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Vitamin C, chlorophylls, carotenoids and xanthophylls content in some basil (*Ocimum basilicum* L.) and rosemary (*Rosmarinus officinalis* L.) leaves extracts

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Abstract

This paper presents the comparative analysis of vitamin C, carotenoids, chlorophylls and xanthophylls content in some basil (Ocimum basilicum) and rosemary (Rosmarinus officinalis) leaves extracts. From each plant material were made three types of extracts: aqueous, alchoholic and acetonic. Vitamin C content was determined by iodometric method and chlorophills, carotenoids, respectively xanthophylls content - by spectrophotometric method. Highest amount of vitamin C was detected in acetonic extract of basil leaves (0.520 mg/ml), followed by the aqueous extract of basil leaves (0,440 mg/ml). In both cases acetonic extracts are the richest in vitamin C and alchoholic extracts are the poorest in vitamin C. The highest content of chlorophyll "a" was found in alcoholic basil leaves extract (32.306 mg/l), followed by acetonic basil leaves extract (27.794 mg/l). The lowest chlorophyll 'a' content was recorded in acetonic extract of rosemary leaves (1.803 mg/l). Chlorophyll "b" is the most abundant in the acetonic extract of basil leaves (12.096 mg/l) and the lowest amount in acetonic extract of rosemary leaves (1,132 mg/l). Carotenes and xanthophylls are best represented in the extracts of basil leaves (63.986 mg/l in the alcoholic extract, respectively 57.396 mg/l in acetonic extract) and the worst in the acetonic extract of rosemary (8.957 mg/l).

Keywords: basil, rosemary, vitamin C, chlorophylls, carotenoids, xanthophylls

1. Introduction

Plants are a rich source of therapeutically active compounds such as antioxidants, dyes, antibiotics, vitamins, organic acids, glycosides, amino acids, proteins, unsaturated fatty acids, and other substances of particular importance to human life. Synthesized organic substances and inorganic substances accumulated in plants are more beneficial than pharmacodynamic activity of many drugs. Almost 12% of native species are used in traditional and scientific medicine [1-3].

Today, there is a great interest among scientists for high capitalization of active compounds from different plant materials, perfecting more and more their separation and purification techiques from plants, for use in food, pharmaceutical and cosmetics industries [1,4,5].

Basil (*Ocimum basilicum*), belonging to the Lamiaceae family, is one of the most popular plants grown extensively in many continents around the world, especially in Asia, Europe and North America. Basil is believed to have originated in Iran and/or India. At least 150 species of the genus *Ocimum* are widely cultivated in other countries of Asia [6]. Although several basil species are found in many regions, the species *O. basilicum* is the most cultivated variety in the world [7]. *Ocimum* is widely cultivated and extensively used for food, perfumery, cosmetics, pesticides, medicine, and

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traditional rituals because of their natural aroma and flavor and other properties [8,9].

Basil contains essential oil (0,10-0,20%) with different chemical composition - linalool, methylcavicol or estragole, cineol, camphor, α-pinene, eugenol, oleanolic acid, anethole, β-sitosterol, methyl cinnamate - saponosids triterpenoid, tanoids, chlorophyll, vitamin C, carotenoids, a wide range of phenolic compounds displaying various antioxidant activities, depending on the basil species and cultivars [10,11]. One hundred grams of fresh basil leaves contain 26 mg of vitamin C, 3.95 mg of carotene, 0.08 mg of thiamin, 0.31 mg of riboflavin, and 1.1 mg of niacin. Its potassium content is 30 times that of sodium. One hundred grams of fresh basil leaves contain 250 mg of calcium, 37 mg of phosphorus, 5.5 mg of iron, and 11 mg of magnesium [12].

