SUPPLEMENTARY MATERIAL

A new monoterpene glucoside and complete assignments of dihydroflavonols of Pulicaria jaubertii: Potential cytotoxic and blood pressure lowering activity

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Abstract

One new monoterpene glucoside and five dihydroflavonols were isolated for the first time from the aerial parts of Pulicaria jaubertii and identified as p-menthane-2-O-\(\beta\)-D-glucopyranoside [1], dihydroquercetin (taxifolin) [2], 7, 3’-di-O-methyltaxifolin [3], 3’-O-methyltaxifolin [4], 7-O-methyltaxifolin (padmatin) [5] and 7-O-methyl-dihydrokampferol (7-O-methylaromadenderin) [6]. The structures of these compounds were unambiguously assigned on the basis of NMR spectroscopic data (\(^1\)H, \(^{13}\)C, DEPT, HSQC, HMBC) and MS analysis. 2D-NMR methods required revision of assignments of H-6 and H-8 for dihydroflavonol compounds. Possible cytotoxic activity as well as blood pressure (BP) lowering activity were tested. The alcoholic extract showed cytotoxic activity against prostate carcinoma (PC-3), breast carcinoma (MCF-7) and hepatocellular carcinoma (HepG-2) human cell lines with IC\(_{50}\) 19.1, 20.0 and 24.1\(\mu\)g, respectively. The higher dose levels of the alcoholic extract significantly reduced normal BP of rats in a dose dependent manner.

Key words: Pulicaria jaubertii, phytoconstituents, cytotoxic activity, blood pressure lowering effect.

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Table S1.1D and selected 2D NMR data of compound [1] (DMSO-$d_6$, 500 MHz)

| Position | $^1$H (J in Hz) | $^1$C | DEPT | COSY | HMBC |
|----------|-----------------|-------|------|------|------|
| 1        | 1.41, m         | 35.91 | CH   | H-2, 6b, 7 |      |
| 2        | 3.79, m         | 75.36 | CH   | H-1, 3a, 3b |      |
| 3a       | 0.91, m         |       |      |       |      |
| 3b       | 1.91, d, 12.5   | 32.31 | CH$_2$ | H-2, 3b, 4 | H-2, 3a |
| 4        | 1.56, m         | 35.99 | CH   | H-3a, 5a, 5b, 8 |      |
| 5a       | 0.93,           |       |      | H-4   |      |
| 5b       | 1.58, m         | 29.06 | CH$_2$ | H-4, 6b |      |
| 6a       | 0.98, m         | 29.32 | CH$_2$ | H-5b, 6b |      |
| 6b       | 1.34,           |       |      | H-5b, 6a |      |
| 7        | 0.88, d, 6.3    | 18.92 | CH$_3$ | H-1 | C-1, 2, 6 |
| 8        | 1.35, m         | 32.36 | CH   | H-4, 9, 10 |      |
| 9        | 0.82, d, 6.7    | 20.08 | CH$_3$ | H-8 | C-4, 8, 10 |
| 10       | 0.82, d, 6.7    | 19.97 | CH$_3$ | H-8 | C-4, 8, 9 |
| 1'       | 4.12, d, 7.7    | 100.42 | CH   | H-2' | C-2 |
| 2'       | 2.95, t, 8.1    | 73.94 | CH   | H-1', 3' |      |
| 3'       | 3.14, t, 8.1    | 77.46 | CH   | H-2', 4' | C-4' |
| 4'       | 3.09, m         | 70.86 | CH   | H-3', 5' | C-3', 5' |
| 5'       | 3.04, m         | 77.25 | CH   | H-4', 6' | C-3' |
| 6'a      | 3.65, d         |       |      |       |      |
| 6'b      | 11.3, 3.38, m   | 61.82 | CH$_2$ | H-6, b | H-6'a, 5' |


