SUPPLEMENTARY MATERIAL

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

Sara Shour$^a$, Milad Iranshahy$^{a*}$, Ngoc Pham$^b$, Ronald J. Quinn$^b$ and Mehrdad Iranshahi$^{a*}$

$^a$Biotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

$^b$Eskitis Institute, Griffith University, Brisbane, Australia.

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 IC$_{50}$ 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 IC$_{50}$ 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 IC$_{50}$ values of 5.44 and 2.44 µg/mL on MCF-7 and DU145 cell lines, respectively. Based on $^1$H NMR and $^{13}$C 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. $^1$H-NMR and $^{13}$C-NMR data for alopecurone A

| Atom number | $^1$H 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, d, 14.5, 3.5) 2.75 eq (1H,dd, 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, d, 2)            | 103.0                           |
| 4'          | -                          | 158.4                           |
| 5'          | 6.47 (1H, dd, 8.3, 2)      | 107.2                           |
| 6'          | 7.41 (1H, d, 7)            | 127.9                           |
| 1"          | -                          | 131.1                           |
| 2"          | 7.23 (1H, d, 8.8)          | 127.9                           |
| 3"          | 6.87 (1H, d, 8.8)          | 115.3                           |
| 4"          | -                          | 158.4                           |
| 5"          | 6.87 (1H, d, 8.8)          | 115.3                           |
| 6"          | 7.23 (1H, d, 8.8)          | 127.7                           |
| 7"          | 5.51 (1H, d, 4.5)          | 94.9                            |
| 8"          | 4.39 (1H, d, 4.5)          | 53.0                            |
| 9"          | -                          | 147.0                           |
| 10"         | 6.18 (1H, d, 1.5)          | 106.9                           |
| 11"         | -                          | 158.3                           |
| 12"         | 6.26 (1H, t, 2)            | 102.0                           |
| 13"         | -                          | 158.4                           |
| 14"         | 6.18 (1H, d, 1.5)          | 106.9                           |
| 1""         | 2.65 (2H, m)               | 27.0                            |
| 2""         | 2.54 (1H, m)               | 46.3                            |
| 3""         | 2.05 (2H, m)               | 32.1                            |
| 4""         | 5.01 (1H, t-like m)        | 123.2                           |
| 5""         | -                          | 131.1                           |
| 6""         | 1.57 (3H, brs)             | 25.4                            |
| 7""         | 1.53 (3H, brs)             | 17.7                            |
| 8""         | -                          | 147.7                           |
| 9""         | 4.57, 4.67 (2H, brs)       | 111.6                           |
| 10""        | 1.65 (3H, brs)             | 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.