Rosemary (*Rosmarinus officinalis* L.) is a spice and medicinal herb widely used around the world. Of the natural antioxidants, rosemary has been widely accepted as one of the spices with the highest antioxidant activity. Rosemary essential oil is also used as an antibacterial, antifungal and anticancer agent [13]. Chemical, leaves and branches with flowers contain volatile oil (0.5 to 9%), consisting of terpene hydrocarbons (α-pinene, β-pinene, camfen, limonene, dipenten, mircen, p-cimen, cariofilen, humulen, santen, α-tujen, fenechen, sabinene, α-and γ-terpinene, α- and β-felandren, tansocimen), alcohols (borneol, α- and β-terpineol, verbenol), oxides (1,8-cineol), ketones (camphor, verberone, octanone). The leaves contain free flavonoids, a series of triterpenes, triterpenic acids, tannin, unspecified saponine, β-sitosterol, choline, glycolic acid, glyceric acid, traces of nicotinic acid, vitamin C, carotenoids, chlorophylls [14]. Thus, according to USDA National Nutrient Database for Standard Reference [15], 100g fresh rosemary leaves contain almost 21.8 mg vitamin C, 0.036 mg thiamin, 0.152 mg riboflavin, 0.912 mg niacin, 0.336 mg vitamin B6, 292.4 IU vitamin A, 317 mg calcium, 6.65 mg iron, 91 mg magnesium, 66 mg phosphorus, 26 mg sodium, 0,93 mg zinc, 2.838 g saturated fatty acids, 1.160 g monounsaturated fatty acisd, 0.901 g polyunsaturated fatty acids.

Vitamin C is of great importance for the body: involved in redox phenomena, is antibiotic, toning, participate in the destruction of toxins, to proper functioning of the adrenal glands. Influence both the functioning of thyroid and corpus luteum in the ovary, maintain and strengthen capillaries [1]. Vitamin C increases the degree of metabolism for a variety of carcinogens, provides protection from ionizing and ultraviolet radiation, showed an high antioxidant activity [16].

Chlorophyll is a specifically pigment of green plants, which plays a key role in photosynthesis (the most important biochemical process on Earth). In plants there are several types of chlorophyll, denoted by letters of the alphabet (a, b, c, d). The most important in photosynthesis is chlorophyll "a", which is darker. Chlorophyll has effects on the human body [17]. External acts as deodorant and skin tonic, internally, stimulates respiration, helps in cleansing waste and help combat anemia. Carotenes and xanthophylls present a very complex biochemical role in the human body. They exhibit anticancer effects, antimicrobial, epithelizant, antioxidant. Carotenoidic compounds involved in the proper conduct of important biochemical processes in animals and human life: in the vision (provitamins A), the growth and reproduction [18].

In the present study aimed to determine the content and comparative analysis of some bioactive compounds (vitamin C, chlorophylls, carotenoids and xanthophylls) from different extracts obtained from leaves of basil and rosemary

2. Materials and Method

It was used raw materials from the domestic market. Extracts were obtained from fresh material using 1 g finely ground material in 10 ml extraction solvent (distilled water, ethanol 96% and acetone p.a.). Extractions were performed at room temperature in Erlenmeyer flask with glass stopper; the extraction time was 1h. Extracts obtained were filtered and then subjected to measurements.

2.1. Determination of vitamin C. In order to determine the C vitamin content, it was used the iodometric method.

The method principle is color reaction between starch and KI + I solution. Determination of C vitamin is made with the aid of $K_2Cr_2O_7$ in the KI-starch presence. Initially, C vitamin is oxidized and then the following reaction takes place:

$$Cr_2O_7^{2-} + 6\,I^- + 14\,H^+ \rightarrow 3\,I_2 + 2\,Cr^{3+} + 7\,H_2O$$

Thus liberated iodine stains starch blue.

Was weighed a sample (0,05-0,1g) on analytical balance, then was brought quantitatively into a titration vessel, were added 10 ml of 2n hydrochloric acid, diluted to 50 ml with distilled water, triturated, added 1 ml of 1% starch solution (freshly prepared) and 1 ml 0.1 N potassium iodide, after which the solution was titrated with 0.1 N potassium dichromate until persistent blue color. Quantification of vitamin C content was done according to the relation:

1 ml 0.1 N potassium dichromate is equivalent to 0.008806 g vitamin [19].

