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TOTAL POLYPHENOLS IN ELDERBERRIES

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***Abstract**: The aim of this study was to investigate the total content of phenolic compounds in wild Elderberry fruits. Black elder, as a shrub has been known since ancient times to have beneficial effects on human health. Flowers and especially elderberries were and are still used today as natural remedies against many diseases. Polyphenols are the main compound that gives them antiviral, antiseptic, and even antitumor properties. They can be extracted from the fresh fruit, but also from the dry one using various extraction methods and various solvents. In the current work, an attempt was made to determine the method that would yield the highest total polyphenol content. The fruits were only from the wild flora, collected in 2021 from the wild elder, which is part of the sub-tree of the deciduous forests around the municipality of Suceava. We used for this research oven-dried fruit at a temperature of 40 degrees Celsius. As "solvents" were: distilled water and 50% ethanol. The samples were sonicated at different amplitudes, pulsations and time intervals. The total phenolic content (TPC) was determined using the Folin-Ciocalteu method. Finding a method of extracting a large amount of polyphenols started from the idea of obtaining a food supplement from elderberries, which would present antiviral properties. Ultrasonication of the extract, which has distilled water as a solvent, for 15 minutes, at an amplitude of 50 and pulses 10:10, proved to be the method by which the largest content of polyphenols is obtained.*

***Keywords:** antioxidant, Black Elder, ultrasonication*

1. Introduction

The healing properties of elderberries have been recognized in traditional medicine for centuries. The European black elder (Sambucus nigra) is found abundantly in the wild flora and can provide us, as a raw material, the elderberries, which have gained attention for their potential health benefits and have become a popular ingredient in natural remedies, dietary supplements and even in skin care products.

Recently, lesser-known fruit species have been gaining importance due to their potential as a source of health-promoting compounds. Wild types or lesser-known berry fruits like sea buckthorn, rose hip, and hawthorn have high levels of bioactive compounds, especially polyphenols, which have strong antioxidant properties. Elderberry is one such species.[1]

Since ancient Egypt, evidence has been found of the use of black elder for treating various diseases and incorporating it into medicinal formulas and food. [2].

European elder is a plant that is originally from Europe, Northern Africa, and Western and Central Asia. Its flowers and berries have been used for a long time in traditional European medicine. Elderberries have also been used to make preserves, wines, winter cordials, and to add flavor and color to other wines. In the United States of America, dietary supplements containing extracts, juices, or syrups made from European elderberries have become popular as

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remedies for treating cold and flu symptoms. These supplements are mainly sold in natural food stores alongside other popular cold and flu herbal remedies.[3] .

Tejero, J. and all [4] have shown that Sambucus contains several organic compounds that are likely important for its medicinal properties. These compounds include simple phenolic acids, as well as complex polyphenols such as flavonoids with anthocyanidins and tannins. Some of the major flavonoids found in elderberries are quercetin-3-O-rutinoside, quercetin-3-O-glucoside, kaempferol-3-O-rutinoside, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucoside and 5-O-caffeylquinic acid. Two flavanol glycosides, isorhamnetin-3-O-monoglycoside and quercetin-3-O-monoglycoside, have been found to have antiulcerogenic activity. Among the anthocyanidins, the most abundant anthocyanins in ripe fruit of S. nigra are cyanidin-3-O-sambubiozides, cyanidin-3-sambubiozides-5-glucosides, cyanidin-3-O-glucoside, and cyanidin-3,5-diglucoside. Cyanidin-3-O-glucoside has been proven to be a compound that inhibits cell growth and has anticancer properties. [4].

Ribosome-inactivating proteins (RIPs) are other valuable chemical components found in elderberries. These enzymes have N-glycosidase activity on the large RNA of ribosomes and, by this mechanism of action, prevent them from participating in protein synthesis. [4].

Numerous studies conducted in laboratory settings and on living organisms have demonstrated that flavonoids and polyphenolic components possess antiviral, antimicrobial, and immune-stimulating effects. [5-10].

