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COMPARATIVE EVALUATION OF THE CONTENT OF ANTIOXIDANT POLYPHENOLIC COMPOUNDS IN SELECTED BULGARIAN MEDICINAL PLANTS

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Abstract. *Polyphenols are strong antioxidants of plant origin that exhibit protective role against the development of several chronic degenerative diseases. Quantitative data on the content of polyphenolic antioxidants in Bulgarian medicinal plants are sporadic and unsystematic.* ***The aim*** *of the study is to determine polyphenols content in series of extracts, infusions and decocts of selected Bulgarian medicinal plants and fruits and to compare their polyphenolic profile.* ***Materials and Methods:*** *In the present study 18 Bulgarian medicinal plants and fruits have been analyzed for Total Phenolic and Total Flavonoids content, along with HPLC analysis for their level of the individual flavonoids (+)-Catechin, (-)-Epicatechin and Rutin. Comparative evaluation of the content of antioxidant polyphenolic compounds was performed by hierarchical cluster analysis.* ***The results*** *show that infusions of Hawthorn (Crategus monogyna) flowers have the highest values of Total Phenolic and Total Flavonoids content, forming a separate Cluster in the analysis of the Agglomeration schedule coefficients. Within fruits, data for Total Phenolics and Total Flavonoids content are more homogenic as the cluster analysis shows distribution of 6 elements in a high mean value group. Elderberry (Sambicus nigra) flowers and fruits are the richest source of Rutin in the selected plants studied.* ***Conclusion:*** *In the present study new data for polyphenolic content in selected Bulgarian medicinal plants and fruits are presented and their degree of similarity in methanolic extracts, infusions and decocts have been assessed in order to provide new data for development of effective antioxidant medicinal plant compositions.*

Key words: *Total Phenolic Total Flavonoids, Catechins, Rutin, medicinal plants*

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INTRODUCTION

Oxidative stress is a physiological condition associated with development of a number of degenerative diseases. Damage caused by reactive oxygen species can affect all organs and systems. For example, LDL oxidation is the initial step towards the development of atherosclerosis, and the oxidation of DNA bases is related with the process of mutagenesis and cancer development [1-3]. The intake of exogenous antioxidants is known to play an important role in the reduction of oxidative stress. Polyphenols, carot-

enoids, vitamin C and vitamin E are the largest contributors to the antioxidant dietary intake [4]. In recent decades, polyphenols, namely flavonoids are the focus of extensive scientific research as potent scavengers of free radicals and preventive antioxidants toward the initiation stage of lipid peroxidation [5-6]. Furthermore, a number of epidemiological studies have revealed that polyphenols belonging to the group of flavonoids have a protective role against development of a number of chronic degenerative diseases like cardiovascular disease, cancer, obesity, diabetes, and others [7-13]. The richest dietary sources of polyphenols are medicinal plants, fruits and vegetables [4]. According to the WHO, between 65% and 80% of the population of developing countries currently uses medicinal plants as remedies [14]. The Bulgarian flora offers a great variety of plants – over 4000 higher plants are known, and at least 800 of them are known to have phytotherapeutic properties. It is known that medicinal plants and fruits that are widely used in folk medicine are a rich source of antioxidant polyphenols and in particular flavonoids, such as *Pentace burmanica* [15]; Dandelion (*Taraxacum officinale*), Hawthorn (*Crategus spp.*), Rose hips (*Rosa rugosa*) [16]; leaves of Common rue (*Ruta graveolens*), Buckwheat blossom (*Fagopyrum esculentum*), Rose blossom (*Rosa rugosa*), etc. [17]. However, quantitative data on the content of polyphenolic antioxidants in Bulgarian medicinal plants and their fruits are sporadic and unsystematic. There are published data on the content of catechins, flavonols and flavones in Bulgarian fruits, vegetables and wines, determined by validated HPLC methods [18-21]. Results for the flavonol Rutin in medicinal plants were provided by Atanasova and Georgieva in Lemon balm (*Melissa officinalis*), Sage (*Salvia officinalis*) and Mint (*Mentha piperita*), which was determined by spectrophotometric methods [22], though a systematic data for polyphenols and individual flavonoids in Bulgarian medicinal plants has not been identified.

The **aim** of the present study is to determine the polyphenols content – Total Phenolic, Total Flavonoids and the individual flavonoids (+)-Catechin, (-)-Epicatechin and Rutin in a series of extracts, infusions and decocts of Bulgarian medicinal plants and their fruits and to assess their degree of similarity by hierarchical cluster analysis approach. This would allow better characterization of their polyphenols profile and provide new data for elaboration of effective antioxidant plant compositions.

