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Short communication

# Identification and quantitative HPLC analysis of the main flavonoids present in weld (*Reseda luteola* L.)

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**Abstract**

In this paper we present a more sensitive quantification HPLC method for analyzing the main flavonoids of weld (*Reseda luteola* L.). Several extraction temperatures and solvent mixtures have been compared, and the extraction kinetics of the three main flavonoids of weld (luteolin, luteolin-7-glucoside and luteolin-3′,7-diglucoside) are provided. After a 15 min extraction in a methanol/water mixture we obtained 0.448% luteolin, 0.357% luteolin-7-glucoside and 0.233% luteolin-3′,7-diglucoside (yields reported by mass of dried plant).


*Keywords:* *Reseda luteola* L.; Luteolin; Luteolin-7-glucoside; Luteolin-3′,7-diglucoside; Natural dyes; HPLC

## 1. Introduction

Since prehistoric times natural dyes have been used for many purposes: coloring natural fibers such wool, cotton and silk, as well as fur and leather. They also served to color cosmetic products and to produce inks, watercolors and artist's paints.

In the mid 1960s an international awareness of environment, ecology and pollution control has created an upsurge in the interest in natural dyes. Recently, the dye industry is more and more forced to reduce toxic effluents and to stop the production of potentially dangerous dyes or pigments [1].

The great abundance and wide geographic distribution over the world (Europe, Western Asia and North America) made of weld (*Reseda luteola* L.) one of the first and most used yellow dyes [2].

While their methods of application, the kind and the quantities of mordant used may have differed widely in the countries where weld was employed, their results appear to have been about the same: both vegetable and animal fibers could be given a fast yellow color, with shades from gold to olive [3].

The first serious competitor for weld was quercitron (oak bark) and later came the aniline dyes, which caused weld to be almost abandoned before 1880.

In 1833 Chevreul [4] investigated the nature of the organic coloring matters in *R. luteola*. He pointed out that the active principle is a yellow

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crystalline substance, which he called luteolin (5,7,3′,4′-tetrahydroxyflavone). In 1896 Perkin [5] confirmed the presence of luteolin in weld.

In 1955 Paris [6] isolated a luteolin glycoside as the main flavonoid present in the floriferous stems of fresh weld. This flavone was characterized and identified later as luteolin-7-glucoside [7].

A new diglycoside from *R. luteola* was isolated in 1961 by Goris et al. [8]: luteolin-3′,7-diglucoside. Its structure, as well as the presence of another monoglycoside, luteolin-3′-glucoside, were confirmed later [9].

The chemical structures of luteolin and its mono and diglycosides identified in *R. luteola* are presented in Fig. 1.

Whereas information on flavonoids present in weld is available, only few reports on the quantitative determination of these compounds have been done. In 1972 flavonoids of *R. luteola* were separated by Thin Layer Chromatography (TLC) and quantified by direct densitometry determination [10]. In 1993, quantitative determination of flavonoid content of weld was carried out using a spectrophotometric assay [11].

None of the techniques used heretofore has been accurate enough for the flavonoid quantitative determination. In this paper we present a sensitive quantification method for analyzing the main flavonoids of weld (*R. luteola* L.), based on High Performance Liquid Chromatography (HPLC). Several extraction temperatures and solvent mixtures have been compared, and the extraction kinetics for the three main flavonoids of weld (luteolin, luteolin-7-glucoside and luteolin-3′,7-diglucoside) are presented.

## 2. Experimental

### 2.1. Materials

The aerial parts of weld (*R. luteola* L.) collected in Ariege (South of France) were used. After drying, the plant was ground in a laboratory mill and then sifted.

The three flavonoids used for the standard curves as well as the internal standard, rutin, all being of analytical grade, were purchased from Extrasynthese (France).

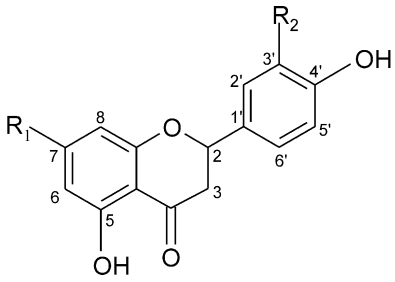
### 2.2. Methods

#### 2.2.1. Flavonoid extraction

The extraction of flavonoid compounds from weld was realized in different solvents: methanol, ethanol, water, a methanol/water mixture (8/2 v/v) and an ethanol/water mixture (8/2 v/v). After HPLC analysis of the extracts, the most appropriate extraction solvent was chosen. Extraction was realized at two temperatures: room and boiling temperatures. The extraction time, varying between 5 and 240 min, was optimized according to the HPLC quantitative analysis results.