All determinations were performed in triplicate, calculating their arithmetic mean.

2.2. Determination of chlorophyll pigments, carotenes and xanthophylls. For determination of chlorophyll, carotenoids and xanthophylls in the aqueous, alcoholic and acetonic extracts of basil and rosemary leaves, were triturated with quartz sand 2.5 g plant material in the presence of the suitable solvent.

Homogenate obtained was then centrifuged at 3000 rpm. and the supernatant was collected in glass containers. The precipitate was treated with solvent until the solvent extract not present any color (colorless). Supernatants were collected and were colorimetrically at 646 nm, 663 nm and 470 nm to an UV-VIS spectrophotometer Perkin Elmer Lambda 25. According to McKinney-Arron relationship chlorophyll content was measured as [20]:

$$\text{Chl a} = 12.21 \cdot (A_{663}) - 2.81 \cdot (A_{646}),$$

$$\text{Chl b} = 20.13 \cdot (A_{646}) - 5.03 \cdot (A_{663}),$$

$$\text{Chl}_{total} = 17.32 \cdot (A_{646}) + 7.18 \cdot (A_{663}),$$

where:

Chl a – chlorophyll a, in mg/l,

Chl b – chlorophyll b, in mg/l,

Chl_{total} – total chlorophylls content, in mg/l,

A_{663} –sample absorbance at 663 nm,

A_{646} – sample absorbance at 646 nm.

For xanthophylls and carotenoids determination was use the relationship [21]:

$$\text{Carotenes and xanthophylls} = [(1000 \cdot A_{470}) - (3,27 \cdot \text{Chl a}) - (1,04 \cdot \text{Chl b})] / 229,$$

where:

Chl a – chlorophyll a, in mg/l,

Chl b – chlorophyll b, in mg/l,

A_{470} –sample absorbance at 470 nm.

Determinations concerning the content of chlorophylls, carotenoids and xanthophylls were made only on alcoholic and acetonic extracts of basil and rosemary leaves and not on the aqueous extracts, knowing that these compounds are insoluble in water.

3. Results and Discussion

3.1. Vitamin C level

In the Tables 1 and 2 (Figures 1 and 2) are the results on vitamin C content of extracts and raw materials.

Table 1. Vitamin C content of raw materials

Sample	Vitamin C content (mg /100g)
Basil leaves	27.05
Rosemary leaves	18.51

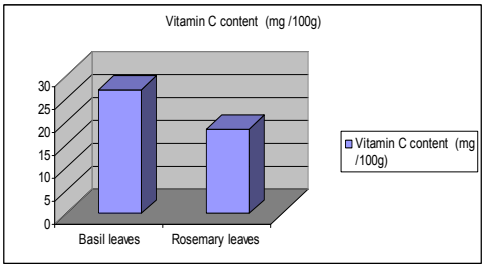

Figure 1. Vitamin C content of raw materials

It could be observed that the basil leaves are richer in vitamin C (27.1 mg/100g) than rosemary leaves (18.5 mg/100g). This results are well corelated with the literature data [12].

Table 2. Vitamin C content of fruits extracts

Sample	Vitamin C (mg/100ml)
Basil aqueous (aq) extract	0.44
Rosemary aqueous (aq) extract	0.26
Basil alcoholic (alc) extract	0.35
Rosemary alcoholic (alc) extract	0.34
Basil acetonic (ac) extract	0.52
Rosemary acetonic (ac) extract	0.36

