

DETERMINATION OF TOTAL FLAVONOID CONTENT OF URTICA DIOICA L. BY A NEW METHOD

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Abstract

Flavonoids have been shown to play a number of biologically important roles such as antioxidant activity. Antioxidants may serve the task of reducing oxidative damage in humans induced by free radicals and reactive oxygen species under 'oxidative stress' conditions. The aim of this work is to develop a sensitive, diversely applicable and simple indirect spectrophotometric and fluorometric method for the determination of total flavonoids content in several plants. The method is based on the oxidation of flavonoids with Ce(IV) salts at room temperature. The spectrophotometric determination of the remaining Ce(IV) or fluorometric determination of the formed Ce(III) was performed after reaction with flavonoids. Quercetin was used as flavonoid standard. The procedure was successfully applied to the assay of total flavonoids in the arial parts of *Urtica Dioica L.* The obtained results were statistically compared with those obtained by known methods.

Introduction

Antioxidants are vital in combating the free radicals which damage human cells under 'oxidative stress' conditions, and an imbalance of free radicals may cause grave disturbances in cell metabolism. Free radicals are instable species because they have unpaired electrons and seek stability through electron pairing with biological macromolecules. The proteins, lipids, and DNA of healthy human cells are good sources for these pairing electrons. Thus oxidative stress conditions can cause DNA and protein damage, lipid peroxidation, cancer, ageing, and inflammatory activities [1,2]. Sources of free radicals include metabolism by-products, neutrophils, UV radiation, air and water pollutants, fatty foods, hazardous chemicals, and cigarette smoke.

Natural antioxidants such as dietary plant flavonoids are increasingly attracting attention. They are natural disease-preventing, health-promoting, and anti-ageing substances. Flavonoids are essentially ingested through food rather than being metabolically synthesized.

There have been an increasing number of reports that directly contradict the putative role of flavonoids as antioxidant and anti-cancer agents [3]. Flavonoids are plant secondary metabolites widely distributed in the plant kingdom. More than 6000 flavonoids have been identified in plants [4]. *Urtica dioica L.* has become a source of folk medicine for the treatment of many diseases. The aqueous extract of the aerial parts of *urtica dioica L.* have been occasionally used as a herbal medicine by cancer patients in Turkey [5]. In the present study, a sensitive and simple spectrophotometric method was developed for the determination of total flavonoid content of *Urtica dioica*. This method is based on the oxidation of flavonoids with Ce(IV) at room temperature, and the absorbance of unreacted Ce(IV) is measured at 320 nm. The reducing flavonoid content of the test sample may be expressed as quercetin equivalents.

Materials and Methods

Quercetin dihydrate, Ce(IV) sulfate tetrahydrate, Ce(III) nitrate hexahydrate, ethyl alcohol were purchased from Sigma, Merck and Riedel, respectively. All spectrophotometric measurements were made using a Philips 8700 UV-spectrophotometer. All fluorometric measurements were carried out on a Perkin Elmer 204 fluorescence spectrophotometer with a 150 W xenon arc lamp. The optical absorbances and fluorescence intensities of solutions were measured in 1.00 cm quartz cuvettes.

Plant Material and Extraction

Dried nettle was obtained from a spice-seller in Istanbul, Turkey. The dried sample was pounded into small parts with a porcelain mortar. The dried powdered aerial parts of the plants were extracted with boiling distilled water and were mixed with magnetic stirrer for fifteen minutes. The extract was filtered through a Whatman filter paper. The aqueous extract of nettle was prepared just before the experiments so as to prevent any undesired degradation reactions.

Analytical procedure for the spectrophotometric method

0.3 ml of 0.025 M Ce(IV) solution was added to 1 ml of quercetin solution of approximate concentration $1.0 \cdot 10^{-4}$ M, and the mixture was diluted to 10 ml with distilled water. After shaking for a few minutes, the solution was let to stand for 15 minutes at room temperature. The absorbance of the reaction mixture was measured at 320 nm against a water blank. Similarly, 1 ml water extract of nettle was reacted with 0.3 ml of 0.025 M Ce(IV) solution for the assay of total flavonoid content as described above. The difference absorption due to Ce(IV) between the blank (containing the same amount of Ce(IV)) and flavonoid reacted solutions was recorded.

Analytical procedure for the fluorometric method

The fluorescence of the accordingly prepared solutions (see above procedure) were measured upon excitation at 260 nm and emission at 360 nm wavelengths. For determination of total flavonoid content in nettle, 0.3 ml of 0.025 M Ce(IV) solution was added to 1 ml water extract of nettle, the mixture was diluted to 10 ml with distilled water, and fluorescence due to formed Ce(III) (as a result of Ce(IV) oxidation of flavonoids) was measured.

Results and Discussion

Spectrophotometric Method

The developed indirect spectrophotometric method is based on the redox reaction between flavonoids and Ce(IV) at room temperature. Quercetin was used to construct the calibration curve. Ce(IV) is a strong oxidizing agent capable of reacting quantitatively with flavonoids. Approx. 0.5 ml of 0.025M Ce(IV) solution was added to quercetin solutions in the concentration range of $2.60 \cdot 10^{-6}$ M – $2,30 \cdot 10^{-5}$ M. Quercetin solution and the mixture was diluted to 10 ml with distilled water. Similarly, water extract of nettle was reacted with Ce(IV) for the determination of total flavonoid content as described above (Fig.1).

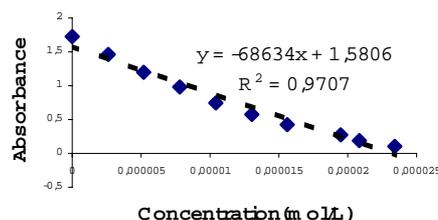


Fig. 1 .Calibration curve of Quercetin

Flavonoid Content (mass,%) =((quercetin equivalent concn. of test sample (in g/L) X total volume of water extract (L) / sample weight (in g)) X 100

Spectrofluorometric Method

This method is based on the oxidation of flavonoid with Ce(IV) and determination of resulting Ce(III) ions by flurometric method. Ce(IV) is non-fluorescent oxidizing agent. Thus total flavonoids assay is possible simply by measuring the fluorescence of the Ce(III) produced. The fluorescent species have excitation and emission maxima at 260 nm and 360 nm, respectively.

Quercetin solution was used in the concentration range between $2.61 \cdot 10^{-6} \text{M}$ and $2.61 \cdot 10^{-5} \text{M}$ to build up the calibration curve.(Fig. 2.) The water extract of nettle was subjected to a similar procedure as described above.

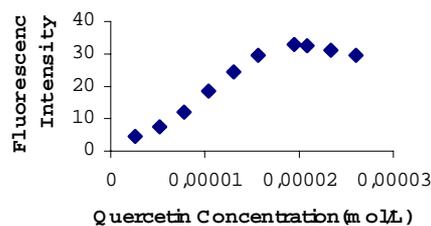


Fig. 2. Change in fluorescence intensity with the concentration of Quercetin

Interferences

In principle, plant pigments absorbing at 320 nm or emitting at 360 nm should not interfere with the proposed methods as long as they do not involve in a redox reaction with Ce(IV) salts, as difference spectrophotometric or fluorometric readings of the original and redox- reacted sample solutions were recorded, the difference corresponding to the consumed Ce(IV) or formed Ce(III) concentrations. However, other reducing agents capable of reacting with Ce(IV) – if present – may interfere.

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