Quercetin from methanol extracts of the leaves of *Ficus benghalensis*

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Abstract:
Flavonoids are ubiquitous in photosynthesising cells and are commonly found in fruit, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey. For centuries, preparations containing these compounds as the principal physiologically active constituents have been used to treat human diseases. Increasingly, this class of natural products is becoming the subject of anti-infective research and many groups have isolated and identified the structures of flavonoids. In this study we concerned on determination flavonoids content from methanolic extracts of *Ficus benghalensis* using catechol and quercetin as standard. The result shows high content of quercetin in methanolic extracts.

Keywords: flavonoids, quercetin, moraceae, extracts.

Introduction
Quercetin belongs to a group of plant pigments called flavonoids that give many fruits, flowers and vegetables their colors. Flavonoids, such as quercetin, are antioxidants. They scavenge particles in the body known as free radicals which damage cell membranes, tamper with DNA and even cause cell death. Antioxidants can neutralize free radicals. They may reduce or even help prevent some of the damage free radicals cause. Quercetin may help protect against heart disease and cancer and it can also help stabilize the cells that release histamine in the body and thereby have an anti-inflammatory and antihistamine effect. It is categorized as a flavonol, one of the six subclasses of flavonoid compounds. The name has been used since 1857 and is derived from quercetum. It is a naturally occurring polar auxin transport inhibitor (Davis et al., 2009; Aguirre et al., 2011; Fischer et al., 1997). The common forms of quercetin were shown in Figure 1.

Quercetin is an aglycone lacking an attached sugar. A quercetin glycoside is formed by attaching a glycosyl group (a sugar such as glucose, rhamnose or rutinosine) as a replacement for one of the OH groups (commonly at position 3). The attached glycosyl group can change the solubility absorption and in vivo effects. As a general rule of thumb, the presence of a glycosyl group (quercetin glycoside) results in increased water solubility compared to quercetin aglycone (Ross & Kasum, 2002; Hollman et al., 1999). Quercetin-type flavonols (primarily as quercetin glycosides), the most abundant of the flavonoid molecules are widely distributed in plants. They are found in a variety of foods including apples, grapes, onions, shallots, tea and tomatoes, as well as many seeds, nuts, flowers, barks and leaves. It is also found in medicinal botanicals, including *Ginkgo biloba*, *Hypericum perforatum* and *Sambucus canadensis* (Häkkinen et al., 1999; Williamson & Manach, 2005; Wiczkowski et al., 2008). In red onions, higher concentrations of quercetin occur in the
outermost rings and in the part closest to the root, the latter being the part of the plant with the highest concentration (Smith et al., 2003). One study found that organically grown tomatoes had 79% more quercetin than chemically grown fruit (Mitchell et al., 2007). Quercetin is present in various kinds of honey from different plant sources (Petrus, Schwartz, & Sontag, 2011). Food-based sources of quercetin include vegetables, fruits, berries, nuts, beverages and other products of plant origin (Tutel’ian & Lashneva, 2013). In the determined food, the highest concentration is 234 mg/100 g of edible portion in capers (raw), the lowest concentration is 2 mg/100 g of edible portion in black or green tea (Bhagwat, Haytowits & Holden, 2011).

Dietary intake of quercetin was different in several countries. The estimated flavonoid intake ranges from 50 to 800 mg/day (quercetin accounts for 75%), mostly depending on the consumption of fruits and vegetables and the intake of tea (Chun, Chung & Song, 2007; Sun et al., 2013).

Figure 1. Flavonoids Compounds from Petroselinum crispum L.
al., 2015; Zhang et al., 2010). Onions, tea and apples contained high amounts of quercetin (Sampson et al., 2002). In Japan, the average and median quercetin intakes were 16.2 and 15.5 mg/day, respectively, the quercetin intake by men was lower than that by women and the quercetin intakes showed a low correlation with age in both men and women. The estimated quercetin intake was similar during summer and winter. In Australia, black and green teas were the dominant sources of quercetin. Other sources included onion, broccoli, apple, grape and beans (Nishimuro et al., 2015; Somerset, S. & Johannot, 2008). In Spain, the average daily intake of quercetin is 18.48 mg/day, which is significantly higher than that in the United States (9.75 mg/day), based on sources like tea, citrus fruits and juice, beers and ales, wines, melon, apples, onions, berries and bananas (Zamora et al., 2010).

