SUPPLEMENTARY MATERIAL

Role of the flavonoid-rich fraction in the antioxidant and cytotoxic activities of *Bauhinia forficata* Link. (Fabaceae) leaves extract

Natalizia Miceli a*, Luigina Pasqualina Buongiorno a, Maria Grazia Celi a, Francesco Cacciola b, Paola Dugo acd, Paola Donato b, Luigi Mondello acd, Irene Bonaccorsi e and Maria Fernanda Taviano a

a “Scienze del Farmaco e Prodotti per la Salute” Department, University of Messina, Viale Annunziata, 98168 Messina, Italy
b “Scienze dell'Ambiente, della Sicurezza, del Territorio, degli Alimenti e della Salute” Department, University of Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy.
c Chromaleont s.r.l., c/o “Scienze del Farmaco e Prodotti per la Salute” Department, University of Messina, Viale Annunziata, 98168 Messina, Italy.
d Centro Integrato di Ricerca, University Campus Bio-Medico of Rome, Via Álvaro del Portillo 21, 00128 Roma, Italy
e “Patologia Umana” Department, University of Messina, A.O.U. Policlinico "G.Martino" Via Consolare Valeria 1, 98124 Messina, Italy

ABSTRACT

*Bauhinia forficata* Link. is utilized as an antidiabetic in Brazilian folk-medicine; further, its antioxidant properties suggest a potential usefulness in the prevention of diabetes complications associated with oxidative stress. The contribution of a flavonoid-rich fraction (FRF), HPLC-PDA-ESI-MS characterized, to the antioxidant and cytotoxic properties of *B. forficata* hydro-alcoholic leaves extract was evaluated for the first time. Both extract and FRF showed radical scavenging activity and reducing power with a strong relationship with the flavonoid content found; hence, flavonoids are mainly responsible for the primary antioxidant activity of *B. forficata* extract. The extract significantly decreased FO-1 cells viability at the higher concentrations. FRF didn’t exert any effect; thus, flavonoids seem not responsible for the cytotoxicity of the extract. The extract resulted virtually non-toxic against both *Artemia salina* and normal human lymphocytes, demonstrating potential selectivity in inhibiting cancer cell growth. Finally, no antimicrobial activity was observed against the bacteria and yeasts tested.

Keywords: *Bauhinia forficata* Link.; Flavonoid-rich fraction; Phenolic profile; Antioxidant activity; Cytotoxic activity.
Contents of supplementary data:

**Figure S1.** HPLC-PDA-ESI-MS polyphenolic fingerprint of *B. forficata* leaves hydro-alcoholic extract and flavonoid-rich fraction (FRF).

**Figure S2.** Free radical scavenging activity of *B. forficata* leaves extract, *n*-hexane fraction (HF) and flavonoid-rich fraction (FRF).

**Figure S3.** Reducing power of *B. forficata* leaves extract, *n*-hexane fraction (HF) and flavonoid-rich fraction (FRF).

**Figure S4.** Chelating activity of *B. forficata* leaves extract, *n*-hexane fraction (HF) and flavonoid-rich fraction (FRF).
Figure S1. HPLC-PDA-ESI-MS (negative ionization mode) polyphenolic fingerprint of (A) B. forficata leaves hydro-alcoholic extract and (B) flavonoid-rich fraction (FRF). Column: C18, 15 cm x 4.6 mm, 2.7 μm particles (Ascentis® Express). For peak identification, see Table 1.
Figure S2. Free radical scavenging activity of *B. forficata* leaves extract, *n*-hexane fraction (HF) and flavonoid-rich fraction (FRF), measured by the DPPH method. The results were obtained from the average of three independent experiments and are expressed as the mean percentage (%) ± SD.
Figure S3. Reducing power of *B. forficata* leaves extract, *n*-hexane fraction (HF) and flavonoid-rich fraction (FRF), evaluated by spectrophotometric detection of Fe^{3+}-Fe^{2+} transformation method. The results were obtained from the average of three independent experiments and are expressed as the mean absorbance ± SD.
Figure S4. Chelating activity of *B. forficata* leaves extract, *n*-hexane fraction (HF) and flavonoid-rich fraction (FRF), measured by inhibition of ferrozine-Fe$^{2+}$ complex formation. The results were obtained from the average of three independent experiments and are expressed as the mean percentage (%) ± SD.