#### 2.2.2. HPLC analysis

HPLC analysis was performed using a LDC Milon Roy CM 4000 gradient pump coupled to a Hewlett Packard 1100 diode-array detector. Flavonoid separation was carried out in a 5 μm Chrompack C18 column 250 mm×4.6 mm, protected by a Chrompack C18 pre-column 3.0 mm×10 mm. Plant extracts were eluted at 1 ml/min




luteolin [491-70-3] : $R_1$ = OH et $R_2$ = OH

luteolin-7-glucoside [5373-11-5] : $R_1$ = O-glucose et $R_2$ = OH

luteolin-3'-glucoside [5154-41-6]: $R_1$ = OH et $R_2$ = O-glucose

luteolin-3',7-diglucoside [52187-80-1] : $R_1$ = $R_2$ = O-glucose

apigenin [520-36-5] : $R_1$ = OH et $R_2$ = H

Fig. 1. Chemical structure of the main flavonoids of weld (*Reseda luteola* L.).

(20 μl injection volume) using as mobile phase a binary solvent system consisting in methanol and a water/acetic acid mixture (100/1 v/v). The gradient scheme is presented in Table 1.

Flavonoids were monitored by UV absorbance at 350 nm. Their UV/visible absorption spectra are presented in Fig. 2. All chromatographic procedures were performed at 25 °C. The amount of luteolin, luteolin-7-glucoside and luteolin-3′,7-diglucoside were estimated from standard curves obtained by analysis of various doses of authentic compounds. Hewlett Packard Chemstation software analyzed our results.

## 3. Results and discussions

The HPLC analysis showed the presence of three main flavonoid compounds. The bibliographical data, the UV/VIS spectra and the retention times permitted the identification of these flavonoids: luteolin, luteolin-7-glucoside and luteolin-3′,7-diglucoside.

The study showed that the main flavonoid of the weld extract is luteolin and not the glycosidic compounds as expected. This fact can be explained by a possible enzymatic hydrolysis during the pre-treatment of the aerial plant parts (drying, grinding). Two other flavonoids, luteolin-3′-glucoside and apigenin, reported in the literature as weld flavonoid compounds [12], were not identified in our extracts.

In this study the quantification of the flavonoids of weld (*R. luteola* L.) was carried out by HPLC coupled to a diode-array detector. Previous studies used as analytic method the thin-layer chromatography (TLC) coupled to a Densitometer [10] and the UV/VIS spectrophotometry [11]. These two methods are less accurate than HPLC.

However, the results obtained in this work can not be compared with the precedent ones. The first quantification study, by TLC/densitometry, used the inflorescence and the roots of weld, and not the entire plant, so the flavonoid content is higher (1.82% luteolin and 6.41% luteolin-7-glucoside) [10]. In addition to this, the analytic method is not

Table 1
HPLC gradient scheme

| Time/min | Initial | 25 | 40 | 50 | 55 | 60 |
|---|---|---|---|---|---|---|
| MeOH/% | 5 | 40 | 60 | 90 | 90 | 5 |

