

## **Effect of five coumarins and standardized extracts from *Tagetes lucida* on motor impairment and neuroinflammation induced with cuprizone.**

**Gabriela Castro-Martínez<sup>1,2</sup>, Maribel Herrera-Ruiz<sup>2</sup>, Manases González-Cortázar<sup>2</sup>, Sandra Liliana Porras -Dávila<sup>2</sup>, Julio Cesar Almanza-Pérez<sup>3</sup>, Enrique Jiménez-Ferrer<sup>2\*</sup>**

<sup>1</sup> Doctorado en Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana, Ciudad de México, México. [gcm\\_19@hotmail.com](mailto:gcm_19@hotmail.com)

<sup>2</sup> Centro de Investigación Biomédica del Sur, Instituto Mexicano Del Seguro Social, Argentina No. 1, Col. Centro, Xochitepec, Morelos 62790, México. [gcm\\_19@hotmail.com](mailto:gcm_19@hotmail.com); [cibis\\_herj@yahoo.com.mx](mailto:cibis_herj@yahoo.com.mx); [gmanases2000@gmail.com](mailto:gmanases2000@gmail.com); [davilasp117@gmail.com](mailto:davilasp117@gmail.com); [jimenezferrer\\_mx@yahoo.com](mailto:jimenezferrer_mx@yahoo.com).

<sup>3</sup> Departamento de Ciencias de la Salud, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Iztapalapa, Av. Ferrocarril San Rafael Atlixco 186, Ciudad de México, México. [jcap@xanum.uam.mx](mailto:jcap@xanum.uam.mx)

\* Correspondence: E-J-F [jesus.jimenezf@imss.gob.mx](mailto:jesus.jimenezf@imss.gob.mx); [enriqueferrer\\_mx@yahoo.com](mailto:enriqueferrer_mx@yahoo.com)

### **Identification of coumarins**

#### **7-Isoprenyloxycoumarin (IC)**

**Data S1;** C<sub>14</sub>H<sub>14</sub>O<sub>3</sub>: <sup>1</sup>H NMR (400 MHz, Chloroform-d): δ 6.14 (1H, d, 9.3 Hz, H-3), 7.58 (1H, d, 9.7 Hz, H-4), 7.29 (1H, d, 8.5 Hz, H-5), 6.74 (1H, dd, 2.3, 8.5 Hz, H6), 6.67 (1H, d, 2.3 Hz, H-8), 4.48 (2H, br, d, 6.6 Hz, H-1'a and H-1'b), 5.37 (1H, dd, 6.6, 6.6 Hz, H-2'), 1.71 (3H, s, H-4'), 1.68 (3H, s, H-5'); <sup>13</sup>C NMR (100 MHz, Chloroform-d): δ 160.76 (C-2), 112.27 (C-3), 143.16 (C4), 128.40 (C-5), 112.27 (C-6), 161.62 (C-7), 100.99 (C-8), 155.28 (C-9), 111.95 (C-10), 64.94 (C-1'), 118.31 (C-2'), 138.54 (C-3'), 25.34 (C-4'), 17.80 (C-5').

#### **Herniarin (HN)**

**Data S2;** C<sub>10</sub>H<sub>8</sub>O<sub>3</sub>: <sup>1</sup>H NMR (600 MHz, Chloroform-d): δ 6.24 (1H, d, 9.0 Hz, H-3), 7.63 (1H, d, 9.7 Hz, H-4), 7.37 (1H, d, 9.0 Hz, H-5), 6.83 (1H, dd, 2.0, 8.3 Hz, H-6), 6.80 (1H, d, 2.7 Hz, H-8), 3.87(3H, 7-OCH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, Chloroform-d): δ 161.12 (C-2), 113.04 (C2), 143.36 (C-4), 128.7 (C-5), 112.52 (C-6), 162.79 (C-7), 100.80 (C-8), 155.86 (C-9), 112.48 (C-10), 55.86 (-OCH<sub>3</sub>).