MATERIALS AND METHODS

Plant material

In the present study 18 different medicinal plants and their fruits were selected – Herbs: Elderberry, flower (*Sambicus nigra*); Hawthorn, flower (*Crategus monogyna*); Wild thyme, stalk (*Thymus serpyllum*); Burra gokharu, stalk (*Tribulus terrestris*); Marigold, flower (*Calendula officinalis*); Oregano, stalk (*Origanum vulgare*); St. John's Wort, stalk (*Hypericum perforatum*); Nettle, leaves (*Urtica dioica*); Wild mint, leaves (*Menta piperita*); Lemon balm, leaves (*Melissa officinalis*); Ironwort, stalk (*Sideritis scardic*); Fruits: Elderberry (*Sambucus nigra*); Cranberry (*Vaccinum vitis-ideae*); Hawthorn (*Crategus monogyna*); Dog rose (*Rosa canina*); Dogwoods (*Cornus mas*); Blackthorn (*Prunus spinosa*); Aronia (*Aronia arbutifolia*). Three individual samples (100 g each) from each medicinal plant material were purchased from the pharmacy net in Sofia.

Sample preparation

Preparation of methanolic extracts. A test sample of 0.2500 g grounded dry plant material was weighted and transferred into a 25 ml volumetric flask and polyphenols were extracted with 80% methanol/water (w/w) in an ultrasonic bath for 30 min at 30ºC. The solutions were ultracentrifuged at 10000 rpm for 5 min and filtered through a membrane filter (0.45 μm) prior to analysis.

Preparation of water infusions and decocts. For herbal infusions 1 g of dry plant material was poured with 100 ml of boiling water, left to boil in water bath for 15 min, left to cool for 45 min at room temperature, and filtered. For preparing fruits' decocts: 100 g of distilled water was added to 5 g of dry fruit material. The fruits were boiled for 5-10 minutes, according to the manufacturer's instructions. After boiling, the fruit sample was soaked for 30 minutes, then the water extract was filtered and left to cool. The samples were stored at 4ºC before analysis, for not more than 24 h, without addition of preservatives.

Determination of Total Phenolic Content

Polyphenols in plant extracts react with specific redox reagents (Folin-Ciocalteu reagent) to form a blue complex that can be quantified by visible-light spectrophotometry at λ = 750 nm. To determine the Total Phenolic content, Folin-Ciocalteu method was applied and gallic acid was used as an external standard [23]. The results are expressed as gallic acid equivalent – mg GAE/g sample.

Determination of Total Flavonoids Content

Total Flavonoids are determined by placing an aliquot of 1 ml of the extracts or standard solutions of Rutin in a 10 ml volumetric flask containing 4 ml of distilled water. 0.3 ml of 5% $NaNO_2$ and after 5 minutes 0.3 ml of 10% AlCl3 was added to the solution. After 6 minutes, 2 ml of 1 M NaOH was added; the solution

was made up to the mark with distilled water, stirred and allowed to stand for 30 minutes [23]. The spectral absorption of the formed pink complex was measured at λ = 510 nm. The Total Flavonoids content is expressed as routine equivalent per gram sample (mg RE/g sample).

HPLC determination of (+)-Catechin, (-)-Epicatechin and Rutin

Chromatographic separation of phenolic compounds – (+)-Catechin, (-)-Epicatechin and Rutin, was carried out using Luna C18 column (3 µm, 150 mm x 4.6 mm, Phenomenex, USA). A gradient elution with 0.9 ml/min flow rate at 30ºC was performed. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). A linear gradient program from 15% solvent B to 35% B in 12 minutes was applied. A Photo Diode Array detection was used at two wavelengths – 280 nm for (+)-Catechin and (-)-Epicatechin and 355 nm for Rutin, with a reference wavelength set at 620 nm [23]. The results of the HPLC analysis are expressed in µg/g sample.

All measurements in the present study were performed in triplicate and the methods were validated, according ISO 17025 requirements [24].

Statistical analysis

A comparative characterization of the selected samples was performed, by the means of calculation methods and cluster analysis. The hierarchical clustering was performed using the Nearest Neighbour clustering method and SED (Squared Euclidian Distance) to measure intervals between classes. The method is sensitive to the local structure of the data, which makes it suitable for solving classification problems using parameters from a certain group, as in this case – analysis of Total Phenolics, Total Flavonoids and Rutin content. The Dendrograms for the different polyphenols groups are presented.

RESULTS

Polyphenols content in extracts, infusions and decocts of selected Bulgarian medicinal plants

In Table 1 the results for Total Phenolic and Total Flavonoids, and the individual flavonoids – (+)-Catechin, (-)-Epicatechin and Rutin content in methanolic extracts of selected medicinal plants (herbs and fruits) are presented. Data are expressed as average values of triplicate analysis along with the standard deviation of the mean.

