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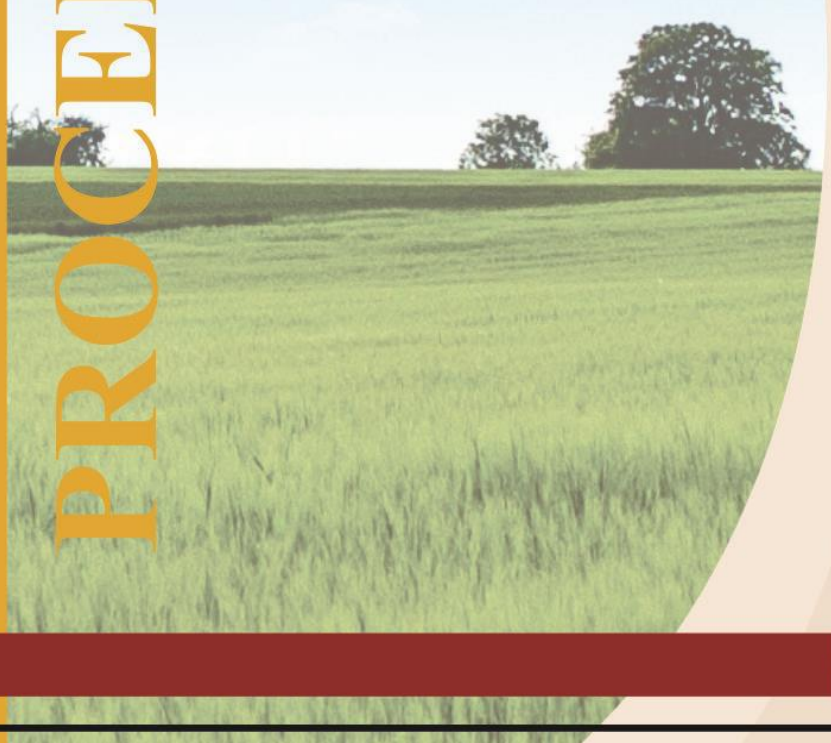

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DETERMINATION OF VITAMIN C CONTENT IN DIFFERENT EXTRACTS OF THE *ALCHEMILLA VULGARIS* L.

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Abstract

In recent years, researchers are interested and focused on the identification of bioactive components in plants and food that affects the health, and may also reduce the risk of some diseases. The research of bioactive components, includes very extensive studies both in conventional breeding and biotechnological researches, with special reference to the possibility to increase their content. *Alchemilla vulgaris* L. is a plant from the *Rosaceae* family. Recent scientific research has shown that the source prevents the growth of many types of bacteria including staphylococci - a bacterium that has become resistant to many antibiotics. Within the experimental part of this final work, the following analyzes were performed: preparation of macerates, extraction of samples in the Soxlet apparatus, ultrasonic extraction and determining the content of vitamin C in the extracts obtained. In determining the content of vitamin C, we noticed that the highest content of this vitamin, determined in the extract obtained by maceration (9.75 mg / 100 g), was slightly lower in ultrasonic extraction (7.50 mg / 100 g), and the smallest content in Soxhlet- of this extract (3.45 mg / 100 g).

Keywords: *Alchemilla vulgaris* L, *maceration, ultrasonic extraction, extraction, vitamin C.*

Introduction

Vitamin C (ascorbic acid) is, by chemical structure,Figure 1., lactone close to L-glucose. It is technically obtained starting from D-glucose, which reduces to D-sorbitol by reduction, and by the action of enzymes of special microorganisms it passes into the L-Sorbose, which with HNO_3 produces 2-keto-L-gluconic acid, the methylester is treated with $NaOC_2H_5$ and then hydrolysed in Vitamin C is formed in the acidic environment. (Duh *et all.*, 1999).