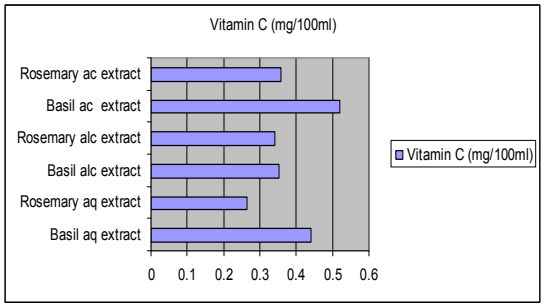

Figure 2. Vitamin C content in fruits extracts

There is a lower concentration of vitamin C in plant extracts than in raw materials, the richest in ascorbic acid being basil leaves acetonic extract (0.52 mg/ml), followed by the aqueous extract of basil leaves (0.44 mg/ml). The aqueous rosemary leaves extract are the poorest in ascorbic acid (0.26 mg/100g). All rosemary leaves extracts have a lower content in vitamin C than the basil leaves corespondent extracts.

3.2. Chlorophylls, carotenoids and xanthophylls level. The obtained results concerning chlorophyll, carotenes and xanthophylls concentration of alcoholic and acetonic extracts of the plants studied are presented in Table 3 (Figure 3) and the absorption spectra recorded, in the Figures 4-7.

It can be seen that the highest chlorophyll "a" content is found in alcoholic basil leaves extract (32.3 mg/l), followed by acetonic basil leaves extract (27.8 mg/l). The lowest chlorophyll "a" content was recorded in acetonic rosemary leaves extract (1.8 mg/l). Chlorophyll "b" is the most abundant in the acetonic basil leaves extract (12.1 mg/l), followed by alcoholic basil leaves extract (8.9 mg/l). Acetonic extract of rosemary leaves is the poorest in chlorophyll "b" content (1.1 mg/l). Carotenes and xanthophylls are best represented in the extracts of basil leaves (64.0 mg/l in the alcoholic extract, respectively 57.4 mg/l in acetonic extract) and the worst, in the acetonic extract of rosemary leaves (9.0 mg/l).

Therefore, both the chlorophylls and carotenoidic pigments have been found in highest quantities in the extracts obtained from basil leaves, the lowest concentrations of pigments being in the rosemary leaves extracts in acetone. For all the two plants studied, it is found that the alcoholic extracts are richer in chlorophylls and carotenoidic pigments than acetonic extracts, the biggest difference being in the rosemary leaves extracts.

Table 3. Content of chlorophylls, carotenes and xanthophylls in the alcoholic and acetonic extracts of basil and rosemary leaves

Plant extract	Basil leaves in alcohol	Basil leaves in acetone	Rose-mary leaves in alcohol	Rose-mary leaves in acetone
A470	3.05	2.73	1.45	0.42
A645	1.17	1.24	0.51	0.10
A663	2.92	2.56	1.28	0.17
Chl.a (mg/l)	32.3	27.8	14.1	1.8
Chl.b (mg/l)	8.9	12.1	3.9	1.1
Chl.a+Chl.b (mg/l)	41.2	39.9	18.0	2.9
Caro-tenes and xanthophylls (mg/l)	64.0	57.4	30.6	9.0

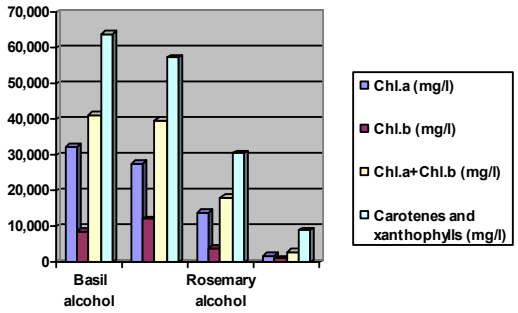

Figure 3. Content of chlorophylls, carotenes and xanthophylls in the alcoholic and acetonic extracts of basil and rosemary leaves