So, the pharmaceutical and food industry is greatly interested in the total phenolic compounds and their properties as natural agents.

2. Materials and methods

2.1 Plant material

The elderberries used in this investigation were picked from the wild elder variety *Sambucus nigra*, found in abundance in the spontaneous flora of Suceava County**.**

In the surroundings of the municipality, there is both vegetation specific to the hills area, as well as that characteristic of the meadow area, and the city of Suceava and the surrounding territory (Dragomirna and Ciungilor-Ilisesti hill) belong entirely to the deciduous forest area. The coppice of these forests consists of rosewood (*Rosa canina*), black elder (*Sambucus nigra*), bloodtwig dogwood (*Cornus sanguinea*), cornelian cherry dogwood (*Cornus mas*), wild hazel (*Corylus colurna*), etc. representing a potential source of raw material for a product on elderberries base.

The ripe berry bunches were cut from the branches, and then, a few hours after being harvested, the fruits were preserved in the refrigerator for 24 hours. Fruit samples (infructescence) were collected when they were fully mature in the year 2021. The first ripe fruit was collected in September, and the last one was collected in October. Only fruits that were well-ripened, black in color, and tasted sweet were collected. Each sample was labeled and stored in a plastic bag in the freezer at a temperature of 4 degrees Celsius for one day.

After harvesting, the processes of biological degradation of the active principles and other biochemical constituents of the plant begin. Under the action of endogenous enzymes, temperature and sunlight, reactions occur to split the hydrocarbon chains of heterosides, ethers, and esters, oxidation reactions, polymerization, etc. As a result, less physiologically active or even inactive compounds result. In order to avoid these processes or to stop them as soon as possible, the activity of the enzymes must be stopped. The process is called stabilization.[11]

To stabilize the product, it was subjected to artificial drying in an oven at 40 degrees

Celsius for 24 hours the day after it was picked. After drying, a quality control of the product was carried out by removing any remaining or excessively dry products.
Then the vegetable product was fragmented to uniform sizes, by shredding with an electric grinder.

2.2. Extraction of phenolic compounds
5 grams of the crushed product were mixed with 100 mL of distilled water or 50% ethanol. After one hour, the mixture was centrifuged for 5 min at 4000 rpm.

Fig.1. **"Crushed" elderberries mixed with distilled water or 50% ethanol**

After that, the mixture was divided into several samples that were ultrasounded at different amplitudes, pulsations, and time periods, according to the Table 1. The device used for ultrasound of the samples was the Optic IVYMEN CY-500 system.
Ultrasound is a new, clean, and environmentally friendly extraction technology used for various molecules and biomaterials. These include polysaccharides, essential oils, proteins, peptides, fine chemicals such as dyes and pigments, and bioactive molecules that are commercially, pharmaceutically, or analytically important.[12]
The mechanical effects of ultrasonic cavitation cause the cell wall to be disrupted. This can be observed through light or scanning electron microscopy of the matrices. These studies have shown the formation of small cracks, cell disruption, and the creation of pores on the surfaces. As a result, the permeability of the matrix is improved. The sonicated cellular matrix allows the solvent to more easily access the internal structure, which facilitates the release of the target compound. This is achieved through the formation of small cracks and channels that enhance the penetration of the solvent into the matrix. By modifying the surface of the matrix, the solvent is able to more effectively reach the internal structure, allowing the target compound to dissolve in the solvent. [12]

Table 1.
Ultrasound conditions

Sample	Time(min)	Amplitude (% ultrasonic energy of 20Hz)	Pulse(sec)
1	30	30	0:00
2	30	50	0:00
3	30	80	0:00
4	30	99	0:00
5	30	30	10:10
6	30	50	10:10
7	30	80	10:10
8	30	99	10:10
9	15	30	0:00
10	15	50	0:00
11	15	80	0:00
12	15	99	0:00
13	15	30	10:10
14	15	50	10:10
15	15	80	10:10
16	15	99	10:10
17	5	30	0:00
18	5	50	0:00
19	5	80	0:00
20	5	99	0:00
21	5	30	10:10
22	5	50	10:10
23	5	80	10:10
24	5	99	10:10

After ultrasonication, each sample was filtered and then centrifuged at 4000 rpm for 5 min. The supernatant was analyzed, and the content of total phenols was determined.