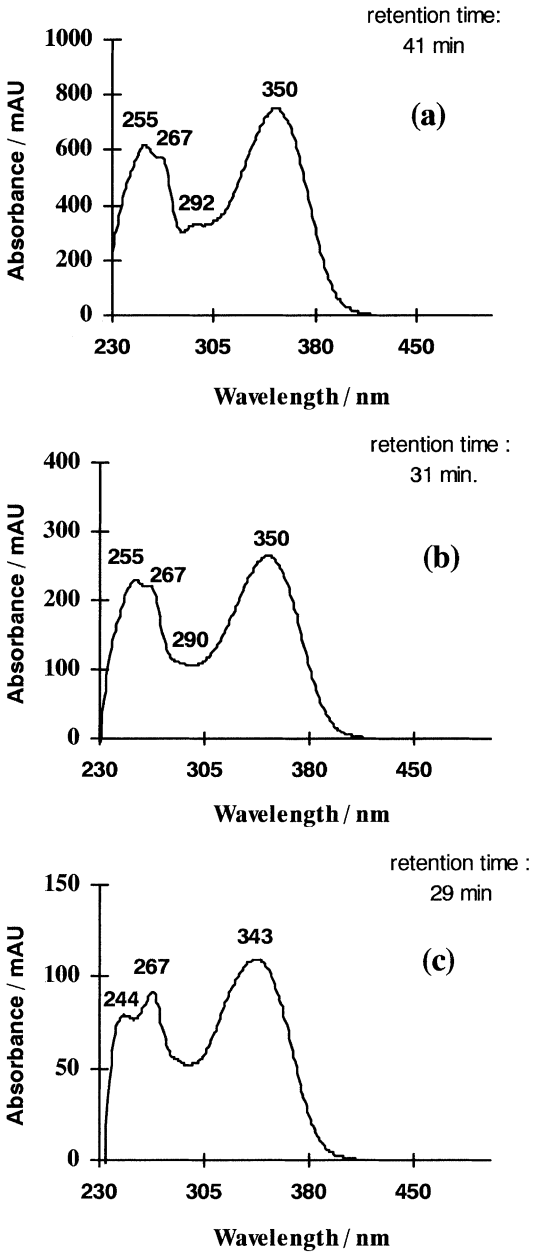



Fig. 2. UV/VIS spectra and the retention time for the three main weld flavonoids. (a) luteolin-3′,7-diglucoside; (b) luteolin-7-glucoside; (c) luteolin.

very exact because it is possible to obtain an absorbance that is caused by the presence of more than one compound absorbing at the same spot. The second quantification study, by a UV/VIS spectrophotometer, presented a flavonoid content of 1.9% [11]. This study was a global one, reported for a unique compound—luteolin—so, once again, the quantification was not very accurate for the same reasons invoiced for the TLC quantification.

The HPLC quantification using an internal standard is, for the moment, the most precise

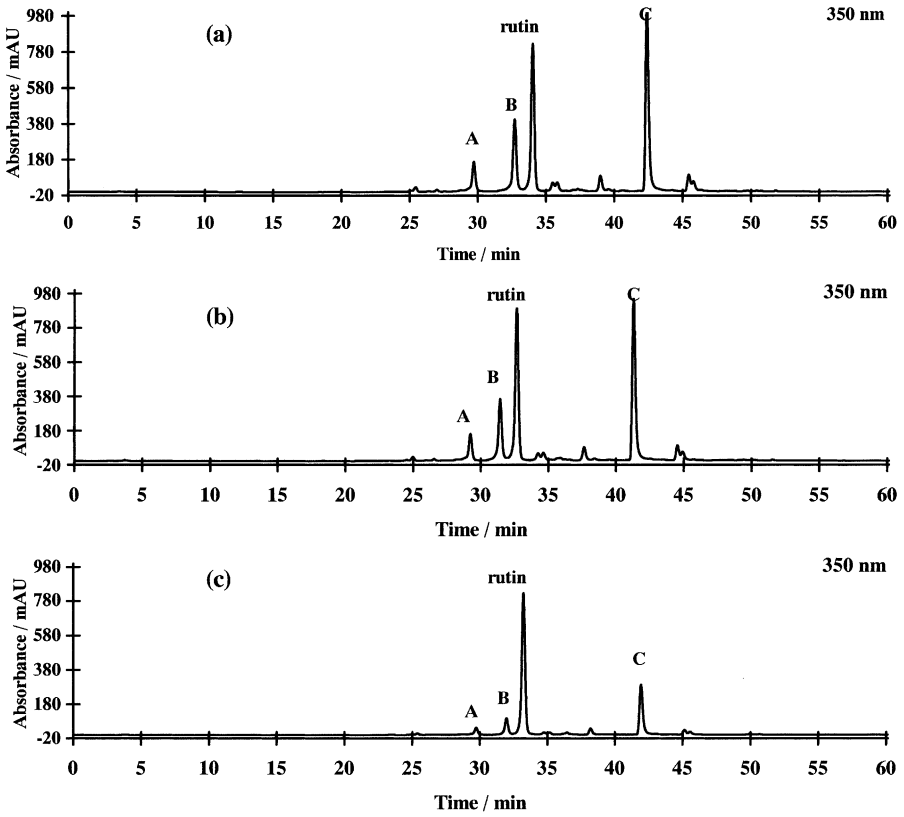



Fig. 3. HPLC analysis of (a) a methanol extract, (b) a methanol/water extract and (c) a water extract. (A) Luteolin-3′,7-diglucoside; (B) luteolin-7-glucoside; (C) luteolin.