Table 1. Total Phenolic, Total Flavonoids, (+)-Catechin, (-)-Epicatechin and Rutin content in methanolic extracts of selected Bulgarian medicinal plants

Plant Material	Samples *Latin name*	Total Phenolic mg GAE/g	Total Flavonoids mg RE/g	(+)-Catechin µg/g	(-)-Epicatechin µg/g	Rutin µg/g
Herbs				**Extracts**		
Elderberry, flower	*Sambucus nigra*	43.07 ± 0.24	34.61 ± 0.63	–	–	18584.6 ± 376.1
Hawthorn, flower	*Crategus monogyna*	92.08 ± 0.98	45.27 ± 1.06	–	–	425.8 ± 4.30
Wild thyme, stalk	*Thymus serpyllum*	92.26 ± 2.08	62.39 ± 1.39	–	–	866.3 ± 11.13
Burra gokharu, stalk	*Tribulus terrestris*	8.21 ± 0.07	4.45 ± 0.02	–	–	248.7 ± 4.87
Marigold, flower	*Calendula officinalis*	20.75 ± 0.10	16.93 ± 0.09	–	140.9 ± 2.59	445.7 ± 3.04
Oregano, stalk	*Origanum vulgare*	88.08 ± 1.56	77.08 ± 0.63	–	–	252.8 ± 2.32
St. John's Wort, stalk	*Hypericum perforatum*	103.37 ± 2.62	97.33 ± 1.58	–	4378.9 ± 104.38	8185.5 ± 123.17
Nettle, leaves	*Urtica dioica*	17.89 ± 0.48	13.53 ± 0.31	–	–	1777.0 ± 39.61
Wild mint, leaves	*Menta piperita*	67.38 ± 1.21	54.59 ± 0.55	–	–	7332.5 ± 1.91.03
Lemon balm, leaves	*Melissa officinalis*	65.17 ± 1.00	57.76 ± 1.10	–	–	402.5 ± 5.21
Ironwort, stalk	*Sideritis scardic*	40.51± 1.11	27.70 ± 0.72	–	–	953.6 ± 25.77
Fruits				**Extracts**		
Elderberry	*Sambucus nigra*	16.88 ± 0.44	16.24 ± 0.44	149.57 ± 3.84	–	2818.7 ± 70.94
Cranberry	*Vaccinum vitis-ideae*	18.42 ± 0.38	17.41 ± 0.42	260.1 ± 8.5	504.51 ± 11.2	25.9 ± 0.7
Hawthorn	*Crategus monogyna*	12.49 ± 0.41	7.08 ± 0.20	–	363.2 ± 11.98	36.8 ± 0.76
Dog rose	*Rosa canina*	17.22 ± 0.33	16.16 ± 0.27	–	–	–
Dogwoods	*Cornus mas*	10.41 ± 0.68	9.51 ± 0.35	–	–	40.8 ± 0.61
Blackthorn	*Prunus spinosa*	3.34 ± 0.12	3.10 ± 0.08	–	–	54.3 ± 1.29
Aronia	*Aronia arbutifolia*	17.76 ± 0.44	15.84 ± 0.61	755.4 ± 3.59	–	67.1 ± 0.67

(-) – non detected

The results show that the higher Total Phenolic level in methanolic extracts was determined in St. John's Wort (stalk), followed by Wild Thyme and Hawthorn – 103.37, 92.26 and 92.08 mg GAE/g respectively. The richest sources of Total Flavonoids among medicinal plants are St. John's Wort, Wild Thyme and Oregano – 97.33, 77.08 and 62.39 mg RE/g. The lowest polyphenol content was found in Burra Gokharu (stalk). The results for Total Phenolic and Total Flavonoids in fruits are generally lower, reaching higher values in Cranberry (18.42 mg GAE/g and 17.41 mg RE/g).

Catechins were detected only in few plant species. (+)-Catechin was found only in fruits with maximum amount determined in Aronia (755.4 µg/g). (-)-Epicatechin was found in two herbal and two fruit samples with highest value of 4378.9 µg/g in St. John's Wort. Rutin was detected in all medicinal plant samples with the exception of Dog rose. The results show an extremely high Rutin level in Elderberry flowers – 18584.6 µg/g.

The results for polyphenols content for infusions and decocts are presented in Table 2. The data show that the highest values for Total Phenolic and Total Flavonoids contents in water infusions are found again in St. John's Wort (103.61 mg GAE/G and 76.77 RE/g). In decocts (+)-Catechin was determined only in Aronia and (-)-Epicatechin only in Hawthorn. Rutin content in Elderberry infusions and decocts reaches very high levels of 20343 µg/g and 2147.6 µg/g, respectively. Rutin is present in all herbal infusions, however has not been detected in Cranberry, Dog rose, Dogwoods and Blackthorn decocts.