#### **7-O-prenylscopoletin (PE)**

**Data S3;** C<sub>15</sub>H<sub>15</sub>O<sub>4</sub>: <sup>1</sup>H NMR (600 MHz, Chloroform-d): δ 6.24 (1H, d, 9.5 Hz, H-3), 7.60 (1H, d, 9.5 Hz, H-4), 6.84 (1H, s, H-5), 6.81 (1H, s, H-8), 4.64 (2H, d, 6.6 Hz, H-1'a and H-1'b), 5.47 (1H, dd, 6.6, 7.0 Hz, H-2'), 1.77 (3H, s, H-4'), 1.76 (3H, s, H-5'), 3.88 (3H, -OCH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, Chloroform-d): δ 161.40 (C-2), 113.19 (C-3), 143.26 (C-4), 108.12 (C-5), 146.62 (C-6), 152.11 (C-7), 101.09 (C-8), 149.85 (C-9), 111.25 (C-10), 66.18 (C-1'), 118.53 (C-2'), 138.86 (C-3'), 25.71 (C4'), 18.24 (C-5'), 56.29 (OCH<sub>3</sub>).

#### **Dimethylfraxetin (DF)**

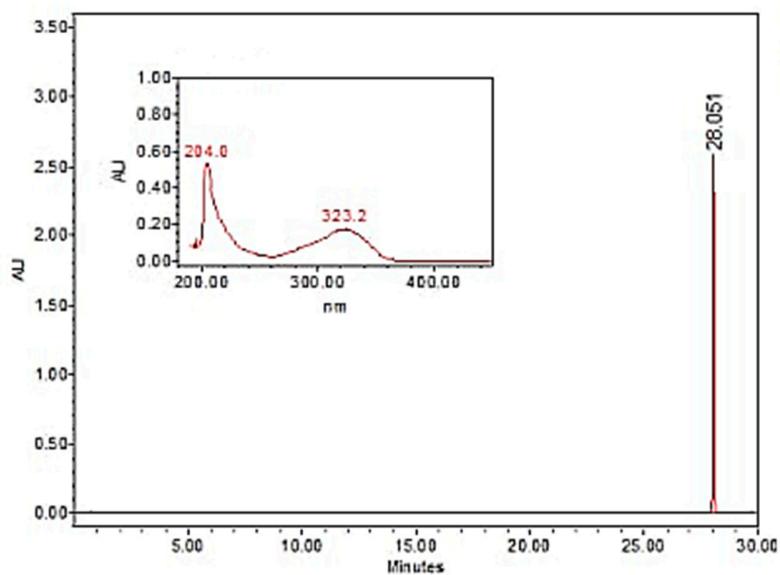
**Data S4;** C<sub>12</sub>H<sub>12</sub>O<sub>5</sub>: <sup>1</sup>H NMR (600 MHz, Chloroform-d): δ 6.32 (1H, d, 9.1 Hz, H-3), 7.60 (1H, d, 9.5 Hz, H-4), 6.67 (1H, s, H-5), 3.89 (3H, s, 6-OCH<sub>3</sub>), 3.98 (3H, s, 7- OCH<sub>3</sub>), 4.02 (3H, s, 8-OCH<sub>3</sub>); <sup>13</sup>C NMR (100

MHz, Chloroform-d):  $\delta$  160.39 (C-2), 114.3 (C-2), 143.38 (C-4), 103.8 (C-5), 150.11 (C-6), 145.96 (C-7), 141.14 (C-8), 143.03 (C-9), 115.12 (C-10), 56.31(6-OCH<sub>3</sub>), 61.44 (7- OCH<sub>3</sub>), 61.77 (8-OCH<sub>3</sub>).

