Research Article

Screening of medicinal plant extracts for quercetin-3-rutinoside (rutin) in Bosnia and Herzegovina

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ABSTRACT

The flavonoid rutin (quercetin-3-rutinoside), is a flavonol glycoside comprised of the flavonol quercetin and the disaccharide rutinose. In this study, using HPLC-ED system, quantification of rutin was carried out in different extracts of medicinal plants. Analyses of rutin were performed with leaves and flowers of 50 medicinal plants of rue, buckwheat, rose, sage, calendula, chamomile, elder, dandelion, feverfew, lemon balm, linden, thyme, valerian, stinging nettle, cloves, dog rose, pansy, parsley, cowslip, rose etc. Rutin was extracted with hot water. Supernatant was used for analyses. The standard solution was 0.2 µg/20 µL rutin. Content of rutin (mg/g) was highest in the leaves of rue (86.0) followed by flowers of buckwheat (53.5), the leaves from buckwheat (20.0), flowers of pansy (33.5) and flowers of rose (10.0). In all other plants, the content of rutin was lower than 0.5 mg/g. The lowest content rutin (0.25 mg/g) was found in the leaves of lemon balm. The high concentration of rutin in flowers and leaves of rue, buckwheat, pansy and rose provided more importance to rue, buckwheat, pansy and rose as medicinal and diet plants. **[Medicinal Plants 2010; 2(2) : 97-102]**.

Keywords : Rutin, HPLC-ED, medicinal plants, rue, buckwheat, rose

INTRODUCTION

The flavonoid rutin (quercetin-3-rutinoside), is a flavonol glycoside comprised of the flavonol quercetin and the disaccharide rutinose (Fig. 1). It is found in many medicinal plants. Its name comes from the name of *Ruta graveolens*, a plant that also contains rutin (Shafi and Ikram, 1982). Rutin is well known as antioxidant, and as natural compound with wide range of medicinal properties (Choi *et al.*, 2009).

Ruta graveolens L. (Rutaceae) is grown as both a medicinal herb and as a condiment. In European folk medicine, R. *graveolens* is used to relieve gas pains and colic, improve appetite and digestion, and promote the onset of menstruation and uteral contractions (Kuštrak, 2005). The rhamno-glucoside of quercetin rutin is found in many plants such buckwheat, *Fagopyrum esculentum* and *Fagopyrum sagittatum* G. (Polygonaceae).

Flour of buckwheat, citrus and other fruits have long included in the human diet (Kuhnau, 1976) and, in addition to ascorbic acid and other compounds, provide flavonoids such rutin, which decrease capillary fragility and therefore, biologically active substance

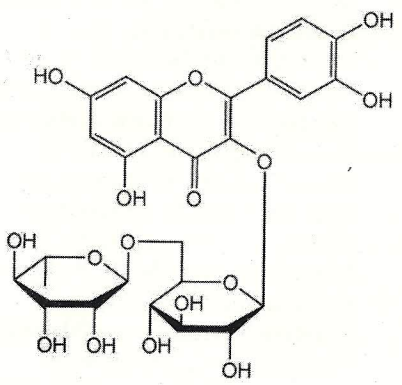

Fig. 1. Chemical structure of rutin

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Table 1. List of examined plant species in the study