Comparative evaluation of polyphenols content by Hierarchical cluster analysis

A comparative evaluation of polyphenols content in the selected medical plant and fruits was performed, by applying Agglomerative hierarchical cluster analysis. For implementing the Agglomerative hierarchical clustering, a successive merging of different clusters was performed until one cluster including all elements is formed. The algorithm for agglomerative hierarchical clustering includes: Step 1 Initialization – At this stage the initial set of clusters is defined, and each element is considered as a separate cluster. Step 2 Proximity matrixes – calculates the similarity of one cluster with all other clusters. At this stage, those clusters that have the greatest similarity are identi-

Table 2. Total Phenolics, Total Flavonoids, (+)-Catechin, (-)-Epicatechin and Rutin content in infusions and decocts of selected Bulgarian medicinal plants

Plant Material	Samples *Medical plant, Latin name*	Total Phenolics mg GAE/g	Total Flavonoids mg RE/g	(+)-Catechin µg/g	(-)-Epicatechin µg/g	Rutin µg/g
Herbs				**Infusions**		
Elderberry, flower	*Sambucus nigra*	55.90 ± 0.69	53.53 ± 0.52	–	–	20643.0 ± 573.2
Hawthorn, flower	*Crategus monogyna*	191.35 ± 2.91	178.31 ± 2.56	–	–	374.9 ± 5.63
Wild thyme, stalk	*Thymus serpyllum*	33.42 ± 0.62	30.37 ± 1.03	–	–	624.4 ± 9.47
Burra gokharu, stalk	*Tribulus terrestris*	11.68 ± 0.08	4.06 ± 0.06	–	–	2789.0 ± 64.86
Marigold, flower	*Calendula officinalis*	17.45 ± 0.11	11.15 ± 0.08	–	–	10860.3 ± 275.96
Oregano, stalk	*Origanum vulgare*	90.80 ± 1.16	78.92 ± 0.41	–	–	307.8 ± 4.52
St. John's Wort, stalk	*Hypericum perforatum*	103.61 ± 2.75	76.77 ± 1.11	–	5115.2 ± 78.93	10528.9 ± 257.29
Nettle, leaves	*Urtica dioica*	16.82 ± 0.37	13.95 ± 0.26	–	–	1675.8 ± 28.01
Wild mint, leaves	*Menta piperita*	67.65 ± 1.04	56.16 ± 0.46	–	–	9352.0 ± 209.25
Lemon balm, leaves	*Melissa officinalis*	64.51 ± 0.74	50.54 ± 0.87	–	–	684.7 ± 8.43
Ironwort, stalk	*Sideritis scardic*	43.21 ± 1.23	31.27 ± 0.51	–	–	1097.6 ± 13.67
Fruits				**Decocts**		
Elderberry	*Sambucus nigra*	16.63 ± 0.40	14.17 ± 0.37	–	–	2147.6 ± 56.70
Cranberry	*Vaccinum vitis-ideae*	1.07 ± 0.01	0.77 ± 0.00	–	–	–
Hawthorn	*Crategus monogyna*	7.11 ± 0.18	1.60 ± 0.05	–	30.5 ± 0.67	34.7 ± 1.17
Dog rose	*Rosa canina*	33.30 ± 0.10	17.09 ± 0.65	–	–	–
Dogwoods	*Cornus mas*	8.79 ± 0.02	2.53 ± 0.13	–	–	–
Blackthorn	*Prunus spinosa*	0.49 ± 0.02	0.49 ± 0.02	–	–	–
Aronia	*Aronia arbutifolia*	6.99 ± 0.05	0.65 ± 0.65	89.6 ± 1.05	–	15.9 ± 0.23

(-) – non detected

fied. Step 3 Grouping/Merging of clusters which are very similar or close to each other. At this stage, the two closest clusters merge to form a new common cluster. Step 2 and step 3 are to be repeated till one cluster including all elements is obtained.

In Figure 1 the dendrograms from cluster analysis for Total Phenolic content in medicinal plants – herbs (a) and fruits (b) are presented.

The analysis of Agglomeration schedule coefficients, measuring the distance between clusters in different stage of the hierarchical cluster analysis, shows that an option of 7 clusters is suitable for the hierarchical cluster classification of Total Phenolic content in the selected medicinal plants (see Fig. 1a). In table 3 the number of elements and the mean value of Total Phenolic in every cluster are presented.

