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PHENOLIC COMPOUDNS IN TRADITIONAL BULGARIAN MEDICAL PLANTS

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ABSTRACT

A number of plants and plant products have medicinal properties that have been validated by recent scientific developments throughout the world, owing to their potent pharmacological activity, low toxicity and economic viability. In recent years, the use of natural antioxidants present in traditional medicinal plants has become of special interest in the scientific world due to their presumed safety and nutritional and therapeutic value. In this present study, comparative phenolic compounds in traditional Bulgarian medical plants. The medical plants were analyzed for their tannins content by titrimetric method; rutin was determined spectrophotometrically by using ammonium molybdate; the total phenolics content was determined by using Folin-Ciocalteu assay and the total flavonoids were used the colorimetric reaction with aluminum (III) chloride. The present paper shown by the results of total phenolic and total flavonoid contents, and rutin and tannins in medical plants that they must be relatively safe for the patient.

Keywords: chicory(Cichoriumintybus L.),white birch (Betula pendula),Cotinuscoggygria, Geranium sanguineumL. and rose hip fruits (Rosa caninaL.), total phenolics, total flavonoids, rutin and tannins

INTRODUCTION

The World Health Organization (WHO) has estimated that almost 80% of the earth's inhabitants believe in traditional medicine for their primary health care needs, and that most of this therapy involves the use of plant extracts and their active components (**Kishore Dubey *et al*., 2015; Winston, 1999**). A number of plants and plant products have medicinal properties that have been validated by recent scientific developments throughout the world, owing to their potent pharmacological activity, low toxicity and economic viability (**Kishore Dubey *et al*., 2015**) .In recent years, the use of natural antioxidants present in traditional medicinal plants has become of special interest in the scientific world due to their presumed safety and nutritional and therapeutic value (**Kishore Dubey *et al*., 2015; Ajila *et al*., 2007**). The majority of the antioxidant activity of plants is due to the presence of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids (**Kishore Dubey *et al*., 2015**).

Bulgaria is situated in the Balkan Peninsula, South-East Europe, Mediterranean and continental climates. The relief of the country is quite diverse ranging from plains to low hills and high mountains. The climate is moderate continental to modified continental, but in southern regions reflects rather a strong Mediterranean influence. As a result of this climatic condition, the Bulgarian flora is remarkable for its diversity (3500 plant species including 600 known medicinal plants) (**Ivancheva and Stancheva, 2000 ;Ivancheva *et al*., 2006**).

Traditional Bulgarian medicinal plants have been used to treat human diseases in Bulgaria for thousands of years, and people are becoming increasingly interested in them because of their good health effects and low toxicity. In recent years, studies on the antioxidant activities of Traditional Bulgarian medicinal plants have increased remarkably in light of the increased interest in their potential as a rich source of natural antioxidants. Several studies have indicated that Traditional Bulgarian medicinal plants possess more potent antioxidant activities than common dietary plants, and contain a wide variety of natural antioxidants, such as total phenolics, flavonoidsand tannins (**Atanassova *et al*., 2011**).

In recent years, interest in plant-derived food additives has grown. Furthermore plant extracts of Bulgarian white birch (*Betula pendula*L.) leaves have been shown to possess health-promoting properties. The white birch leaves extract were strong diuretic and have effect at nephrolithiasis and urinary bladder lythiasos, sedative effect on spasms of smooth muscle. It might be used in following conditions: kidney diseases, ischia nerve inflammation and podagra and atherosclerosis and also it has an antimicrobial effect (**Christova-Bagdassrian *et al*., 2014; Harbone, 1993**).

The extracts from the leaves of the white birch significantly increase diuresis, and with this and the emission of sodium and chloride ions, ie act as salidiuretik. Until recently it was assumed that the diuretic action is due to the presence of resinous substances. Therefore birch buds were preferred because they are rich in resins. However, it is clear that flavonoids have a greater role in the diuretic effect. They are contained mainly in the leaves. In addition, the leaves contain potassium nitrate, which enhances the diuretic effect of the flavonoids. This effect was related to total flavonoids (**Christova-Bagdassrian *et al*., 2014; Neoretal , 2006**).

