz) at δ; 7.94 (2H, d, J = 5.4 Hz, H-2’, H-6’), 6.9 (2H, d, J = 5.4 Hz, H-3’, H-5’), 6.5 (1H, d, J = 1.2 Hz, H-8), 6.2 H, d, J = 1.2 Hz, H-6). The EI-MS spectrum showed the molecular ion peak as the base peak (M+) at m/z 287 (84%) corresponding to molecular formula C15H11O6 together with fragments at m/z 269, 259 (12%), 258 (15%), 153 (42%) and 133 (48%).

Compound (V)

Compound is colorless crystals that gave violet color with ferric chloride solution with melting point 215-216 ° C. The 1H-NMR (CD3OD, 300 MHz) showed signals at δ; 7.58 (2H, d, J = 2.1 Hz, H-2, 6), 6.92 (2H, d, J = 1.2 Hz, H-3, 5).

Compound (VI)

Compound VI is identified by co-chromatography with authentic using paper chromatography with solvent system butanol: acetic acid: water (5:4:1) and acetic acid 6%. Spots visualized under UV light show violet color and gave blue color with FeCl3 with Rs = 0.53 and 0.56 respectively.

Table 1: of isolated compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>λmax (nm)</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound (I)</td>
<td>265, 294, 322, 367</td>
<td>0.53, 0.56</td>
</tr>
<tr>
<td>Compound (II)</td>
<td>265, 294, 322, 367</td>
<td>0.53, 0.56</td>
</tr>
<tr>
<td>Compound (III)</td>
<td>265, 294, 322, 367</td>
<td>0.53, 0.56</td>
</tr>
<tr>
<td>Compound (IV)</td>
<td>265, 294, 322, 367</td>
<td>0.53, 0.56</td>
</tr>
</tbody>
</table>

4. Biological activities

4.1 Evaluation of anti-inflammatory activity

The accumulation of nitrite, an indicator of NO synthesis was measured by Griess reagent (Gerhauser, et al., 2003). A standard curve was plotted using serial concentration of sodium nitrite (Fig. 1). The NO inhibition percentage was calculated by treating the nitrite content of the cell supernatant of cultures submitted the nitrite content of the cell supernatant of cultures treated with DMSO (control) LPS or LPS/treated compounds (Fig. 2).
Fig 1: The level of Nitric oxide in RAW 264.7 cells supernatant after the treatment with the samples (50 µg/ml) compared with LPS-treated cells (200 µg/ml), as measured by Griess assay.

Fig 2: The percentage of inhibition of Nitric oxide in LPS-activated RAW 264.7 cells supernatant after the treatment with the samples (50 µg/ml) compared to LPS treated cells, as measured by Griess assay.

4.2 Evaluation of antioxidant activity
DPPH is a deep violet radical due to its unpaired electron, which in the presence of antioxidant radical scavenger (donate an electron to DPPH) decolorizes to the pale yellow non-radical form [30]. The change in the colorization and subsequent fall in absorbance were monitored spectrophotometrically at 520 nm. A standard calibration curve was plotted using serial dilutions of ascorbic acid in concentrations ranging from 0 to 100 µg/ml in distilled water (fig. 3). The half maximal scavenging capacity (SC₅₀) values for each tested sample and ascorbic acid were estimated via dose curve and calculated from curve equation (fig. 4).
4.3 Evaluation of cytotoxic activity
Cytotoxicity of the tested extract was measured against Hep-
G2 cells using MTT cell viability assay, which is based on
ability of active mitochondrial dehydrogenase enzymes of
living cells to cleave the tetrazolium rings of the yellow MTT
and form dark blue insoluble formazan crystals which are
largely impermeable to cell membranes, results in its
accumulation within healthy cells (result in the liberation
of crystals, which are then solubilized). The number of viable
cells is directly proportional to the level formazan dark blue
color. The extent of reduction of MTT was quantified by
measuring the absorbance at 570 nm \[^{14}\]. The percentage of
relative viability was calculated using the following equation
(absorbance of treated cells / absorbance of control cell)*100.
Then the half maximal inhibitory concentration (IC\(_{50}\)) was
calculated from the equation of dose response curve. Results
showed that butanol extracts had weak cytotoxicity activity
against Hep-G2 cells, IC\(_{50} = 568.5 \mu g/ml\) (fig. 5), (Olajire and
azeez, 2011).
Fig 5: Cytotoxic effect of different samples against Hep-G2 cells using MTT assay (n=4), data expressed as the mean value of cell viability (% of control) ± S.E.

5. References