The distribution of herbal medicinal plants to clusters is as follows:

- Cluster 1 – Elderberry, flower (extract); Ironwort, stalk (extract); Wild Thyme, stalk (infusion); Ironwort, stalk (infusion);
- Cluster 2 – Hawthorn, flower (extract); Wild Thyme, stalk(extract); Oregano, stalk (extract); Oregano, stalk (infusion);
- Cluster 3 – Burra Gokharu, stalk (extract); Marigold, flower (extract); Nettle, leaves (extract); Burra Gokharu, stalk (infusion); Marigold, flower (infusion); Nettle, leaves (infusion);
- Cluster 4 – St. John's Wort, stalk (extract); St. John's Wort, stalk (infusion);
- Cluster 5 – Wild mint, leaves (extract); Lemon balm, leaves (extract); Wild mint, leaves (infusion); Lemon balm, leaves (infusion);
- Cluster 6 – Elderberry, flower (infusion);
- Cluster 7 – Hawthorn, flower (infusion).

The analysis of Agglomeration schedule coefficients for Total Phenolic content in fruits shows that a distribution in 5 clusters is a suitable choice (see Fig. 1b). The intersection between the blue vertical line and the horizontal lines represents the elements in clusters. For example, Dogwoods (extract) (10.41 ± 0.68 mg GAE/g) and Hawthorn (extract) (12.49 ± 0.41 mg GAE/g) build group Cluster 2 (11.45 ± 1.04 mg GAE/g). It is shown that within Cluster 1 (17.48 ± 0.28 mg GAE/g) there are six elements – extract and decocts of Elderberry and Dog rose, Aronia (extract) and Cranberry (extract). In Cluster 3 (3.64 ± 0.30 mg GAE/g) alight Blackthorn (extracts) (3.34 ± 0.12 mg GAE/g) and Dogwoods (infusions) (3.94 ± 0.02 mg GAE/g). In Table 4 the number of elements in every cluster and the mean value of their Total Phenolic content are presented.

In Figure 2 the dendrograms of the hierarchy cluster analysis for assessment of degree of similarity of the experimental data for Total Flavonoids content are presented.

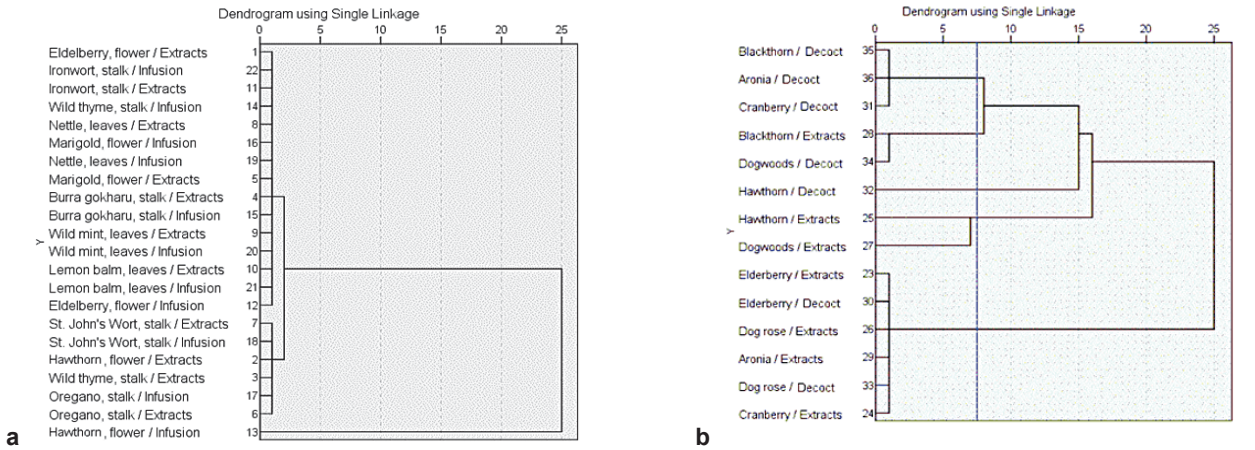


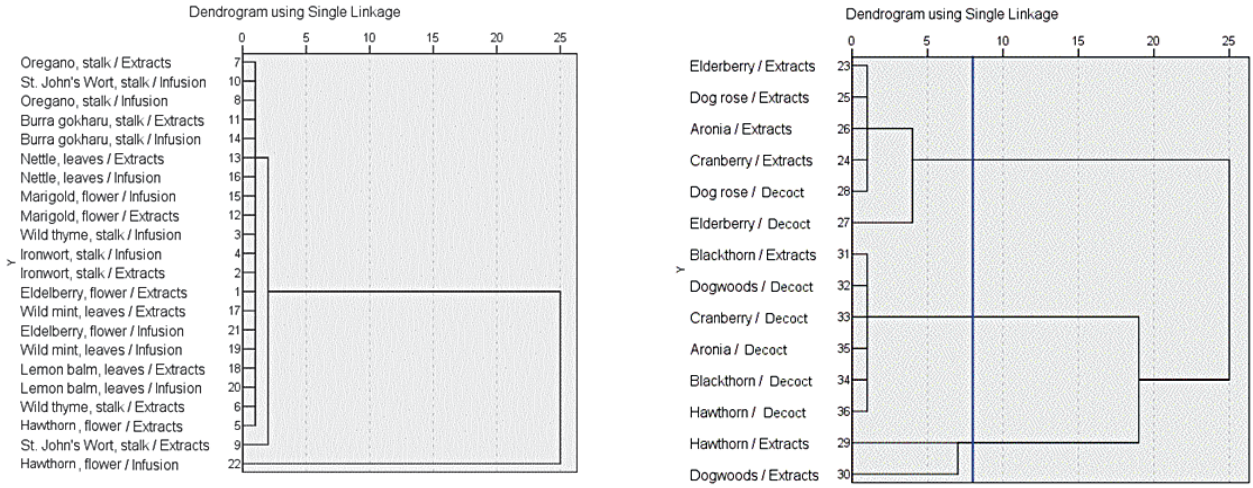
Fig. 1. Hierarchical cluster analysis for Total Phenolics: a) Herbs, b) Fruits