*Cotinus coggygria*is one of two species constituting a minor genus of the family *Anacardiaceae*, viz., *Cotinus coggygria*Scop. (syn.: *Rhus cotinus* L.) Itself and *Cotinusobovatus* Raf., the American smoketree. Its wide distribution extends from southern Europe, the Mediterranean, Moldova and the Cauca-sus, to central China and the Himalayas (**Christova-Bagdassarian *et al*., 2016; Novakovic *et al*., 2007; *Matic et al*., 2011**). *C. coggygria*is a common medicinal plant (well known as 'smradlika' or 'tetra') in the Bulgarian folk medicine for outer use predominantly (**Christova-Bagdassarian *et al*., 2016; Landzhev Chemical, I., 2010; Ivanova *et al*., 2013).**

Plants of the family *Anacardiaceae* have a long history of use by various peoples for medicinal and other purposes. *Rhus glabra*is traditionally used in the treatment of bacterial diseases such as syphilis, gonorrhea, dysentery and gangrene, while *R. coriaria*, besides its common use as a spice consisting of ground dried fruits with salt, is also widely used as a medicinal herb, particularly for wound healing (**Christova-Bagdassarian *et al*., 2016; Matic et al., 2011; Rayne and Mazza, 2007**)

In folk medicine, *Cotinus coggygria*is routinely used as an antiseptic, anti-inflamatory, antimicrobial and antihaemorragic agent in wound-healing (**Christova-Bagdassarian *et al*., 2016; Rayne and Mazza, 2007; Demirci *et al*.,**

2003), as well as for countering diarrhea, paradontosis, and gastric and duodenal ulcers (**Christova-Bagdassarian *et al*., 2016; Rayne and Mazza, 2007; Ivanova *et al*., 2007**). However, these by-products are still a good and cheap source of high-quality polyphenolic compounds which can be used in different therapeutic procedures with the purpose of free radical neutralisation in biological systems (**Christova-Bagdassarian *et al*., 2016; Bucić-Kojić *et al*., 2007; Heim *et al*., 2002; Yilmaz and Toledo, 2004)**

Cichorium intybus L., commonly known as chicory, belongs to family *Asteraceae* and widely distributed in Asia and Europe(**ZahidKhorshid *et al*., 2015; Bais *et al*., 2001**). All parts of this plant possess great medicinal importance due to the presence of a number of medicinally important compounds such as alkaloids, inulin, sesquiterpene lactones, coumarins, vitamins, chlorophyll pigments, unsaturated sterols, flavonoids, saponins and tannins (**ZahidKhorshid *et al*., 2015; Bais *et al*., 2001, Atta *et al*., 2010; Molan *et al*., 2003; Muthusamy *et al*., 2008; Nandagopal *et al*., 2007**). The whole plant has numerous applications in food industry and medicine (**Denev *et* al., 2014; Ilaiyaraja *et al*., 2010**). Its dried roots were used as a substitute or adulterant in coffee powder (**Denev *et* al., 2014; Jung *et al*., 1994**). The young leaves can be added tosalads and vegetable dishes, while chicory extracts are used for the production of invigorating beverages (**Denev *et* al., 2014**).Leaves of chicory are good sources of phenols, vitamins A and C as well as potassium, calcium, and phosphorus (**ZahidKhorshid *et al*., 2015; Muthusamy *et al*.,2008**) *C. intybus* has been traditionally used for the treatment off ever, diarrhea, jaundice and gallstones **(ZahidKhorshid *et al*., 2015; Abbasi *et al*., 2009; Afzal *et al*., 2009**).During the past decade, there is a growing interest in natural plant extracts with potential antioxidant activity, because of their improved healthy effect (**Denev *et al*., 2014; Alexieva *et al*., 2013; Mihaylova *et al*., 2013**). The expanded application is due to their protective properties against oxidative stress disorders, as well asoxidative damage in food products (**Denev *et al*., 2014; Ivanov et al., 2014**).It is well known that polyphenols from plant extracts possessed strong antioxidant activities. Their presence in medicinal plant that are natural source of inulin-type fructans prebiotics additionally increase the biological activity of the obtained extracts (**Denev *et al*., 2014; Petkova *et al*., 2012; Vrancheva *et al*., 2012**).