Table S2. $^1$H NMR data of compounds [2-6] (DMSO-$d_6$, 600, 500 and 400 MHz)

| Position | 2       | 3       | 4       | 5       | 6       |
|----------|---------|---------|---------|---------|---------|
| 2        | 4.96, d, 11.0 | 5.07, d, 12.0 | 5.05, d, 11.2 | 5.03, d, 11.2 | 5.09, d, 11.4 |
| 3        | 4.48, d, 11.1 | 4.70, dd, 12.0, 6.0 | 4.67, d, 11.6 | 4.56, d, 11.2 | 4.63, dd, 11.4, 6.0 |
| 6        | 5.85, brs | 6.10, d, 2.4 | 5.92, brs | 6.11, brs | 6.10, d, 2.4 |
| 8        | 5.82, brs | 6.08, d, 2.4 | 5.88, brs | 6.08, brs | 6.08, d, 2.4 |
| 2`       | 6.88, brs | 7.10, d, 1.8 | 7.12, s | 6.90, s | 7.31, d, 8.4 |
| 3`       | -       | -       | -       | -       | 6.77, d, 8.4 |
| 5`       | 6.75, s | 6.77, d, 7.8 | 6.80, d, 8.0 | 6.76, s | 6.77, d, 8.4 |
| 6`       | 6.75, s | 6.90, dd, 7.8, 1.8 | 6.91, d, 7.6 | 6.76, s | 7.31, d, 8.4 |
| 7-OCH$_3$ | -       | 3.77, s | -       | 3.78, s | 3.77, s |
| 3`-OCH$_3$ | -      | 3.76, s | 3.79, s | -       | -       |
| 3$_{eq}$-OH | -     | 5.81, d, 6.0 | 5.78, brs | 5.84, brs | 5.80, d, 6.0 |
| 5-OH      | 11.90, s | 11.87, s | 11.94, s | 11.88, s | 11.86, s |
| 3`-OH      | 9.08, s | -       | -       | 9.06, s |
| 4`-OH      | 9.11, s | 9.14, s | 9.33, s | 9.06, s | 9.56, s |
Table S3. $^{13}$C NMR data of compounds [2-6] (DMSO-$d_6$, 150, 125 and 100 MHz)

| Position | 2     | 3     | 4     | 5     | 6     |
|----------|-------|-------|-------|-------|-------|
| 2        | 82.44 | 83.30 | 83.65 | 83.67 | 83.01 |
| 3        | 70.96 | 71.43 | 71.87 | 72.11 | 71.51 |
| 4        | 196.96| 198.51| 198.31| 198.84| 198.53|
| 5        | 162.74| 162.94| 163.78| 162.95| 162.97|
| 6        | 95.47 | 94.90 | 96.54 | 95.31 | 94.89 |
| 7        | 166.58| 167.52| 167.05| 167.95| 167.53|
| 8        | 94.47 | 93.77 | 95.53 | 94.23 | 93.78 |
| 9        | 162.08| 162.47| 163.02| 163.47| 162.52|
| 10       | 99.75 | 101.33| 100.89| 101.83| 101.36|
| 1\(^{\circ}\) | 127.46| 127.86| 128.25| 128.34| 127.38|
| 2\(^{\circ}\) | 114.75| 112.14| 112.67| 115.84| 129.49|
| 3\(^{\circ}\) | 145.16| 147.31| 147.81| 146.27| 114.88|
| 4\(^{\circ}\) | 144.34| 147.01| 147.49| 145.41| 157.77|
| 5\(^{\circ}\) | 114.53| 114.93| 115.39| 115.58| 114.88|
| 6\(^{\circ}\) | 118.77| 121.21| 121.65| 119.89| 129.49|
| 7-OCH\(_3\) | -     | 55.93 | -     | 56.39 | 55.94 |
| 3'-OCH\(_3\) | -     | 55.66 | 56.17 | -     | -     |
Table S4. Cytotoxicity of the alcoholic extract of *Pulicaria jaubertii* against cultured PC-3, MCF-7 and HepG-2 cancer cell lines.

| Sample            | Growth inhibition constant (IC$_{50}$)$^a$ [μg] |
|-------------------|-----------------------------------------------|
|                   | PC-3                      | MCF-7                  | HepG-2                  |
| Alcoholic extract | 19.1 ± 0.7                | 20 ± 0.3               | 24.1 ± 0.22             |
| Vinblastine$^b$   | 5.5 ± 0.4                 | 4.6 ± 0.18             | 4.6 ± 0.17             |

$^a$ IC$_{50}$ is defined as the concentration that resulted in a 50% decrease in viable cell number.