Table 3. Total Phenolic Content – distribution of herbal medicinal plants within the clusters

Cluster	1	2	3	4	5	6	7
Count of elements	4	4	6	2	4	1	1
Mean value of Total Phenolic, mg GAE/g	40.05 ±2.30	90.80 ±0.96	15.47 ±1.88	103.50 ±0.12	66.18 ±0.79	55.90	191.35

Table 4. Total Phenolics Content – distribution of fruits' elements within the clusters

Cluster	1	2	3	4	5
Count of elements	6	2	2	3	1
Mean value of Total Phenolic, mg GAE/g	17.48 ±0.28	11.45 ±1.04	3.64 ±0.30	0.79 ±0.16	7.11



a b

Fig. 2. Hierarchical cluster analysis for Total Flavonoids: a) Herbs, b) Fruits

In Table 5 the number of elements in every cluster and the mean value of Total Flavonoids content in medicinal plants are presented. Based on analysis of agglomeration schedule coefficient, 6 clusters are modelled (Fig 2 a).

The distribution of herbal medicinal plants to clusters is as follows:

- Cluster 1 – Elderberry, flower (extract); Ironwort, stalk (extract); Wild thyme, stalk (infusion); Ironwort, stalk (infusion);
- Cluster 2 – Hawthorn, flower (extract); Wild thyme, stalk (extract); Wild mint, leaves (extract); Lemon balm, leaves (extract); Wild mint, leaves (infusion); Lemon balm, leaves (infusion); Elderberry, flower (infusion)
- Cluster 3 – Oregano, stalk (extract); Oregano, stalk (infusion); St. John's Wort, stalk (infusion);
- Cluster 4 – St. John's Wort, stalk (extract);
- Cluster 5 – Burra gokharu, stalk (extract); Marigold, flower (extract); Nettle, leaves (extract); Burra gokharu, stalk (infusion); Marigold, flower (infusion); Nettle, leaves (infusion);
- Cluster 6 – Hawthorn, flower (infusion).

Data show that representatives in Cluster 6 and Cluster 4 are the richest source of Total Flavonoids with statistically significant higher values than other plants studied. We have to point out that both clusters contain only one representative each – Hawthorn, flowers (infusion) and St. John's Wort, stalk (methanol extract), correspondingly in Cluster 6 and Cluster 4.

The analysis of Agglomeration schedule coefficients for Total Flavonoids in fruits shows that the choice of only 3 clusters is adequate. In Table 6 the number of elements in every cluster and the mean value of Total Flavonoids content are presented.

Table 5. Total Flavonoids Content – distribution of herbal medicinal plants within clusters

Cluster	1	2	3	4	5	6
Count of elements	4	7	3	1	6	1
Mean value of Total Flavonoids, mg RE/g	30.99 ±1.42	54.32 ±2.05	77.59 ±0.67	97.33	10.68 ±2.17	178.31

Table 6. Total Flavonoids Content – distribution of fruits' within clusters

Cluster	1	2	3
Count of elements	6	2	6
Mean value of Total Flavonoids, mg RE/g	16.15 ±0.47	8.30 ±1.22	1.53 ±0.44

The distribution of fruits to clusters is as follows:

- Cluster 1 – Elderberry (extract); Cranberry (extract); Dog rose (extract); Aronia (Extract); Dog rose (decoct); Elderberry (decoct);
- Cluster 2 – Hawthorn (extract); Dogwoods (extract);
- Cluster 3 – Blackthorn (extract); Dogwoods (decoct); Cranberry (decoct); Blackthorn (decoct); Aronia (decoct); Hawthorn (decoct).