Wild growing rose hip fruits (*Rosa canina* L.) are widespread plant in Bulgaria with great importance in herbal medicine. The *Rosa canina* fruits are a valuable source for food and pharmaceutical industry. They contain a wide variety of biologically and physiologically active ingredients, such as vitamins (C, B, P, PP, E, K), flavonoids, carotenes, carbohydrates (mono- and oligosaccharides), organic acids (tartaric, citric), trace elements and others (**Taneva *et al*., 2016; Ognyanov *et al*., 2014; Mihaylova *et al*., 2015**).

These compounds play an important role in maintaining fruit quality and determining nutritive value. Rose hips are also well known to have the highest vitamin C content (300–4000 mg/100 g) among fruits and vegetables (**Taneva *et al*., 2016; Demir *et al*., 2001**). In Bulgaria rose hip fruits are typically consumed as infusion. It was found that juice and aqueous extracts from rose hip possessed exceptional antioxidant activity (**Taneva *et al*., 2016; Demir *et al*., 2001)**. This makes them suitable for use both in the fresh or dry state, or in the form of extracts in food products and cosmetics (**Taneva *et al*., 2016; Ognyanov *et al*., 2014; Mihaylova *et al*., 2015**). According to some authors, the higher values of antioxidant activity of rose hip extracts due to synergism between polysaccharides and organic acids (gallic, cinnamic, ellagic), with phenolic antioxidants: flavonoids (rutin, kaempferol, quercetin) (**Taneva *et al*., 2016; Ognyanov *et al*., 2014; Mihaylova *et al*., 2015**).

The most common antioxidants contained in fruits are ascorbic acid, carotenoids and polyphenol substances with proven antioxidant capacity (**Taneva *et al*., 2016; Mihaylova *et al*., 2015**).

Geranium macrorrhizum L.is a perennial herb native from the Balkans, occurring occasionally also in the Carpathian Mountains and in the Alps. It is known as "Zdravets" which means "healthy" in Bulgarian folk medicine. A methanol extract from leaves possesses strong hypotensive activity, cardiotonic, capillary anticomplementary and sedative action as well (**Ivancheva *et al*., 2006; Genova *et al*., 1989; Ivancheva and Wollenweber, 1989; Ivancheva *et al*., 1992**). Central depressive action of methanol extracts has also been demonstrated. The whole plant is rich in tannins with more in the stems than in the green foliage. No alkaloids and cardiological glycosides have been found. The presence of six flavonol aglycones in aerial parts of *G. macrorrhizum* has been established (kaempferol 3-methylether (*isokaempferide*), kae-3,7-dimethylether (*kumataketin*), kae-3,4'- dimethylether (*ermanin*), quercetin, qu-7,3'-dimethylether and qu-3,7,3',4'-tetramethylether (*retusin*)); two of these, namely ermanin and retusin were said to be present in the roots too (**Ivancheva *et al*., 2006**). The focus in the present study is a comparative evaluation of the total phenolic and total flavonoid contents, rutin and tannins in traditional Bulgarian medical plants as sources for human health.

MATERIAL AND METHODS

Plant material

The leaves from chicory (*Cichorium intybus* L.), white birch (*Betula pendula*), zdravec (*Geranium macrorrhizum* L.), smradlika (*Cotinus coggygria*, syn.: *Rhus cotinus* L.) and rose hip fruits (*Rosa canina* L.) were harvested from different regions of Bulgaria. All sample data are stated in the sampling protocol. The dried leaves and rose hip fruit were kept in a dry place until further use.

Sample preparation

A dry simple of 0.5 g was weighted and phenolic and flavonoid compounds were extracted with 50 mL80% aqueous methanol on an ultrasonic bath for 20 min. An aliquot (2 mL) of the extracts was ultracentrifugated for 5 min at 14 000 rpm. The extract prepared in this way was used for further spectrophotometric determination of polyphenols.

