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<tr>
<td><strong>Title</strong></td>
<td>Isolation, characterisation and antioxidative capacity of flavonoids from balsam flowers and laksa leaves</td>
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Summary

Modern scientific researches have proven that plants contain plenty of antioxidants that can combat over-produced reactive oxygen species. It has been reported that the roles of fruits, vegetables and red wine in disease prevention have been attributed, in part, to the antioxidant properties of their constituents including vitamins C and E, carotenoids, phenolic acids, flavonoids and so on.

Flavonoids possess unusual structures that contribute to their characteristic chemical properties so as to become promising antioxidants. Their diversity and abundance in plants perhaps would give us a chance to find out certain potential applications in the fields of biology and pharmacy. Fortunately, Singapore, located in the tropics and richly endowed by nature, is full of diverse and numerous kinds of plants. These cheap and plentiful plants/herbs offer us so much convenience to undertake studies on antioxidants from the plants. Thus, our research project was started with a study on flavonoids from local plants.

The main objectives of this study are (a) to isolate and purify flavonoids from two selected local plants, Balsam flowers and Laksa leaves, (b) to identify the main flavonoids present in each of the above-mentioned plants, and (c) to measure the antioxidative capability of the identified flavonoids by ESR and ABTS-UV/VIS evaluations.

Isolation, purification and identification of flavonoids from plants were time & labor-consuming work, but they are indispensable and very important. A series of applicable experiments were conducted for this purpose: (1) In order to check
component composition of plants and to determine which method was used for further isolation, a screening by a HPLC-online-UV assay was carried out before relative larger scale isolation. (2) For a material with simple composition, such as Balsam flowers, a direct preparative HPLC isolation was undertaken; while for a material with complex composition such as Laksa leaves, pre-isolation was done by using classical polyamide chromatograph column prior to subsequent preparative HPLC isolations. (3) Some compounds isolated by previous procedures needed further purification although the purity of most isolated compounds was satisfactory for follow-up work. (4) Identification of final products was completed by elucidating various data from HPLC-online-UV spectrometry, LC-MS and NMR based on the knowledge on chemistry, phytochemistry and botany.

As a result, four flavonoids belonging to flavon-3-ol family were isolated and identified from dried Balsam flowers. While, ten flavonoids belonging to flavonol and flavone were also isolated from dried Laksa leaves and identified by the same elucidation approaches. They are:

**From dried Balsam flowers:**
- Kaempferol (Aglycone)
- Kaempferol-3-glucoside
- Kaempferol-3-p-coumaroyl glucoside
- Kaempferol-3-glucosyl rhamnoside

**From dried Laksa leaves:**
- 3-O-α-L-rhamnopyranosyloxy-3',4',5,7-tetrahydroxyflavone (quercitrin)
- 3-O-β-D-glucopyranosyloxy-4',5,7-trihydroxyflavone (kaempferol-3-glycoside; Astragalin)
Scutillarein 7-O-β-D-glucopyranoside or 6-hydroxyapigenin
6'-O-(3,4,5-trihydroxybenzoyl) 3-O-β-D-glucopyranosyloxy-3', 4', 5, 7-tetra-hydroxyflavone (kaempferol-3-glycoside; Astragalin)
Scutillarein (aglycone)
6-hydroxyluteolin; 3',4',5,6,7-pentahydroxyflavone
6-hydroxyluteolin-7-O-β-D-glucopyranoside
Quercetin 3-O-β-D-glucopyranoside or quercetin-3-glucuronide
2''-O-(3,4,5-trihydroxybenzoyl) quercitrin; galloyl quercitrin
Quercetin

Antioxidative property of isolated flavonoids is the main concern of this study. Several methods were used for evaluating antioxidative activity of the flavonoids. The data from ESR assays in PBN-AI and Et-H$_2$O$_2$-HRP systems showed that the flavonoids possess strong antioxidative activity. Moreover, in the quantitative ABTS UV-VIS measurements all these flavonoids showed more powerful antioxidative capability than Trolox (an antioxidant standard equivalent to vitamin C on a molar basis in aqueous solution in vitro).

Quantitative measurement of antioxidative capability is essential to evaluate an antioxidant. A convenient and easy-controlling method for assaying Trolox equivalent antioxidant capability (TEAC) of antioxidants (ABTS UV-VIS spectrometry) was utilized for this propose after the analytical condition was optimized entirely. In terms of TEAC values, the antioxidative capabilities of various isolated flavonoids were compared on a molar basis.
Certain conclusions were drawn by elucidating the relationship between structures of the isolated flavonoids and the antioxidative capability. The following are the major ones:

(1) For the kaempferol compounds (isolated from Balsam flowers), their TEAC values are nearly the same, although kaempferol aglycone showed a bit higher TEAC value than its derivatives. This implies that, in the view of molecular structure, a water-soluble group such as commonly occurring sugar or sugars attached to 3 position linked by a \(-O-\) bond has less effect on antioxidative potential, although solubility of the kaempferol derivatives in water was improved by attaching some water-soluble groups (e.g. sugars) as compared with kaempferol aglycone.

(2) A very powerful antioxidant, 2’’-O-(3,4,5-trihydroxybenzoyl) quercitrin (TEAC=6.14, the TEAC value of vitamin C is 0.99) was found in dried Laksa leaves.

(3) Esterification of flavonoids, where gallic acid is attached to flavonoid nuclei at 3-position by ester linkage via a sugar, can enhance the antioxidative activity.

(4) A combination of two flavonoids cannot significantly improve the antioxidative potential of the mixture, that is, synergic antioxidative activity of two flavonoid compounds is not remarkable.