Chapter 2
Experimental

2.1 Reagents

2,2-Di(tert-octylphenyl)-1-picryhydrazyl (DOPH) was purchased from Aldrich (Milwaukee WI, USA). DPPH, rutin hydrate, and quercetin dihydrate were purchased from Sigma-Aldrich Chemie GmbH (Germany). Ascorbyl palmitate and trolox were from purchased Fluka (Switzerland). Potassium dihydrogen phosphate, AR grade, was purchased from Merck (Germany). Phosphoric acid 85%, AR grade, was purchased from Carlo Erba (Italy). Acetonitrile and methanol, both HPLC grade, were purchased from Labscan Asia Co. Ltd. (Thailand). All water used was deionized.

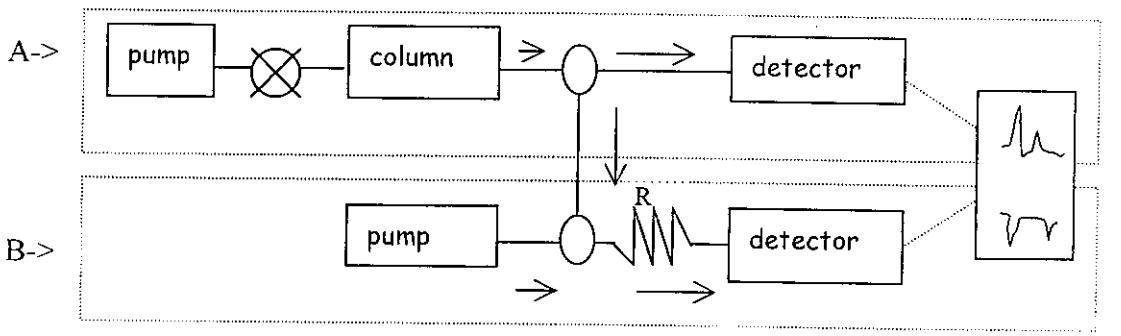
2.2 Apparatus

The HPLC was coupled on-line with colorimetric detection for antioxidant activity as shown in Fig 1. The apparatus is described as follows.

Line A, HPLC system, consisted of a pump, type 3200 (TSP, USA), an injector, type 7725i with 10 µl loop (Rheodyne, USA), a UV-VIS detector model 785A (Perkin Elmer, USA) and a flatbed, double pen recorder, model BD 112□(Kipp & Zonen, the Netherlands). The I.D. of the post-column tubing was 0.254 mm. A 20-cm length of 0.064 mm I.D. PEEK tubing coupled the line A to the line B by a T-connector; the column effluent was split into two streams with the flow ratio of 8:2, with the minor stream going to line B.

Line B represents the flow injection analysis (FIA) system for antioxidant activity detection. It consisted of a pump, type 3500 (TSP, USA), a home-made knitted-shape reactor, UV-VIS detector model 9050 (Varian, USA), which was connected to the double pen recorder of line A.

Fig.1 Scheme of the HPLC coupled with FIA detection system; A is the HPLC-line; B is the FIA-line. R is the reactor. The arrows indicate flow directions.



2.3. Conditions of HPLC and FIA systems

Line A, HPLC system: a LiChrospher 60 RP-8 select B column (125 x 4 mm, 5 µm, Merck, Germany) was used, combined with a precolumn, (45 x 3.9 mm), home-packed with LiChrosorb RP-18, 10 µm, (Merck, Germany). The mobile phase was MeCN:MeOH:25mM KH_2PO_4 pH 3.0 20:15:65 (v/v/v) or 0:50:50 (v/v/v). The pump in the HPLC-line was set at 1ml min^{-1}. The detector was set at 220 nm.

LineB, FIA system: After optimization, the conditions for the FIA were as follows: the reactor consisted of a 70 cm long, 0.8 mm I.D., knitted PTFE tubing. The carrier stream solution was 0.1 mM DOPH or DPPH in MeOH. The pump was set at

0.5 ml min^{-1}. The detector in line B was set at 515 nm for the experiments using DPPH and at 538 nm for the experiments using DOPH as the FIA reagent.

2.4. Sample Preparation

Sophora japonica L. dried flowers and *Morus alba* L. dried leaves were bought from the local market in Phitsanulok, Thailand. The plant materials were ground and macerated with methanol at ambient temperature (about 28° C) for 3 days. The ratio of methanol:plant material was 50 ml g^{-1}. The plant extracts were filtered and evaporated to dryness under reduced pressure at temperature below 50° C. The dried extracts were dissolved in methanol to a concentration of 10 mg ml^{-1} and filtered over 45 μm nylon membrane before injected into HPLC system.