Determination of total phenolics assay

The total phenolic contents of medicinal plants were determined by using the Folin-Ciocalteu assay.An aliquot (1 mL) of extracts or standard solution of gallic acid (10, 20, 40, 60, 80, 100 and 120 mg/L) was added to 25 mL volumetric flask, containing 9mL of distilled deionised water (dd H_2O). A reagent blank using dd H_2O was prepared. One milliliter of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 mL of 7%Na_2CO_3 solution was added to the mixture. To the solution the dd H_2O was added up to volume of 25mL and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with an UV-Vis Spectrophotometer BOECO – Germany. All samples were analyzed in duplicates (**Marinova *et al*., 2005**).

Determination of total flavonoids assay

The total flavonoid contents were measured by aluminum chloride colorimetric assay. An aliquot (1 mL) of extracts or standard solution of catechin (10, 20, 40, 60, 80, 100 and 120 mg/L) wasadded to 10 mL volumetric flask, containing 4 mL of distilled deionised water (dd H_2O). To the flask was added 0.3 mL 5% $NaNO_2$. After 5 min, 0.3 mL of 10% $AlCl_3$ was added. At 6thmin, 2mL 1 M NaOH was added and the total volume was made up to 10 mL with dd H_2O. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm. UV-Vis Spectrophotometer BOECO – Germany. All samples were analyzed in duplicates (**Marinova *et al*., 2005**).

Rutin assay

The analyses of rutin content in Bulgarian medicinal plants were performed according to The International Pharmacopoeia and AOAC method, after modified methods with using 80% aqueous methanol. Pipet 2 mL aliquots solution into 50 mL volumetric flask was added to 2 mL deionized water (dd H2O) and 5 mL ammoniummolybdat. The solution was added volume (50mL) with dd H2O and mixed. Was prepared standard solution of rutin (0.0200 g dissolved into 2 mL methanol) was added volume (50 mL) with 80% aqueous methanol. An aliquot (1 mL) of standard solution into 50 mL volumetric flask and dilute to volume with distilled deionized water (dd H_2O). A reagent blank using dd H_2O was prepared. The absorbance against prepared reagent blank was determined at 360 nm with an UV-Vis Spectrophotometer BOECO – Germany. All samples were analyzed in duplicates (**Atanassova *et al*., 2009a**).

Calculations

Calculations are based on averaging results from analyses of duplicate samples. Calculate content (%) of rutin (R) in sample as follows:

$$R(\%) = \frac{A_{sample} \times C \times 50 \times 100}{A_{stand} \times W \times 2}$$

Where:
A_{sample} - Absorbance of sample was determined at 360 nm;
A_{stand}- Absorbance of standard solution was determined at 360 nm;
C – Concentration of standard solution of rutin (g/mL);
W – weight (g) of sample for analyses;
2 – Volume (mL) of sample for analyses;
100 – Percent, %.

Tannins assay

The analyses of tannins content in traditional Bulgarian medicinal plantswere performed according to The International Pharmacopoeia and AOAC method, after modified methods. Measured 25 mL of this infusion into 1 L conical flask and add 25 mL indigo solution and 750mL distilled deionized water (dd H_2O). Titred with 0.1 N water solution of $KMnO_4$ until blue solution changes to green, then add a few drops at time until solution becomes golden yellow. Was prepared standard solution of Indigo carmine (dissolve 6 g indigo carmine in 500mL distilled deionized water (dd H_2O) by heating, cool add 50 mL 96% - 98% H_2SO_4, diluted to 1 L and then filtered. For the blank similarly titred mixture of

25 mL indigocarmine solution and 750mL ddH_2O. All samples were analyzed in duplicates (**Atanassova *et al*., 2009b**).