### Scoparone (SC)

**Data S5;** C<sub>11</sub>H<sub>10</sub>O<sub>4</sub>: <sup>1</sup>H NMR (600 MHz, Chloroform-d):  $\delta$  6.24 (1H, d, 9.5 Hz, H-3), 7.59 (1H, d, 9.5 Hz, H-4), 6.84 (1H, s, H-5), 6.80 (1H, s, H-8), 3.88 (3H, s, 6- OCH<sub>3</sub>), 3.91 (3H, s, 7-OCH<sub>3</sub>); <sup>13</sup>C NMR (100 MHz, Chloroform-d):  $\delta$  161.26 (C-2), 113.38 (C-2), 143.22 (C-4), 108.07 (C-5), 146.3 (C-6), 152.83 (C-7), 99.83 (C-8), 149.94 (C-9), 111.37 (C-10), 56.28(6-OCH<sub>3</sub>), 56.26 (7- OCH<sub>3</sub>).

### Standardization chromatograms



**Figure S1:** Standardization chromatogram and uv spectra of IC.

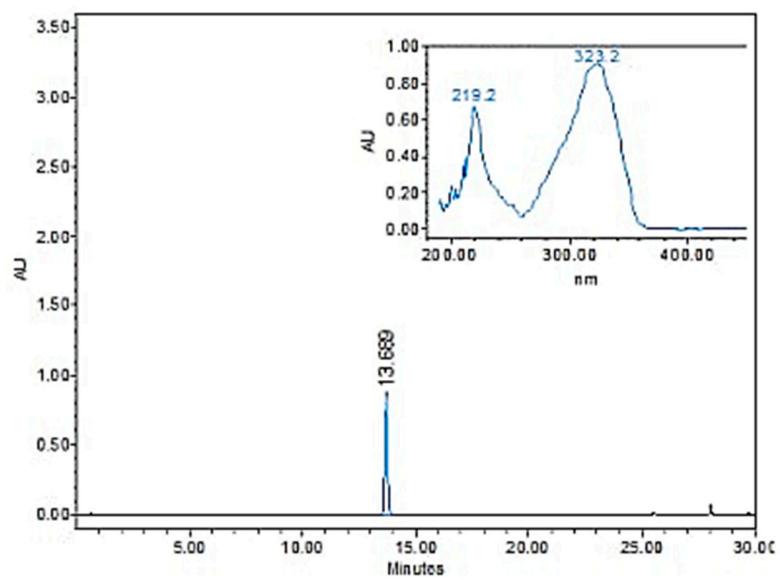


Figure S2: Standardization chromatogram and uv spectra of HN.