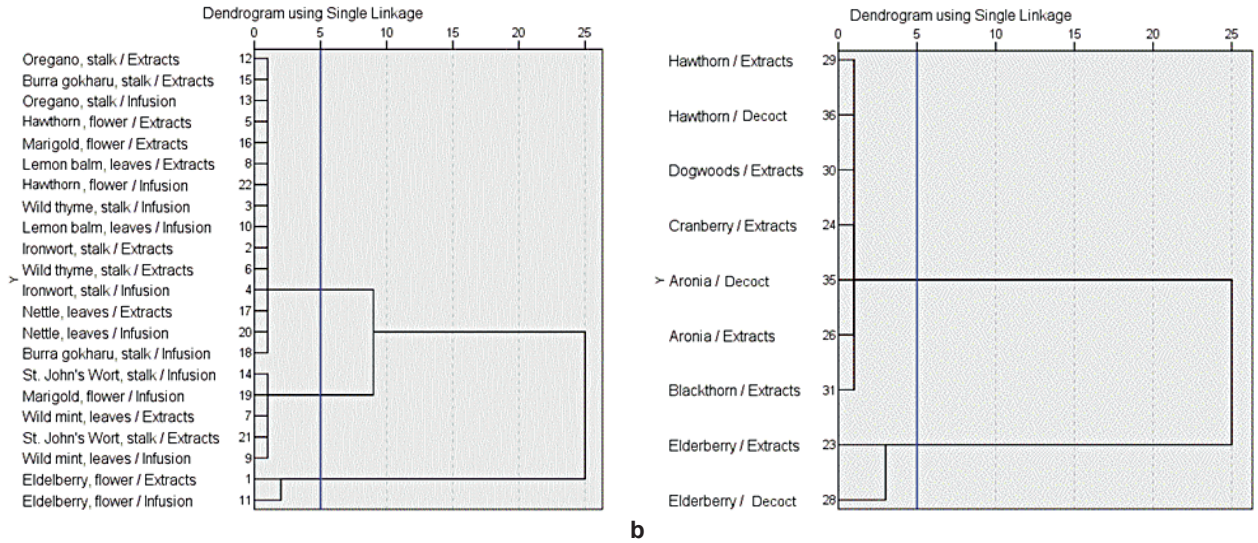
The intersection between the blue vertical line and horizontal lines shows the elements in each cluster. For example the Total Flavonoids content in methanolic extract of Dogwoods (7.08 ± 0.20 mg RE/g) and Hawthorn (9.51 ± 0.35 mg RE/g) are included in Cluster 2 (8.30 ± 1.22 mg RE/g). The degree of similarity for Total Flavonoids, according the hierarchical cluster analysis, show that for fruits Cluster 1 (with the highest mean values) contains 6 elements in contrary to data for herbal medical plant where for Clusters 4 and 6 only one representative was determined to have the higheest values of Total Flavonoids (Table 5).

In Figure 3, the dendrogram of the Hierarchical cluster analysis for Rutin in the selected medicinal plants is presented.

In Table 7 the number of elements in every cluster and the mean value of Rutin content in medicinal plants are presented.

The analysis of Agglomeration schedule coefficients for herbs shows that distribution in 3 clusters is a good choice, as follows:

- Cluster 1 – Elderberry, flower (extract); Elderberry, flower (infusion);
- Cluster 2 – Ironwort, stalk (extract); Wild thyme, stalk (infusion); Ironwort, stalk (infusion); Hawthorn, flower (extract); Wild thyme, stalk (extract); Lemon balm, leaves (extract); Lemon balm, leaves (infusion); Oregano, stalk (extract); Oregano, stalk (infusion); Burra gokharu, stalk (extract); Marigold, flower (extract); Nettle, leaves (extract); Burra gokharu, stalk (infusion); Nettle, leaves (infusion); Hawthorn, flower (infusion);
- Cluster 3 – Wild mint, leaves (extracts); Wild mint, leaves (infusion); St. John's Wort, stalk (infusion); Marigold, flower (infusion); St. John's Wort, stalk (extracts)



a b

Fig. 3. Hierarchical cluster analysis for Rutin: a) Herbs, b) Fruits

Table 7. Rutin content – distribution of herbal medicinal plants within clusters

Cluster	1	2	3
Count of elements	2	15	5
Mean value of Rutin, µg/g	19613,8 ±1029,1	861,8 ±185,6	9251,8 ±672,6

The hierarchy cluster analysis for Rutin content in fruits shows that chose of 2 clusters is adequate (Fig 3 b). The intersection between blue vertical line and horizontal lines shows the elements in clusters. For instance, Elderberry extracts and decocts (2818.7 ± 70.94; 2147.6 ± 56.70 μg/g), respectively comprise Cluster 1 (2483.2 ± 39.4 μg/g). In Table 8 the number of elements in every cluster and the mean value of Rutin content are presented.