Calculations

Calculations are based on averaging results from analyses of duplicate samples. Calculate content (%) of tannins (T) in sample as follows:

$$T(\%) = \frac{(V - V_0) \times 0.004157 \times 250 \times 100}{g \times 25}$$

Where:
V – Volume of 0.1 N water solution of $KMnO_4$ for titration of sample, mL;
V_0 – Volume of 0.1 N water solution of $KMnO_4$ for titration of blank sample, mL;
0.004157 – Tannins equivalent in 1 mL of 0.1 N water solution of $KMnO_4$;
g – Mass of the sample for analyses, g;
250 - Volume of volumetricflask, mL;
100 – Percent, %.

Statistical analysis

All experiments were performed in triplicates. Analysis at every time point from each experiment was carried out induplicate or triplicate. The statistical parameters are calculated in terms of the reproducibility of the experimental data using a statistical package universal ANOVA.

RESULTS AND DISCUSSION

Different phytochemicals have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well being. Methanolic extract of rose hip fruits (*Rosa canina* L.), chicory (*Cichorium intybus* L.), zdravec (*Geranium macrorrhizum* L.), smradlika (*Cotinus coggygria*, syn.: *Rhus cotinus* L.) and white birch (*Betula pendula* L.) were analyzed for phytoconstituents. The quantitative estimation of phenolic compounds of white birch (*Betula pendula* L.), smradlika (*Cotinus coggygria*, syn.: *Rhus cotinus* L.), zdravec (*Geranium macrorrhizum* L.), chicory (*Cichorium intybus* L.) and rose hip fruits (*Rosa canina* L.) show that the traditional Bulgarian medical plants are rich in total phenolics, total flavonoids according to the data shown in the Table1 and Figure 1. It is well that plant flavonoids and phenols in general, are highly effective free radical scavenging and antioxidants. The phenolic constituents found in herbs have attracted considerable attention as being the main agents of antioxidant activity, although they are not the only ones. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygenquenchers. In addition, they have a metal chelation potential. Hence, the antioxidantactivity of phenolics plays an important role in the adsorption or neutralization of free radicals (**Dutra *et al*., 2008; Laguerre *et al*., 2007**).
The major compounds that are related to the antioxidant potential of a plant (or plant part) are the total phenolics and, more specifically, the flavonoids (**Dutra *et al*., 2008; Kumar Ashok *et al*., 2012**), though there is no consensus as to whether these are the sole substances in plants that act in scavenging free radicals. It is well known that plant phenolics, in general are highly effective in free radical scavenging and they are antioxidants. The presence of these phytochemicals in medical plants is thus a significant finding of the present study. The content of total phenolics and total flavonoids in white birch (*Betula pendula* L.), varying between 5256,30 mg GAE/100g dw to 2245,70 mg CE/100g dw, was found to be much higher than and in rose hip fruits (*Rosa canina* L.) - 406,79 mg GAE/100g dw to 290,13 mg CE/100g dw, respectively, as shown in Table 1 and Figure 1 with gallic acid and catechin as standards. These results indicate that the higher antioxidant activity of the white birch (*Betula pendula* L.) methanol extract, compared to the rose hip fruits (*Rosa canina* L.) methanol extract, may be correlated to the phenolic and flavonoid content of respective medical plant extract.

Table 1 Total phenolics and total flavonoids in studied Traditional Bulgarian medical plants

Bulgarian medical plants	Total phenolics, (mg GAE/100g dw)	Total flavonoids, (mg CE/100g dw)
Rose hip fruits (*Rosa canina* L.)	406,79÷0,02 (RDS 0,03; n=3)	290,13÷0,01 (RDS 0,03; n=3)
Chicory (*Cichorium intybus* L.)	635,87÷0,05 (RDS 0,07; n=3)	315,15÷0,04 (RDS 0,01; n=3)
Zdravec (*Geranium macrorrhizum* L.)	1530,70÷0,02 (RDS 0,01; n=3)	110,20÷0,06 (RDS 0,05; n=3)
Smradlika (*Cotinus coggygria*, syn.: *Rhus cotinus* L.)	2581,60÷0,02 (RDS 0,08; n=3)	810,40÷0,04 (RDS 0,04; n=3)
White birch (*Betula pendula* L.)	5256,30÷0,04 (RDS 0,08; n=3)	2245,70÷0,04 (RDS 0,01; n=3)