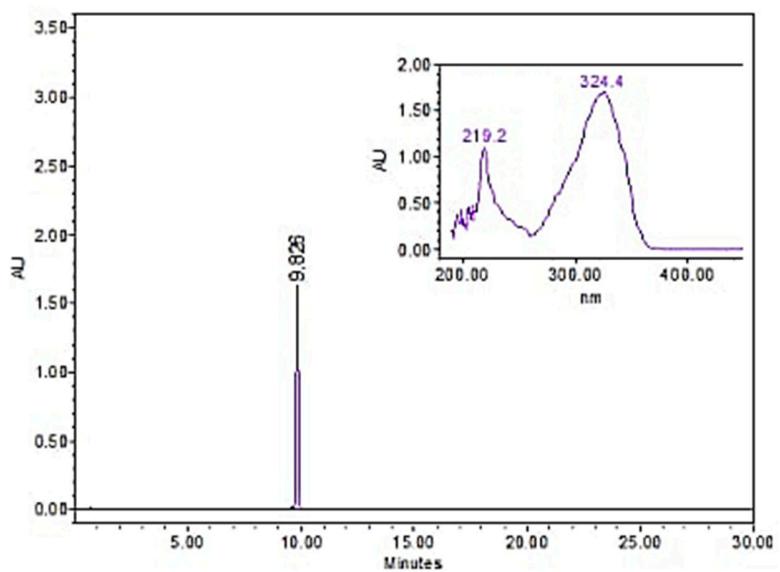


Figure S3: Standardization chromatogram and uv spectra of PE

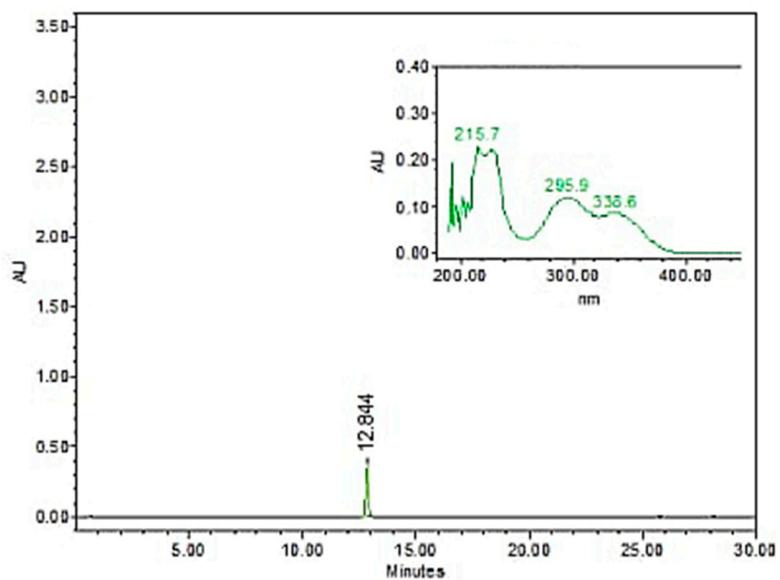


Figure S4: Standardization chromatogram and uv spectra of DF

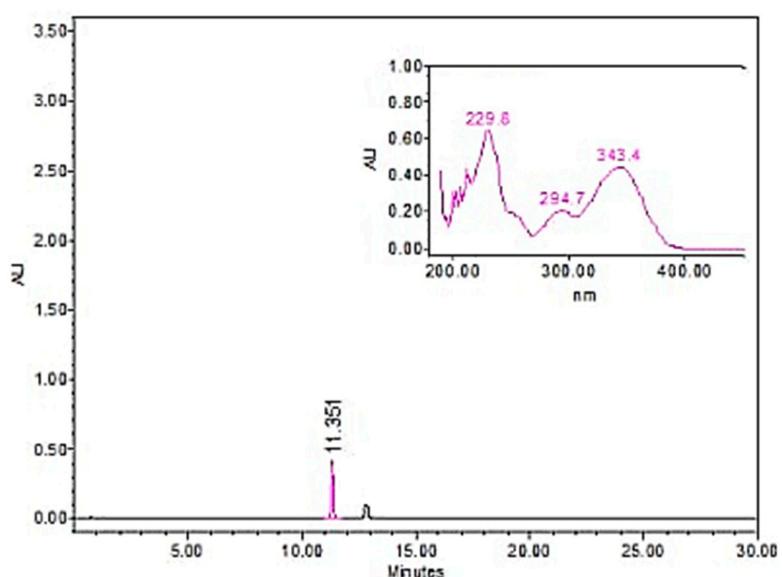


Figure S5: Standardization chromatogram and uv spectra of SC.

## Statistical análisis

**Table S1:** ANOVA test of OFT

Table Analyzed	croosings to the periphery number	Center croosings number	Total croosings number	Rearing	Number of grooming	Stool number
<b>ANOVA summary</b>						
F	16.7	10.7	16.72	49.16	57.26	7.627
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P value summary	****	****	****	****	****	****
Significant diff. among means (P < 0.05)?	Yes	Yes	Yes	Yes	Yes	Yes
R squared	0.6845	0.5815	0.6847	0.8646	0.8815	0.4976
<b>Brown-Forsythe test</b>						
F (DFn, DFd)	2.638 (10, 77)	1.209 (10, 77)	3.300 (10, 77)	1.628 (10, 77)	0.4607 (10, 77)	0.09070 (10, 77)
P value	0.0081	0.2987	0.0013	0.1145	0.9101	0.9999
P value summary	**	ns	**	ns	ns	ns
Are SDs significantly different (P < 0.05)?	Yes	No	Yes	No	No	No
<b>Bartlett's test</b>						
Bartlett's statistic (corrected)	21.93	7.955	32.33	12.31	7.339	3.625
P value	0.0154	0.6332	0.0004	0.2646	0.6931	0.9627
P value summary	*	ns	***	ns	ns	ns
Are SDs significantly different (P < 0.05)?	Yes	No	Yes	No	No	No