$^b$ Positive control substance. Data are represented as mean ± SE of three independent replicates.
Figure S1. Concentration dependant cytotoxicity of alcoholic extract of *Pulicaria jaubertii* towards PC-3, MCF-7 and HepG-2 cell lines.
Figure S2. Effect of single doses treatment with *Pulicaria jaubertii* alcoholic extract (5, 10 and 20 mg/Kg i.v) on SBP of anaesthetized male albino normotensive rats. Values are represented as mean ± SE of the mean.

* Significantly different from control at p<0.05.
S1. **Material and apparatus:** UV spectra were determined with a Shimadzu UV-1650PC spectrophotometer; IR spectra were carried out on a Nicolet 205 FT IR spectrometer connected to a Hewlett-Packard Color Pro. Plotter. The \( ^1H \)- and \( ^{13}C \)-NMR measurements were obtained with a Bruker Avance spectrometer operating at 600 MHz (for \( ^1H \)) and 150 MHz (for \( ^{13}C \)), a Bruker BioSpin AG operating at 500 MHz (for \( ^1H \)) and 125 MHz (for \( ^{13}C \)) and a Bruker Avance III NMR spectrometer operating at 400 MHz (for \( ^1H \)) and 100 MHz (for \( ^{13}C \)) in DMSO-\( d_6 \) solution, and chemical shifts were expressed in \( \delta \) (ppm) with reference to TMS, and coupling constant (\( J \)) in Hertz. \( ^{13}C \) multiplicities were determined by the DEPT pulse sequence (135\(^\circ \)). COSY, HMBC and HSQC NMR experiments were carried out using a Bruker AV-600, a Bruker BioSpin AG 500 and a Bruker AV-400 III spectrometers. EIMS was carried on Scan EIMS-TIC, VG-ZAB-HF, X-mass (158.64, 800.00) mass spectrometer (VG Analytical, Inc.). Si gel (Si gel 60, Merck), was used for open column chromatography. Solid phase extraction was performed on SPE-C\(_{18}\) cartridges (Strata columns). TLC was carried out on precoated silica gel 60 F254 (Merck) plates. Developed chromatograms were visualized by spraying with 1% vanillin-H\(_2\)SO\(_4\), followed by heating at 100 for 5 min.

S2. **Cytotoxicity assays**

The cytotoxicity of the alcoholic extract was tested against three human tumor cell lines; prostate carcinoma (PC-3), breast carcinoma (MCF-7) and hepatocellular carcinoma (HepG-2) cell lines. The cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were grown on Roswell Park Memorial Institute (RPMI) 1640 medium (Nissui Pharm. Co., Ltd., Tokyo, Japan) supplemented with 10% inactivated fetal calf serum and 50\( \mu \)g/mL gentamycin. The cells were maintained at 37\(^\circ \)C in a humidified atmosphere with 5% CO\(_2\) and were sub cultured two to three times a week. The cytotoxic activity was determined by using cell viability assay method as described previously (Mosmann, 1983, Gangadevi and Muthumary, 2007). The experiments were performed in triplicates and the percentage of cell viability was calculated as the mean absorbance of control cells/mean absorbance of treated cells. Concentration-response curves were prepared and the IC\(_{50}\) values were determined. The results are presented in (Table 4)
S3. Evaluation of blood pressure lowering effect

Adult male albino Sprague-Dawley rats weighing 150-200 g were used in our experiments. They were purchased from the animal facility of the pharmacology department, College of Pharmacy, King Abdul-Aziz University, Jeddah, KSA. Animals were housed in cages kept under constant environmental and nutritional conditions throughout the period of investigation. They were allowed a free access to water and diet consisting of standard pellet chow. The study was carried out according to The European Communities Council Directive of 1986 (86/609/EEC) and approved by the Ethical Committee for Animal Experimentation at the Faculty of Pharmacy, Umm Al Qura University, Makkah, KSA.