Table 2
Yields (g/100 g dry plant) of the three weld flavonoids as a function of extraction time, at room temperature and boiling temperature

| | Room temperature | | | Boiling temperature | | |
|---|---|---|---|---|---|---|
| Extraction time/min | Luteolin yield | Luteolin-7-glucoside yield | Luteolin-3′,7-diglucoside yield | Luteolin yield | Luteolin-7-glucoside yield | Luteolin-3′,7-diglucoside yield |
| 5 | 0.194 | 0.151 | 0.044 | 0.364 | 0.294 | 0.171 |
| 15 | 0.202 | 0.165 | 0.079 | 0.448 | 0.357 | 0.233 |
| 30 | 0.238 | 0.183 | 0.086 | 0.456 | 0.363 | 0.239 |
| 60 | 0.346 | 0.226 | 0.124 | 0.443 | 0.366 | 0.241 |
| 120 | 0.370 | 0.292 | 0.167 | 0.429 | 0.359 | 0.246 |
| 240 | 0.396 | 0.271 | 0.161 | 0.422 | 0.358 | 0.242 |

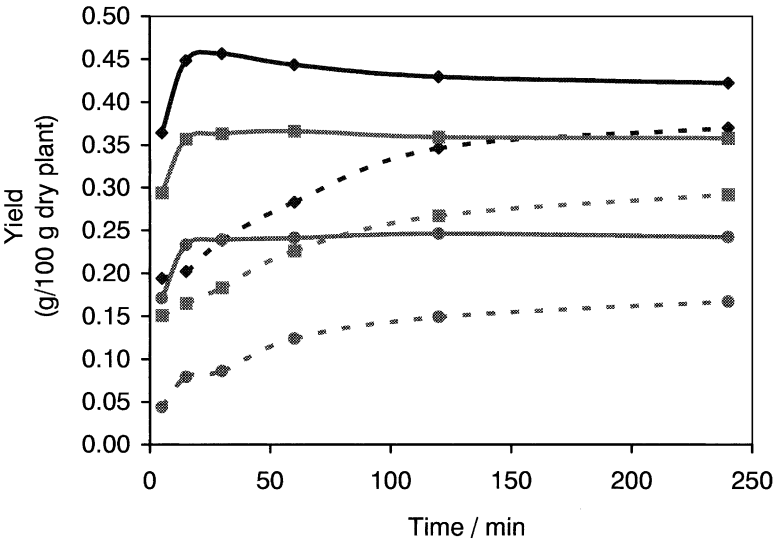



Fig. 4. Luteolin (◆), luteolin-7-glucoside (■) and luteolin-3′,7-diglucoside (●) yields as a function of extraction time at room temperature (- - -) and at the boil (——).

analytical method for the flavonoid compounds of weld (*R. luteola* L.). It allows the identification and the very accurate quantification of the three main flavonoids in the same time.

Fig. 3 presents extract chromatograms obtained in different solvents. The extract composition is the same in each case, but the flavonoid yields are different. After the quantification of the flavonoid compounds in each extract (methanol, ethanol, water, ethanol/water and methanol/water), the methanol/water mixture (8/2 v/v) gave the highest flavonoid yield.

Knowing the extraction environment, we could determinate the extraction time and temperature. In Table 2, we present the extraction yields (g/100 g dry plant) for luteolin, luteolin-7-glucoside and luteolin-3′,7-diglucoside, in function of extraction time and temperature.

The extraction kinetic is presented in Fig. 4. At room temperature, flavonoid extraction yields increased with the extraction time. The best yield was obtained after 240 min. At boiling temperature, after 15 min, the flavonoid extraction yields reach a constant value. A comparison between an extraction at room temperature for 240 min and an extraction at boiling temperature for 15 min showed that the last one permits the extraction of the main coloring matters of weld (flavonoids) with the best yield.

Thus the optimal extraction parameters can be determined: a methanol/water mixture in ratio (8/2 v/v), boiling temperature and a 15 min extraction time. Using these parameters, 0.233% luteolin-3′,7-diglucoside, 0.357% luteolin-7-glucoside and 0.448% luteolin (yields reported at weight of dried plant) can be obtained.

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