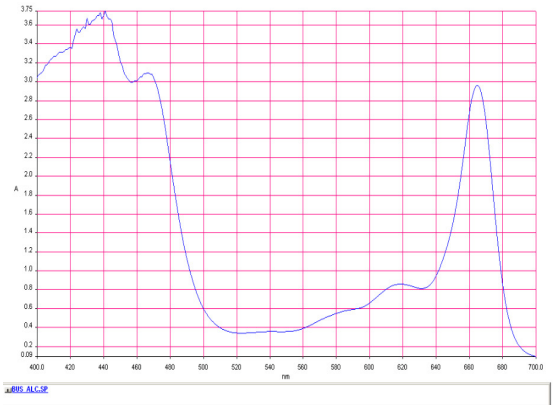

Figure 4. Visible absorption spectrum of basil leaves alcoholic extract

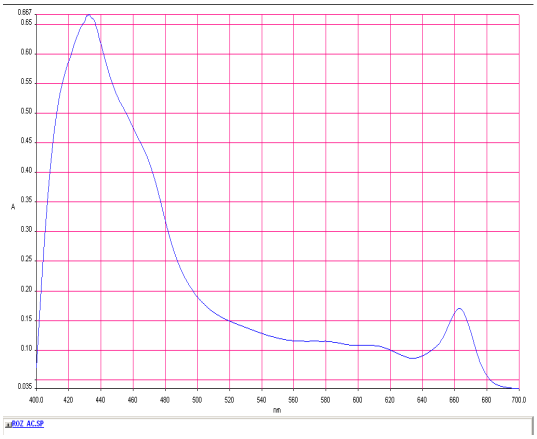

Figure 7. Visible absorption spectrum of rosemary leaves acetonic extract

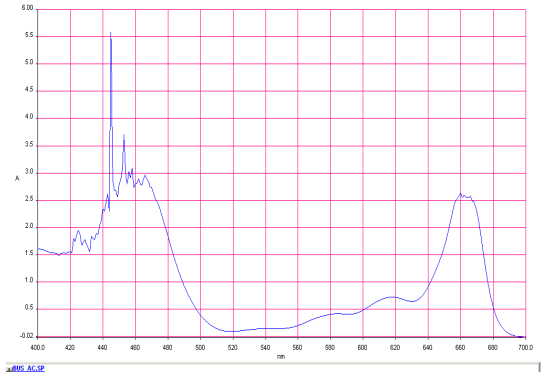

Figure 5. Visible absorption spectrum of basil leaves acetonic extract

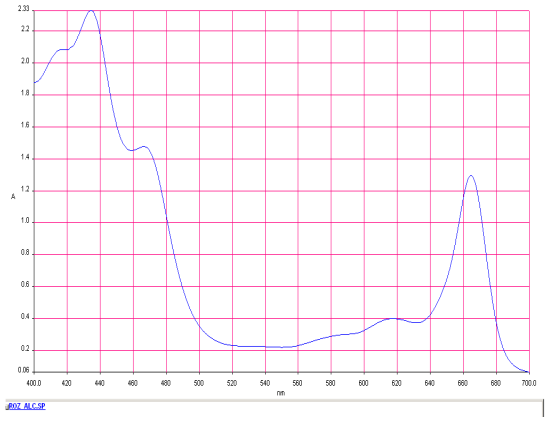

Figure 6. Visible absorption spectrum of rosemary leaves alcoholic extract

4. Conclusion

In this work was determined for each studied plant material, comparative, content of vitamin C, chlorophylls, carotenes and xanthophylls, detaching the following conclusions: (1) the basil leaves are richer in vitamin C than rosemary leaves; (2) there is a lower concentration of vitamin C in plant extracts than in raw materials, the richest in ascorbic acid being basil leaves acetonic extract, followed by the aqueous extract of basil leaves. All rosemary leaves extracts have a lower content in vitamin C than the basil leaves corespondent extracts; (3) the highest chlorophyll "a" content is found in alcoholic basil leaves extract, followed by acetonic basil leaves extract; (4) carotenes and xanthophylls are best represented in the extracts of basil leaves; (5) both the chlorophylls and carotenoidic pigments have been found in highest quantities in the extracts obtained from basil leaves; (6) both for basil leaves and rosemary leaves it is found that the alcoholic extracts are richer in chlorophylls and carotenoidic pigments than the acetonic extracts.

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