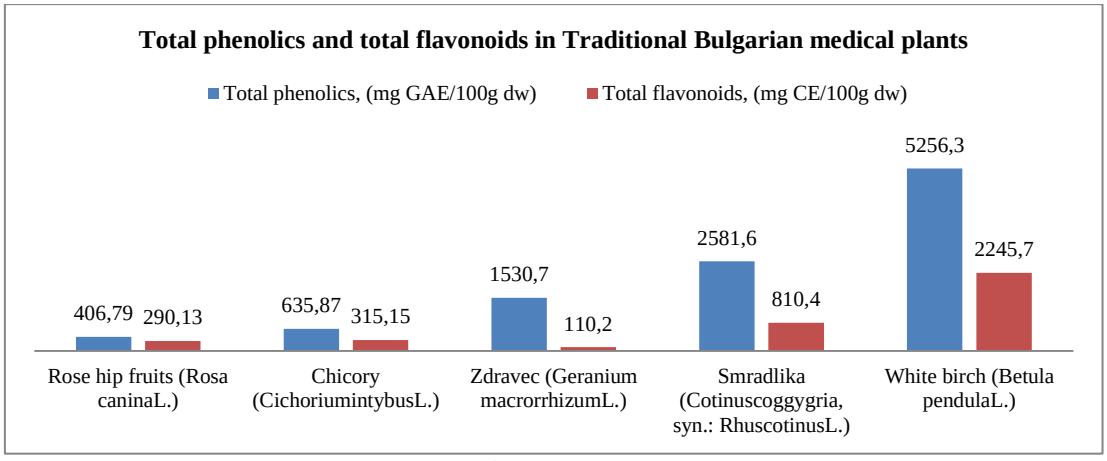

Figure 1 Total phenolics and total flavonoids in Traditional Bulgarian medical plants

The presence of rutin and tannins in chicory (*Cichorium intybus* L.), rose hip fruits (*Rosa canina* L.), zdravec (*Geranium macrorrhizum* L.), white birch (*Betula pendula* L.) and smradlika (*Cotinus coggygria*, syn.: *Rhus cotinus* L.) show that the traditional Bulgarian medical herbs are rich in tannins, rutin according to the data shown in the Table 2 and Figure 2. The phytochemical screening and quantitative estimation of the percentage of chemical constituents of the plants studied showed that the dry herbs were rich in rutin and tannins.
Tannins are distributed all over the plant kingdom (**Bate-Smith, 1962**). The term tannin refers to the use of tannins in tanning animal hides into leather; however, the term is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. Tannins have molecular weights ranging from 500 to over 3000 (**Bate-Smith, 1962; Cheng *et al*., 2003**). Tannins may be employed medicinally in antidiarrheal, haemostatic, and antihemorrhoidal compounds (**Bate-Smith, 1962; Lin et al., 2004**). Tannins can also be effective in protecting the kidneys (52, 55). Tannins are also beneficial when applied to the mucosal lining of the mouth (**Bate-Smith, 1962; Habtemariam and Varghese, 2015**). Rutin is a common dietary flavonoid widely distributed in the plant kingdom. It is also present in plant-derived beverages and foods as well as numerous medicinal and nutritional preparations (**Sando and Lloyd, 1924; Habtemariam and Varghese, 2015**). Today, rutin is among the most popular natural flavonoids known for its multifunctional nutritional and therapeutic uses (**Sando and Lloyd, 1924; Habtemariam and Lenti, 2015**). As far as commercial exploitation of rutin is concerned, however, very few plants store it in large amounts to merit the cost of its extraction from natural sources (**Sando and Lloyd, 1924**). They were known to show medicinal activity as well as exhibiting physiological activity. The presence of these phenolic compounds in traditional Bulgarian medical herbs is a significant finding in this present study. The content for rutin of white birch (*Betula pendula* L.) varied between 6,24 % was found to be much higher than and chicory (*Cichorium intybus* L.) - 2,09 %, respectively as shown in Table 2 and Figure 2 with rutin as
standards. The content for tannins of smradlika (*Cotinus coggygria*, syn.: *Rhus cotinus* L.) varied between 11,15 % was found to be much higher than and chicory (*Cichorium intybus* L.) - 2,26 %, respectively as shown in Table 2 and Figure 2 and KMnO4 as titrate. It is important to notice that the comparison of

the results for rutin and tannin contents in the medical herbs will be not correct because of the different methods of analysis.