Sample	Scientific plant name	Common plant name	Plant part used
1.	*Artemisia absinthium* L. (Asteraceae)	Wormwood	leaves
2.	*Calendula officinalis* L. (Asteraceae)	Calendula	herb*
3.	*Chamomilla chamomilla* (L.) Rydb. (Asteraceae)	Chamomile	leaves
4.	*Chamomilla chamomilla* (L.) Rydb. (Asteraceae)	Chamomile	flowers
5.	*Erica carnea* L. (Ericaceae)	Winter Heath	herb
6.	*Fagopyrum esculentum* Moench (Polygonaceae)	Buckwheat	leaves
7.	*Fagopyrum esculentum* Moench (Polygonaceae)	Buckwheat	flowers
8.	*Fagopyrum esculentum* Moench (Polygonaceae)	Buckwheat	flour
9.	*Fagopyrum esculentum* Moench (Polygonaceae)	Buckwheat	stalk
10.	*Fagopyrum esculentum* Moench (Polygonaceae)	Buckwheat	root
11.	*Hypericum perforatum* L. (Clusiaceae)	St John's Wort	flowers
12.	*Hypericum perforatum* L. (Clusiaceae)	St John's Wort	leaves
13.	*Hypericum perforatum* L. (Clusiaceae)	St John's Wort	root
14.	*Hyssopus officinalis* L. (Lamiaceae)	Hyssop	leaves
15.	*Inula helenium* L. (Compositae)	Horse-heal	leaves
16.	*Inula helenium* L. (Compositae)	Horse-heal	flowers
17.	*Levisticum officinale* L. Koch. (Umbelliferae)	Lovage	herb
18.	*Melissa officinalis* L. (Lamiaceae)	Lemon Balm	leaves
19.	*Melissa officinalis* L. (Lamiaceae)	Lemon Balm	herb
20.	*Mentha piperita* L. (Lamiaceae)	Meant	leaves
21.	*Ocimum basilicum* L. (Lamiaceae)	Basil	flowers
22.	*Ocimum basilicum* L. (Lamiaceae)	Basil	leaves
23.	*Origanum majorana* L. (Lamiaceae)	Sweet Marjoram	herb
24.	*Origanum vulgare* L. (Lamiaceae)	Oregano	herb
25.	*Pelargonium graveoles* L. L'Hér. (Geraniceae)	Geranium	herb
26.	*Petroselinum crispum* Miller (Apiaceae)	Parsley	leaves
27.	*Primula veris* L. (Primulaceae)	Cowslip	flower
28.	*Rosa canina* L. (Rosaceae)	Dog rose	flower
29.	*Rosa damascena* Mill. (Rosaceae)	Damask rose	herb
30.	*Rosmarinus officinalis* L. (Lamiaceae)	Rosemary	herb
31.	*Rosmarinus officinalis* L. (Lamiaceae)	Rosemary	leaves
32.	*Ruta graveolens* L. (Rutaceae)	Rue	herb
33.	*Salvia officinalis* L. (Lamiaceae)	Sage	herb
34.	*Salvia officinalis* L. (Lamiaceae)	Sage	herb
35.	*Sambucus nigra* L. (Adoxaceae)	Elder	flowers
36.	*Satureja montana* L. (Lamiaceae)	Winter Savory	leaves
37.	*Syzygium aromaticum* L. (Myrtaceae)	Cloves	herb
38.	*Tanacetum parthenium* (L.) Sch. Bip. (Asteraceae)	Feverfew	leaves
39.	*Tanacetum parthenium* (L.) Sch. Bip. (Asteraceae)	Feverfew	flowers
40.	*Taraxacum officinale* F.H. Wigg (Asteraceae)	Dandelion	herb
41.	*Thymus serpyllum* L. (Lamiaceae)	Thyme	herb
42.	*Thymus sibthorii* Benth (Lamiaceae)	Thyme	herb
43.	*Thymus vulgaris* L. (Lamiaceae)	Thyme	herb
44.	*Thymus vulgaris* L. (Lamiaceae)	Thyme	leaves
45.	*Tilia tomentosa* L. (Malvaceae)	BasswoodSilver Lime	leaves
46.	*Ulmaria pentapetala* Gilib (Rosaceae)	Meadowsweet	flowers
47.	*Ulmaria pentapetala* Gilib (Rosaceae)	Meadowsweet	leaves
48.	*Urtica dioica* L. (Urticaceae)	Stinging Nettle	herb
49.	*Valeriana officinalis* L. (Valerianaceae)	Valerian	herb
50.	*Viola tricolor* L. (Violaceae)	Heartsease	flower

*herb = leaves, flowers, stems

present in the flowers, leaves, flour, herbage (stalk) and root of buckwheat is rhamno-glucoside (rutin).

Rosmarinus officinalis L. (Lamiaceae) is a woody, perennial herb with fragrant evergreen needle-like leaves. It is native to the Mediterranean region. It is widely used as a medicinal and culinary herb (Kuštrak, 2005).

Pelargonium graveolens L'Hér (Geraniaceae) has great importance in the perfumery industry. It is cultivated on a large scale and its foliage is distilled for its scent. *Pelagronium graveolens* cultivars have a wide variety of smells, including rose, citrus, mint, coconut and nutmeg, as well as various fruits. However, the most commercially important varieties are those that have rose scents (Kuštrak, 2005).