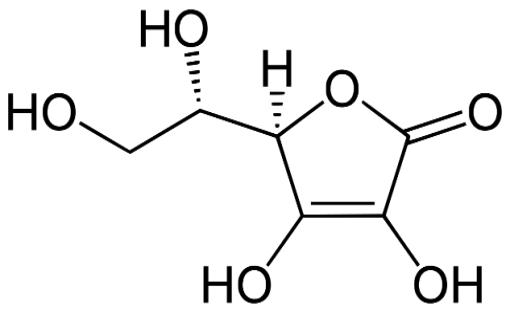

Figure 1. Vitamin C structure

Vitamin C is involved in the construction of cyclic amino acids, steroid hydroxylation, folic acid hydration in tetrahydrofuran, increases breathing intensity, activates many enzymes and improves the acquisition of microelements. Oxidation of L-ascorbic acid first produces monohydroascorbic acid, followed by dehydroascorbic acid. Both have vitamin activity, because they can be converted to ascorbic acid by reduction. L-ascorbic acid is of utmost importance in the metabolism of humans and animals (Tandon *et.*, 1995).

In vitamin C plants is 95% in reduced form, and only 5% is in the oxidized form as dehydroascorbic acid. It is important for the synthesis of collagen and carnitine. Also, the role of vitamin S in the creation of collagen is important for the regeneration of tissue, blood vessels, bones and teeth. The most important group of herbal preparations are extracts, which are obtained by applying different extraction methods, ranging from simpler technologies to advanced techniques. Extraction is the separation and concentration of certain constituents of plant and animal tissues by selective solvents using standard procedures. Depending on consistency, the extracts are divided into liquid, semi-solid and solid. Herbal extracts are obtained by crushing, mainly dry, parts of the plant into contact with the extraction solvent in the appropriate device, the extractor (Daker *et al*.,2008). Extraction material can be: plant, animal and mineral origin. The rate of extraction is influenced by: the size of the contact surface of the solvent and the particulate matter, the thickness of the boundary layer around the particle, and the temperature of the system. In organic laboratories, organic compounds are usually extracted from aqueous solutions using an organic solvent, which is not mixed with water or mixed in part, and two layers are formed. This technique is known as liquid-liquid extraction (Yan *et al.*, 2006).

Material and Methods

As a material in this final work, a virak plant (*Alchemilla vulgaris* L.) was used. The plant material originates from the locality of Cacak, Moravica District, acquired in November 2017. Extracts were obtained from dry plants. In Figure 4, the plant material used for the analysis is shown.All chemicals and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MQ, USA), Aldrich Chemical Co. (Steinheim, Germany) and Alfa Aesar (Karlsruhe, Germany). *Alchemilla vulgaris* L. is a plant from the Rosaceae family. This is a perennial herbaceous plant up to 50 cm high. Blooms from June to September. Ground leaves in the rosette from which the tree grows, more or less covered with hair. The rounded-kernel leaves, starched on the 7-11 lobes, along the entire periphery, are bent or chopped. The stem is upright, developing laterally on the rosette. The leaves on the stem are shaped like rosette leaves, but they are smaller. The flowers are small, yellow-green, naked, without crocheted leaves. The fruit of virka is nuts. The bloom is a wide broom. Three measurements of the fragmented sample were performed. Samples were dried in a drying oven at the prescribed temperature (at 105 ºC) under atmospheric pressure to constant mass. The height of the applied temperature depends on the type of sample whose water content is determined (Hsu *et al*.,2008). Before the samples are put on drying, it is necessary to dry the veggel with a lid in the dryer to a constant mass (at least 1 hour) at the prescribed temperature (105 ºC). The veggle is placed on the test sample and measured. Then it is put on drying in the dryer. During the drying of the vegeglas with the pattern, it must be opened, i.e. The lid is located next to it during drying. After drying, the vegeglas is cooled in a desiccator, and then measured.

Extraction by maceration

The extraction is the uniform separation of one or more constituents of a solid or liquid mixture (starting material) with another solvent which is not mixed or mixed with the solvent of the initial mixture, and the other ingredients are not soluble or less soluble in it.

The milled and homogenized sample (5 g), was poured with a solvent (200 mL 96% ethanol), then left in a closed, protected enamel mist. Maceration is done for five days, with shaking every day twice a day. After five days, the plant material from the maceration was cut through the gauze, and after and through the filter paper, black strip. The solvent is removed by evaporation on an aqueous bath, and the resulting extract is dried to a constant weight at a temperature of 50 ° C (Merken H. M. and Beecher G. R., 2000).