Figure 2. Fresh Leaves of Ficus benghalensis

Materials and methods

Plant Materials

The leaves of Ficus benghalensis were collected from Alkurmah State in the morning, then plant material was dried at room temperature in April 2023. The dried and finely ground samples of the leaves (48g) were extracted with petroleum ether (40-80°C) for defatting of fatty acids then the dried residues was extracted with methanol solvents by maceration (48 hours). The solvents removed under vacuum at temperature below 50°C. Each extract was prepared just before the experiment to prevent any further degradation.

General Material and Methods

Column chromatography and analytical TLC were carried out using silica gel, Merck 230-400 mesh and Merck 70-230 mesh. Melting points were determined on a Kofler hot-stage microscope melting point apparatus and were uncorrected. ¹H and ¹³C-NMR were recorded on JEOL ANM-GSX 400 spectrometer. Mass spectra were obtained using GCMS-QP 5050 A Shimadzu mass spectrometer.

Extraction and Isolation of Quercetin

The leaves of Ficus benghalensis were air-dried, ground and subjected to extraction with petroleum ether (40-80°C) and methanol successively. The solvents were then removed under reduced pressure to give the following extracts as shown in table 1.

Table 1. The Extracts of the Leaves of Ficus benghalensis

<table>
<thead>
<tr>
<th>Material (g)</th>
<th>Solvent</th>
<th>Colour and nature of extracts</th>
<th>Yield of extracts (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves (2400)</td>
<td>petroleum ether</td>
<td>yellowish-brown, semi-solid</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td>methanol</td>
<td>dark-brown, solid</td>
<td>48.4</td>
</tr>
</tbody>
</table>

Isolation of Quercetin

The crude methanol extract (24.4g) was subjected to silica gel column chromatography using gradient solvent mixtures of 100% petroleum ether, petroleum ether/chloroform (1:1), 100% chloroform and chloroform / methanol (5:2) to give 15 fractions. Fractions 8 and 14 which gave the same values (0.35, chloroform:methanol, 3:1) on TLC were combined together and resubjected to column chromatography with chloroform and methanol (1:1) to give quercetin as yellow color (42.3 mg) with melting point 314°C (Scholz and Williamson 2007, 316°C) (Scholz & Williamson, 2007).
Results and Discussion

The present study has shown the compound obtained from methanol extracts of the leaves of *Ficus benghalensis* was being quercetin according to interpretation of the spectral data of 1H-NMR and 13C-NMR as shown in figure 3 and figure 4 respectively.

In 1H-NMR spectrum there were five protons in the aromatic region appearing at singlets at δ 7, 7.1, 7.4, 8.2 and 12 assigned for proton at C-3, C-3', C-4, C-7 and C-5 respectively. The two sharp singlets in the downfield region at δ 8.2 and 12 were assigned to the two chelated hydroxyl groups at positions 5 and 7. The 13C-NMR spectrum indicated the presence of fifteen carbons in the structure. The mass spectrum gave a molecular ion peak at m/z 302 which corresponded to the molecular formula C15H10O7. On comparison the spectral with published data (Day et al., 2000) suggested the structure to be 3, 3', 4', 5, 7-pentahydroxyflvanone (quercetin).

![Figure 3. 1H-NMR for quercetin](image1)

![Figure 4. 13C-NMR for quercetin](image2)
Conclusions

Isolation of phytochemical compounds often leads to possible synthesis in the laboratory and on the industrial scale. The methanol extract of leaves shows higher flavonoids content. Therefore, the plant is an excellent source of quercetin according to the percentage yield.

References