Some studies suggest that flavonoids may be useful in the treatment of many impaired conditions (Cody *et al.*, 1986; Havsteen *et al.*, 1983; Middleton *et al.*, 1984). In fact, many of the medicinal action of herbs, of pollens and propolis are now known to be directly related to their flavonoid content. Over 8000 flavonoids have been characterized and classified according to chemical structure. Rutin as a flavonoid and its aglucone quercetin (P vitamin) have an ability to increase intracellular ascorbic acid levels, decrease capillary permeability and fragility, scavenge oxidants and free radicals, and unique ability to bind to collagen structure directly (prolin-hydroxylation to hydroxyprolin) as well as inhibit destruction of bone (Cody *et al.*, 1986; Middleton *et al.*, 1984).

The increasing interest in powerful biological activities of plant phenolic and flavonoids outlined the necessity of determining their contents in Bosnian fruits, vegetables, and medicinal plants. Our study comprises thirty five medicinal plant species.

METERIALS AND METHODS

Plant materials

Plant materials were collected in Bosnia and Herzegovina during 2009. All examined plant species are listed in Table 1.

High Pressure Liquid Chromatography (HPLC) with Electrochemical Detection (ED)

Water extracts of examined plants were prepared using reflux extraction of fresh leaves, flowers, etc. Afterward, 1 ml of extract was decanted and centrifuged (15000 o/ min), obtaining supernatant which was used for further analysis. Pure rutin (0.01 mg/ml) was used as standard solution, and it was purchased from Merck Chemical Company (Germany). HPLC conditions were following: column ODS Hypersil C-18 mobile phase: methanol-acetonitrile-water-acetic acid (20+10+70+1); electrochemical-detector with range 50 nA, potential +0.840 V, filter 0.02 Hz; flow rate 0.8 ml/min; temperature 25°C.

Determination of rutin was based on a comparison of retention-times obtained from different extract of plants, and standard solution of rutin. Limit of detection was 50 ng.

Calculation of rutin in biological samples

Quantitation of rutin was done by computer using a LabSolution program of Shimadzu (Kyoto, Japan). Concentration of rutin calculated using an equation:

$$\text{Content of rutin} = \frac{A_{sample}}{A_{standard}} \cdot \gamma \text{ (mg/g)}$$

where is: A_{sample} - peak area for sample plant with the same retention time as rutin standard; $A_{standard}$ - peak area for rutin standard; γ - concentration of rutin standard. Injection volume for standard solution and biological samples were 20μL. Concentation of rutin in injected standard solution was 0.2 μg /20 μL. Content of rutin in biological samples was expressed as mass of rutin per mass of fresh tissue $mg/g_{(f.t.)}$.

RESULTS

The present results show particular specificity of distribution of quercetin 3-rutinoside in different parts of the studied medicinal plants (Fig. 2).

The results presented in Table 2, showed the highest rutin content in rue (86.6 mg/g), flowers of buckwheat (53.5 mg/g), flower of pansy (33.6 mg), flowers of rosemary (34.0 mg/g), leaves of thyme (24.9 mg/g), herba of thymus sibthorii (22.6 mg/g), leaves of buckwheat (20.0 mg/g), flower of dog rose (14.0 mg/g), and herba of dandelion (14.0 mg/g). Other examined plant species showed much lower levels of rutin. The lowest content of rutin was established in feverfew (1.4 mg/g) and lemon balm (0.7 mg/g), respectively. In every one buckwheat plant organs rutin was found, such the content of rutin in stalk (5.0 mg/g), seeds-flour (0.12 mg/g), and root (0.02 mg/g).