**Table S2:** ANOVA test of Rr

Table Analyzed	36 RPM	28 RPM	20 RPM
<b>ANOVA summary</b>			
F	25.06	43.27	28.42
P value	<0.0001	<0.0001	<0.0001
P value summary	****	****	****
Significant diff. among means (P < 0.05)?	Yes	Yes	Yes
R squared	0.6193	0.7375	0.6486
 <b>Brown-Forsythe test</b>			
F (DFn, DFd)	0.9986 (10, 154)	1.603 (10, 154)	0.8751 (10, 154)
P value	0.4472	0.1103	0.5579
P value summary	ns	ns	ns
Are SDs significantly different (P < 0.05)?	No	No	No
 <b>Bartlett's test</b>			
Bartlett's statistic (corrected)	15.02	25.03	17.04
P value	0.1313	0.0053	0.0735
P value summary	ns	**	ns
Are SDs significantly different (P < 0.05)?	No	Yes	No

**Table S3:** ANOVA test of EB

Table Analyzed	Evans Blue Brain	Evans Blue Left Kidney	Evans Blue Right Kidney	Evans Blue Spleen
<b>ANOVA summary</b>				
F	105	160.8	112.4	74.55
P value	<0.0001	<0.0001	<0.0001	<0.0001
P value summary	****	****	****	****
Significant diff. among means (P < 0.05)?	Yes	Yes	Yes	Yes
R squared	0.9317	0.9543	0.9359	0.9064
<b>Brown-Forsythe test</b>				
F (DFn, DFd)	2.691 (10, 77)	1.091 (10, 77)	0.5111 (10, 77)	0.8006 (10, 77)
P value	0.007	0.3798	0.8773	0.6284
P value summary	**	ns	ns	ns
Are SDs significantly different (P < 0.05)?	Yes	No	No	No
<b>Bartlett's test</b>				
Bartlett's statistic (corrected)	23.78	13.83	7.087	8.726
P value	0.0082	0.181	0.7172	0.5583
P value summary	**	ns	ns	ns
Are SDs significantly different (P < 0.05)?	Yes	No	No	No

**Table S4:** ANOVA test of ILs.

<b>Table Analyzed</b>	<b>IL-4</b>	<b>IL-10</b>	<b>IL-1<math>\beta</math></b>	<b>TNF-<math>\alpha</math></b>
<b>ANOVA summary</b>				
<b>F</b>	10.42	7.128	76.91	15.33
<b>P value</b>	<0.0001	<0.0001	<0.0001	<0.0001
<b>P value summary</b>	****	****	****	****
<b>Significant diff. among means (P &lt; 0.05)?</b>	Yes	Yes	Yes	Yes
<b>R squared</b>	0.6546	0.5645	0.9333	0.736
<b>Brown-Forsythe test</b>				
<b>F (DFn, DFd)</b>	0.9689 (10, 55)	0.5554 (10, 55)	0.8925 (10, 55)	5.091 (10, 55)
<b>P value</b>	0.4805	0.8425	0.5459	<0.0001
<b>P value summary</b>	ns	ns	ns	****
<b>Are SDs significantly different (P &lt; 0.05)?</b>	No	No	No	Yes
<b>Bartlett's test</b>				
<b>Bartlett's statistic (corrected)</b>	22.26	8.453	10.4	32.09
<b>P value</b>	0.0138	0.5846	0.4063	0.0004
<b>P value summary</b>	*	ns	ns	***
<b>Are SDs significantly different (P &lt; 0.05)?</b>	Yes	No	No	Yes