Table 2 Tannins and rutin in studied Traditional Bulgarian medical plants

Bulgarian medical plants	Tannins, (%)	Rutin, (%)
Chicory (*Cichorium intybus* L.)	2,26÷0,04 (RDS 1,8; n=3)	2,09±0,01 (RDS 0,6; n=3)
Rose hip fruits (*Rosa canina* L.)	4,11±0,09 (RDS 2,3; n=3)	2,16±0,04 (RDS 1,9; n=3)
Zdravec (*Geranium macrorrhizum* L.)	6,08±0,07 (RDS 1,2; n=3)	3,20±0,08 (RDS 2,3; n=3)
White birch (*Betula pendula* L.)	9,04±0,03 (RDS 0,4; n=3)	6,24±0,02 (RDS 0,3; n=3)
Smradlika (*Cotinus coggygria*, syn.: *Rhus cotinus* L.)	11,15±0,06 (RDS 0,5; n=3)	3,06±0,06 (RDS 1,9; n=3)

CONCLUSION

In conclusion, on the basis of the results of this research showed that total phenolic, total flavonoid, rutin and tannin contents are important components in traditional Bulgarian medical plants grown in the country. The use of medical plants as the first choice in self0treatment of minor conditions continues to expand rapidly across the world. This makes the safety of medical plants an important public health issue. The results can be used in public health campaigns to stimulate the consumption of traditional Bulgarian plants as chicory (*Cichorium intybus* L.), rose hip fruits (*Rosa canina* L.), zdravec (*Geranium macrorrhizum* L.), white birch (*Betula pendula* L.) and smradlika (*Cotinus coggygria*, syn.: *Rhus cotinus* L.) which are able to provide significant health protection in order to prevent chronic diseases.

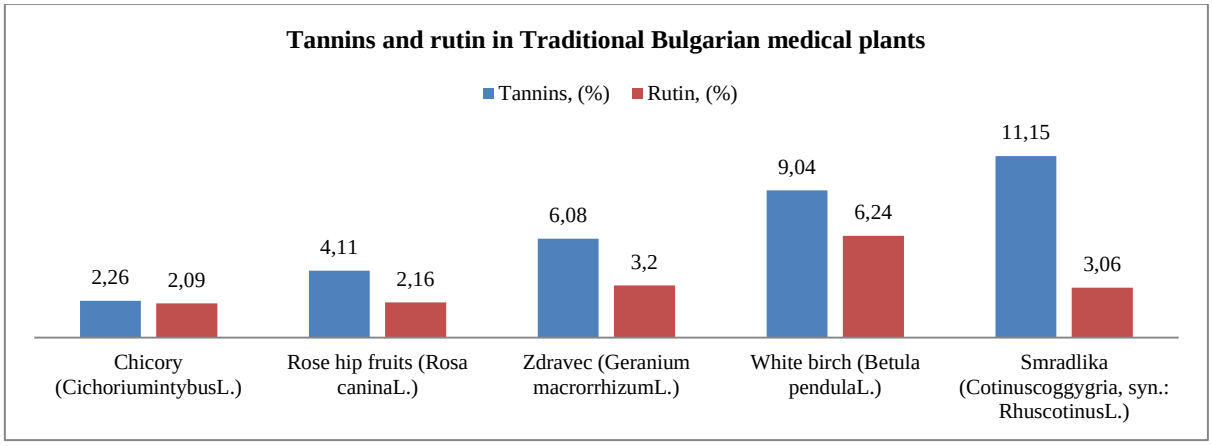

Figure 2 Tannins and rutin in Traditional Bulgarian medical plants

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