SUPPLEMENTARY MATERIAL

Dereplication of cytotoxic compounds from different parts of *Sophora pachycarpa* using an integrated method of HPLC, LC-MS and ¹H-NMR techniques

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Abstract:

Sophora pachycarpa Schrenk ex C.A.Mey. is an annual plant belonging to the family Fabaceae. The cytotoxic activities of methanol-dichloromethane extracts (1:1) of different parts of *S. pachycarpa* were investigated on DU145 (prostate cancer cell line) and MCF-7 (breast cancer cell line) cell lines. The root extract of *S. pachycarpa* was the only extract that showed significant cytotoxic activity with IC₅₀ values of 39.88 and 16.49 µg/mL on DU145 and MCF-7 cell lines, respectively. The root extract was then subjected to RP-HPLC for further fractionations. Among the isolated fractions from root extract, only one of them had remarkable cytotoxic effects with IC₅₀ value of 26.43 on MCF-7 and 7.54 µg/mL on DU145 cell lines. Further purification led to isolation of a compound with IC₅₀ values of 5.44 and 2.44 µg/mL on MCF-7 and DU145 cell lines, respectively. Based on ¹H NMR and ¹³C NMR spectra, together with LC-MS, the structure of the purified compound was assigned as the flavonostilbene alopecurone A.

Keywords: *Sophora pachycarpa*, alopecurone A, flavonostilbene, cytotoxicity

Table S1. ^1H -NMR and ^{13}C -NMR data for alopecurone A

Atom number	^1H NMR(125.7 MHz, δppm)	^{13}C NMR(125.7 MHz, δppm)
2	5.73 (1H, 13.2, 2)	74.1
3	3.14 ax (1H, <i>d</i> , 14.5, 3.5) 2.75 eq (1H, <i>dd</i> , 17.1, 2.5)	41.4
4	-	197.8
5	-	158.4
6	-	108.7
7	-	167.9
8	-	103.0
9	-	162.8
10	-	104.8
1'	-	117.5
2'	-	156.2
3'	6.51 (1H, <i>d</i> , 2)	103.0
4'	-	158.4
5'	6.47 (1H, <i>dd</i> , 8.3, 2)	107.2
6'	7.41 (1H, <i>d</i> , 7)	127.9
1''	-	131.1
2''	7.23 (1H, <i>d</i> , 8.8)	127.9
3''	6.87 (1H, <i>d</i> , 8.8)	115.3
4''	-	158.4
5''	6.87 (1H, <i>d</i> , 8.8)	115.3
6''	7.23 (1H, <i>d</i> , 8.8)	127.7
7''	5.51 (1H, <i>d</i> , 4.5)	94.9
8''	4.39 (1H, <i>d</i> , 4.5)	53.0
9''	-	147.0
10''	6.18 (1H, <i>d</i> , 1.5)	106.9
11''	-	158.3
12''	6.26(1H, <i>t</i> , 2)	102.0
13''	-	158.4
14''	6.18 (1H, <i>d</i> , 1.5)	106.9
1'''	2.65 (2H, <i>m</i>)	27.0
2'''	2.54(1H, <i>m</i>)	46.3
3'''	2.05 (2H, <i>m</i>)	32.1
4'''	5.01 (1H, <i>t</i> -like <i>m</i>)	123.2
5'''	-	131.1
6'''	1.57 (3H, <i>brs</i>)	25.4
7'''	1.53 (3H, <i>brs</i>)	17.7
8'''	-	147.7
9'''	4.57, 4.67 (2H, <i>brs</i>)	111.6
10'''	1.65 (3H, <i>brs</i>)	18.5

Figure. S1. LC/MS spectrum for the cytotoxic fraction (Fr 4).

Figure. S2. LC/MS spectrum of purified cytotoxic compound (alopecurone A).

Figure. S3. Chromatogram of the standard solution at 254nm.

Figure. S4. The HPLC analysis of *S. pachycarpa* extract at 254 nm and the fractionation method.