Table 2. Levels of rutin ($mg/g_{f.t.}$) in the various medicinal plants

Sample	Scientific plant name	Plant organ	Levels of rutin ($mg/g_{f.t.}$)
1.	*Ruta graveolens* L. (Rutaceae)	herb★	86.0
2.	*Fagopyrum esculentum* Moench (Polygonaceae)	flowers	53.5
3.	*Viola tricolor* L. (Violaceae)	flower	33.6
4.	*Rosmarinus officinalis* L. (Lamiaceae)	herb	34.0
5.	*Thymus vulgaris* L. (Lamiaceae)	leaves	24.9
6.	*Thymus sibthorii* Benth (Lamiaceae)	herb	22.6
7.	*Fagopyrum esculentum* Moench (Polygonaceae)	leaves	20.0
8.	*Rosa canina* L. (Rosaceae)	flower	14.0
9.	*Taraxacum officinale* F.H. Wigg (Asteraceae)	herb	14.0
10.	*Pelargonium graveoles* L. L'Hér. (Geraniceae)	herb	14.0
11.	*Petroselinum crispum* Miller (Apiaceae)	leaves	12.0
12.	*Salvia officinalis* L. (Lamiaceae)	herb	11.8
13.	*Urtica dioica* L. (Urticaceae)	herb	11.4
14.	*Origanum vulgare* L. (Lamiaceae)	herb	11.2
15.	*Primula veris* L. (Primulaceae)	flower	11.0
16.	*Inula helenium* L. (Compositae)	flowers	10.7
17.	*Rosa damascena* Mill. (Rosaceae)	herb	10.0
18.	*Chamomilla chamomilla* (L.) Rydb. (Asteraceae)	flowers	9.5
19.	*Thymus serpyllum* L. (Lamiaceae)	herb	9.0
20.	*Hypericum perforatum* L. (Clusiaceae)	root	8.7
21.	*Valeriana officinalis* L. (Valerianaceae)	herb	8.6
22.	*Hypericum perforatum* L. (Clusiaceae)	leaves	8.4
23.	*Hypericum perforatum* L. (Clusiaceae)	flowers	8.0
24.	*Tilia tomentosa* L. (Malvaceae)	herb	8.0
25.	*Calendula officinalis* L. (Asteraceae)	herb	6.6
26.	*Ulmaria pentapetala* Gilib (Rosaceae)	leaves	6.6
27.	*Tanacetum parthenium* (L.) Sch. Bip. (Asteraceae)	flowers	6.5
28.	*Ulmaria pentapetala* Gilib (Rosaceae)	flowers	6.5
29.	*Ocimum basilicum* L. (Lamiaceae)	leaves	6.4
30.	*Syzygium aromaticum* L. (Myrtaceae)	herb	6.0
31.	*Fagopyrum esculentum* Moench (Polygonaceae)	stalk	5.0
32.	*Salvia officinalis* L. (Lamiaceae)	herb	5.0
33.	*Hyssopus officinalis* L. (Lamiaceae)	leaves	4.5
34.	*Levisticum officinale* L. Koch. (Umbelliferae)	herb	4.5
35.	*Thymus vulgaris* L. (Lamiaceae)	herb	4.5
36.	*Origanum majorana* L. (Lamiaceae)	herb	4.4
37.	*Sambucus nigra* L. (Adoxaceae)	flowers	3.9
38.	*Satureja montana* L. (Lamiaceae)	leaves	3.8
39.	*Erica carnea* L. (Ericaceae)	herb	3.6
40.	*Ocimum basilicum* L. (Lamiaceae)	flowers	3.6
41.	*Chamomilla chamomilla* (L.) Rydb. (Asteraceae)	leaves	2.8
42.	*Artemisia absinthium* L. (Asteraceae)	leaves	2.7
43.	*Inula helenium* L. (Compositae)	leaves	2.2
44.	*Mentha piperita* L. (Lamiaceae)	leaves	1.9
45.	*Rosmarinus officinalis* L. (Lamiaceae)	leaves	1.6
46.	*Tanacetum parthenium* (L.) Sch. Bip. (Asteraceae)	leaves	1.4
47.	*Melissa officinalis* L. (Lamiaceae)	herb	0.7
48.	*Melissa officinalis* L. (Lamiaceae)	leaves	0.3
49.	*Fagopyrum esculentum* Moench (Polygonaceae)	flour	0.12
50.	*Fagopyrum esculentum* Moench (Polygonaceae)	root	0.02