Systolic blood pressure (SBP) was measured in urethane anesthetized animals (1.3 g/kg i.p.) directly from the left common carotid artery according to the method of Burden et al. (1979), via a polyethylene cannula attached to a pressure transducer (APT300, Hugo Sachs Elektronik, Germany) connected to an oscilographic recorder (Harvard Apparatus, UK). The right jugular vein was similarly cannulated for intravenous injection of the extract under investigation.

The extract was freshly reconstituted and injected intravenously in the right jugular vein. Three dose levels of the alcoholic extract were used; 5, 10 and 20 mg/Kg. The experiment was repeated in 6 animals. A control group composed of 6 rats was injected with the solvent in the same volume as the treated group. Changes in SBP were recorded and read directly from the tracing that was calibrated at the end of each experiment.
S-4(a) $^1$H NMR spectrum of compound-1

S-4(b) $^1$H NMR spectrum of compound-1
S-5(a) $^{13}$C NMR spectrum of compound-1

S-5(b) $^{13}$C NMR spectrum of compound-1
S-5(c) $^{13}$C NMR spectrum of compound-1

S-6(a) $^{13}$C-DEPT NMR spectrum of compound-1
S-6(b) $^{13}$C-DEPT NMR spectrum of compound-1

S-6(c) $^{13}$C-DEPT NMR spectrum of compound-1
S-7(a) HSQC spectrum of compound-1

S-7(b) HSQC spectrum of compound-1
S-7(c) HSQC spectrum of compound-1

S-8(a) COSY spectrum of compound-1
S-8(b) COSY spectrum of compound-1

S-8(c) COSY spectrum of compound-1
S-8(d) COSY spectrum of compound-1

S-8(e) COSY spectrum of compound-1
S-9(a) HMBC spectrum of compound-1

S-9(b) HMBC spectrum of compound-1
S-9(c) HMBC spectrum of compound-1

S-10 ESIMS spectrum of compound-1
S-11(a) $^1$H NMR spectrum of compound-2

S-11(b) $^1$H NMR spectrum of compound-2
S-12(a) $^{13}$C NMR spectrum of compound-2

S-12(b) $^{13}$C NMR spectrum of compound-2
S-13 HSQC spectrum of compound-2

S-14 HMBC spectrum of compound-2
S-15(a) $^1$H NMR spectrum of compound-3

S-15(b) $^1$H NMR spectrum of compound-3
S-15(c) $^1$H NMR spectrum of compound-3

S-15(d) $^1$H NMR spectrum of compound-3
S-15(e) $^1$H NMR spectrum of compound-3

S-15(f) $^1$H NMR spectrum of compound-3
S-16(a) $^{13}$C NMR spectrum of compound-3

S-16(b) $^{13}$C NMR spectrum of compound-3
S-16(c) $^{13}$C NMR spectrum of compound-3

S-17(a) HSQC spectrum of compound-3
S-17(b) HSQC spectrum of compound-3

S-17(c) HSQC spectrum of compound-3
S-18(a) HMBC spectrum of compound-3

S-18(b) HMBC spectrum of compound-3
S-18(c) HMBC spectrum of compound-3

S-18(d) HMBC spectrum of compound-3
S-19 $^1$H NMR spectrum of compound-4

S-20 $^{13}$C NMR spectrum of compound-4
S-21(a) HSQC spectrum of compound-4

S-21(b) HSQC spectrum of compound-4
S-21(c) HSQC spectrum of compound-4

S-22(a) HMBC spectrum of compound-4
S-22(b) HMBC spectrum of compound-4

S-22(c) HMBC spectrum of compound-4
S-23 $^1$H NMR spectrum of compound-5

S-24 $^{13}$C NMR spectrum of compound-5
S-25(a) HSQC spectrum of compound-5

S-25(b) HSQC spectrum of compound-5
S-25(c) HSQC spectrum of compound-5

S-26(a) HMBC spectrum of compound-5
S-26(b) HMBC spectrum of compound-5

S-26(c) HMBC spectrum of compound-5
S-27(a) $^1$H NMR spectrum of compound-6

S-27(b) $^1$H NMR spectrum of compound-6
S-27(c) $^1$H NMR spectrum of compound-6

S-27(d) $^1$H NMR spectrum of compound-6
S-28(a) $^{13}$C NMR spectrum of compound-6

S-28(b) $^{13}$C NMR spectrum of compound-6