Extraction by Soxlet

The best-known apparatus for the continuous extraction of solids is the descendant of Soklet. Socklet extraction takes place by placing the starting material in the cauldron. The pattern with the sample is then inserted into the middle part of the extractor which is connected to the refrigerator and the balloon. The balloon was pre-dried for 1 hour at 105 ºC and measured on an analytical scale. Using a small funnel on the upper side of the condenser, so much solvent was poured into the apparatus that the extractor was filled and poured into the balloon. Further solvents (96% ethanol) are then added, ensuring that the total amount of solvent does not exceed more than ¾ of the volume of the balloon.

Ultrasonic extraction

Ultrasonic extraction is performed in an ultrasonic water bath (EUP540A, Euinstruments, France). The sample (5 g) was placed in a balloon and poured with 200 mL of 96% ethanol. The mixture was extracted for 30 minutes at a frequency of 40 kHz and ultrasound effect 90% (216 W), (Macheix, J.-J. and Fleuriet, A., 1998). For the quantitative determination of vitamin C, the Tillmans method (Tillmans), based on oxidimetric titration, is used in which L-ascorbic acid is oxidized to dehydroascorbic, while simultaneously reducing the reagent used. Titration with 2,6-dichlorophenolinophenol, i.e. The Tilmans Reagent (TR) is carried out in an acidic medium at pH 4-6. The oxidized form of the Tilmans reagent solution (which also has the role of the indicator) has a dark blue color (at pH 5,2), while in the presence of ascorbic acid, TR passes into its reduced, leuky form.

Calculation

The content of ascorbic acid (in mg / 10 g of extract) = ((V - Vsp) * s * 100) / Val,

V - mean value of the volume of TR solution used for the titration of the test probe (mL),

Vsp - the mean value of the volume of the TR solution consumed for the titration of the blank (mL),

c - titre of TR solution (mg $C_6H_8O_6$ / 1 mL TR solution)

Val - the volume of the aliquot part of the sample (mL).

Results and Discussion

Content of total extracted matter

After completed extractions, the extraction of the obtained herbal extracts to dryness was carried out, and then the measurement of the obtained residues was obtained. The yield of the extraction is calculated. From 5 g of plant material of virka (*Alchemilla vulgaris* L.)

The results obtained in percentages are shown in Table 1.

Table 1. Percentage yield extractions

Sample	Maceration	Soxhlet extraction	Ultrasonic extraction
herbal drug *Alchemilla vulgaris* L.	9.45%	4.72%	32.54%

Based on the results obtained, we can conclude that the smallest yield was obtained by Soxlet extraction, followed by maceration, and the highest yield was obtained by ultrasonic extraction. The method of ultrasonic extraction proved to be the most optimal method for this plant species, because it shortly lasts for maceration and takes place at a lower temperature, which is not the case with the Soxlet method, which occurs at high temperature, and it is assumed that there has been degradation of vitamins and other thermolabile compounds.

Table 2. Vitamin C content

Type of extraction	Vitamin C mg / 100 g
Maceration	9.75
Soxhlet extraction	3.45
Ultrasonic extraction	7.50

In determining the content of vitamin C, Table 2, we concluded that the highest content of this vitamin is determined by maceration (9.75 mg / 100 g), followed by ultrasound (7.5 mg / 100 g) and the smallest in Soxhlet's extract (3.45 mg / 100g). We assume that maceration as an extraction method has proved to be the most optimal, with the highest yield of vitamin C, because the isolation was carried out at room temperature, and we know that vitamin C is thermolabile.

Conclusions

The smallest extraction yield was obtained by Soxlet extraction, slightly higher maceration, and the highest yield was obtained by ultrasonic extraction. In determining the content of vitamin C, we noticed that the highest content of this vitamin, determined in the extract obtained by maceration (9.75 mg / 100 g), is slightly lower in ultrasonic extraction (7.50 mg / 100 g), and the smallest content in Soxhlet extract (3.45 mg / 100 g). The maceration extraction method proved to be the most optimal and with the highest yield of vitamin C, since the isolation was carried out at room temperature, bearing in mind the fact that vitamin C is thermolabile.

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