★herb = leaves, flowers, stems

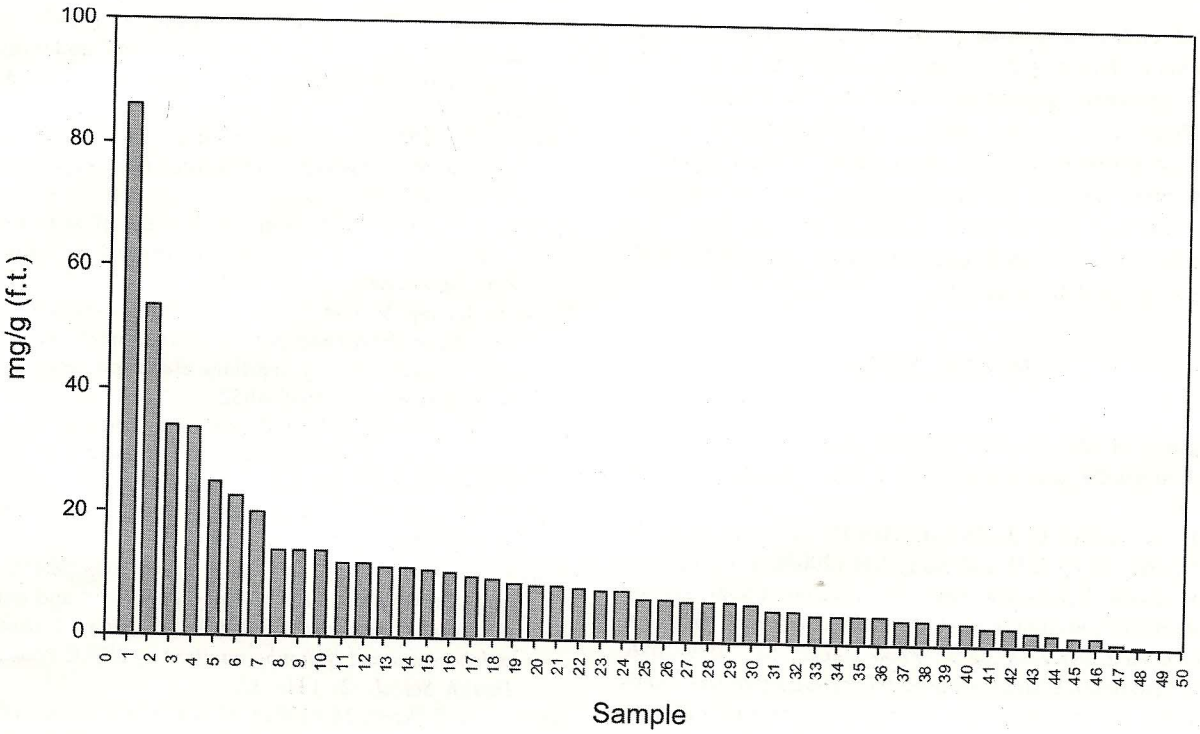

Fig. 2. Levels of rutin ($mg/g_{f.t.}$) in the various medicinal plants.

DISCUSSION

In various plant organs obtained from medicinal plants the presence of rutin was revealed by HPLC-ED method developed in our laboratory. In the chromatographic conditions used, the retention time (Rt) in biological samples corresponding to this rutin was 17 min and retention time (Rt) of standard was similar to that obtained in standard solution of rutin (Fig. 3). Table 2 showed the levels of the rutin in the biological samples. It can be seen that results agrees with earlier reports (Sofic *et al.*, 2009; Atanassova and Bagdassarian, 2009; Samee and Vorarat, 2007; El-Sherbeny *et al.*, 2007; Kreft *et al.*, 1999). The variation of content of rutin in various medicinal plants from Bosnia showed lower levels of rutin (about 12-100%) of the levels recorded for rue, buckwheat, pancy and rose. The results of screening of medicinal plant extracts from Bosnia

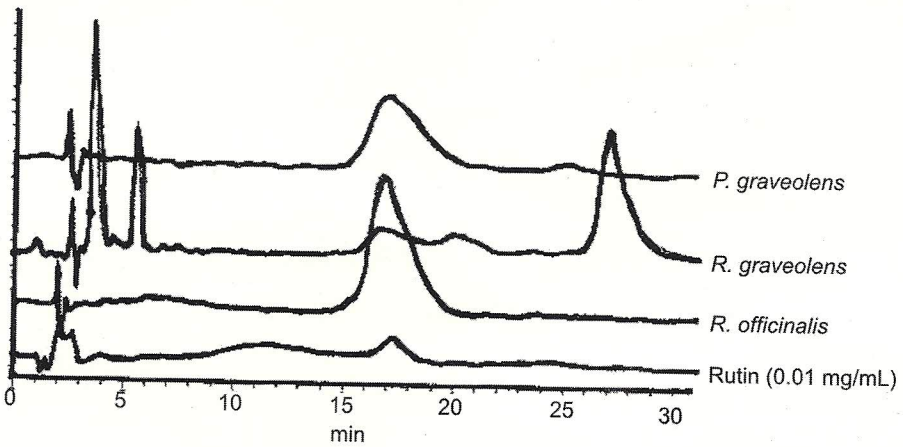

Fig. 3. Chromatograms of standard solution of rutin and some plant extract.

for quercetin-3-rutinoside (rutin) revealed that only rue, buckwheat, thyme, pancy and rose contains high levels of this phenolic glycosides. The results indicate that buckwheat, rue, thyme, pansy, and rose could be an important nutritional sources of flavonoids, especially in countries with a low mean daily flavonoids intake. Furthermore, developed HPLC-ED method for determination of rutin is easy and sensitive, and it will be used in further research.

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