Table 8. Rutin content – distribution of fruits' within clusters

Cluster	1	2
Count of elements	2	7
Mean value of Rutin, μg/g	2483.2 ± 39.4	335.6 ± 6.5

The distribution of fruits to clusters is as follow:

- Cluster 1 – Elderberry (extracts); Elderberry (decoct);
- Cluster 2 – Cranberry (extracts); Aronia (extracts); Hawthorn (extracts); Dogwoods (extract); Blackthorn (extract); Aronia (infusion); Hawthorn (infusion)

DISCUSSION

The results of the current study for the polyphenols content in selected medicinal plants show that there is lack of correlation between data for methanolic extracts and water infusions and decocts. This could be attributed to multiple factors affecting the process of solid/solvent distribution of biologically active substances during extraction, such as mass transfer coefficient, herbs saturation, concentration gradient, contact surface, duration of the process, temperature, viscosity of the medium, etc. However a general tendency of higher polyphenols values in methanol extracts in fruits in comparison to decocts samples has been observed, probably due to the rough structure of dry fruits cell walls that restricts water penetration to a greater extend. As a general rule the finer the plant particles are, the faster and more complete the extraction process is. However, higher degree of sample comminution implies a greater number of destroyed cell walls, which allows direct dissolution of larger amounts of concomitant compounds in the extract. Therefore an optimal particle dispersion of ground plant material is important for each specific medicinal plant. In cases where the particle size of a specific plant material is not specified, the particle size of ground flowers, leaves and grasses samples is 3-5 mm, for stems, roots and rhizomes – 1-3 mm, and for fruits and seeds – 0.3-0.5 mm [25].

According to our knowledge, this is the first detailed comparative evaluation of the polyphenols content of selected Bulgarian medicinal plants, based on original data. On the basis of the cluster analysis, quantitative data for the richest sources of polyphenols in Bulgarian medicinal plants with significance for the elaboration of phytotherapeutic agents and food supplements formulations with antioxidant properties could be provided. Our analysis has shown that infusion of Hawthorn flowers (Crategus monogyna) is the single richest source of Total Phenolics and Total Flavonoids among the selected medicinal plants, forming a single cluster with statistically significant distinction. In Bulgarian traditional medicine fruits and leaves infusions of Hawthorn are used against cardiovascular diseases. However, we have determined that the polyphenols content in Hawthorn flowers infusions are about 26 times higher in Total Phenolics and about 110 times higher in Total Flavonoids than the Hawthorn fruit decocts. These results could provide a basis for appraise the application of different parts of the Hawthorn plant and favourable wider inclusion of their flower in antioxidant phytochemical formulations.

St. John's stalk extracts and infusions are the second richest source of polyphenols, forming an own cluster of Total Phenolic content (Cluster 4, see Table 4). The Total Flavonoids content in methanolic extracts of St. John's Wort is higher than those in infusions with a statistically significant level. The level of Total Flavonoids in St. John's Wort is similar to those in Oregano stalk (extracts and infusions). In Bulgarian traditional medicine St. John's Wort is a very popular medicinal plant, while oregano is used mainly as a leafy condiment. Our results might substantiate the promotion of wider application of oregano as a phytotherapeutic agent.

Our data have shown unambiguously that Elderberry flowers extracts and fruits decocts are the richest source of Rutin among the studied samples. Considering the results for Elderberry flowers and fruits we have found that the polyphenols level is higher in flowers in comparison of fruits, with an exception of data for (+)-Catechin. The Rutin content in extracts of Elderberry flowers is approximately 6 times higher than those found in fruits, suggesting that flavonoids level decreases with the process of fruit development. This result is in tune with the literature data, showing that the accumulation of flavonoids in fruits is strongly influenced by the physiological state of the fruit with the general trend that flavonoid are generally higher in immature fruits and plant tissues [26, 27]. These results could also suggest wider use of Elderberry flowers as a phytotherapeutic agent

along with the long-established in Bulgarian market variety of food supplements, containing Elderberry fruits extracts.

CONCLUSION

In the present study 18 Bulgarian medicinal plants and fruits were studied for their Total Phenolic and Total Flavonoids content, applying two different extraction techniques. Furthermore, HPLC data for the level of (-)-Catechin, (-)-Epicatechin and Rutin were also presented. The degree of similarity of the polyphenols content of medicinal plants and their fruits has been assessed using cluster analysis. The results show that infusions of Hawthorn flowers (Crategus monogyna) have the highest values of Total Phenolic and Total Flavonoids, forming a separate cluster in the analysis of Agglomeration schedule coefficients. Among fruits there is no single leader regarding Total Phenolics and Total Flavonoids content, while cluster analysis show distribution of 6 elements in a high mean value cluster. The analysis of data shows that Elderberry (Sambicus nigra) flowers and fruits are the richest source of Rutin in the selected plants studied. The current study could provide data for the identification and development of new, more effective antioxidant plant sources and combinations.

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