2.3. Total phenolic content (TPC)

The phenolic content was determined using the Folin–Ciocalteau method as follows: 0.2 mL of extract (prepared as described in section 2.2) was mixed with 2 mL of Folin–Ciocalteau reagent, which was diluted 1:10, and 1.8 mL of sodium carbonate 7.5% (w/v) in a tube. [13] The mixture was left for 30 min at room temperature in the dark. The TPC was determined at 750 nm wavelength using a UV-VIS-NIR spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The calibration curve of the polyphenols was created by using gallic acid at concentrations of 10–500 mg/L with the regression coefficient $R^2 = 0.9967$ and equation $y = 0.0048x + 0.1659$. The samples were analyzed in duplicate.

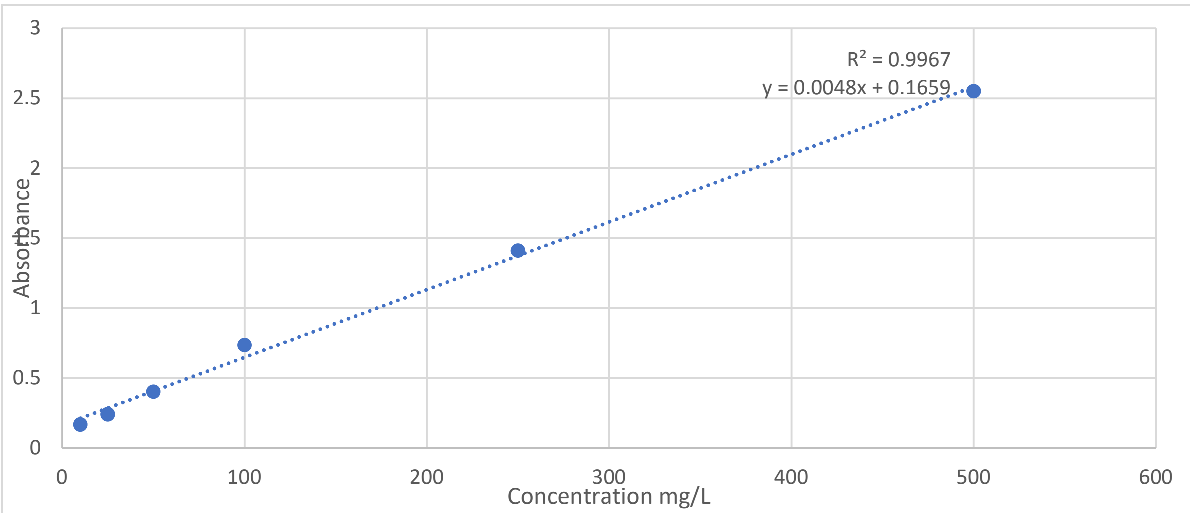

Fig 2. Gallic acid calibration curve

3. Results and discussions

Lee & Finn [14] and Duymuş et al. [15] reported that TPC varied between 4917–8974 mg GAE/100g extract DW in the elderberries analyzed in their studies. In this study, TPC varied between 3471-8987 mg GAE/100g DW extract.

a. Water extract

As can be seen in Figure 3 in the extracts where water was used solvent, the ultrasound time is a very important parameter. The time of 15 min for ultrasonication is the optimal time to obtain a high content of polyphenols."

Along with the sonication time, amplitude and pulsation are two other important parameters for the extraction of polyphenols from elderberries.

It is observed that the samples subjected to 10:10 pulses had a higher content of polyphenols, regardless of time or amplitude. The ideal amplitude to obtain the highest content of polyphenols was 50, with 10:10 pulses.

For the samples that were extracted in water, the highest content of polyphenols was obtained after 15 min of ultrasonication, at 50 amplitude and 10:10 pulses (8987 mg GAE/100 g DW extract).

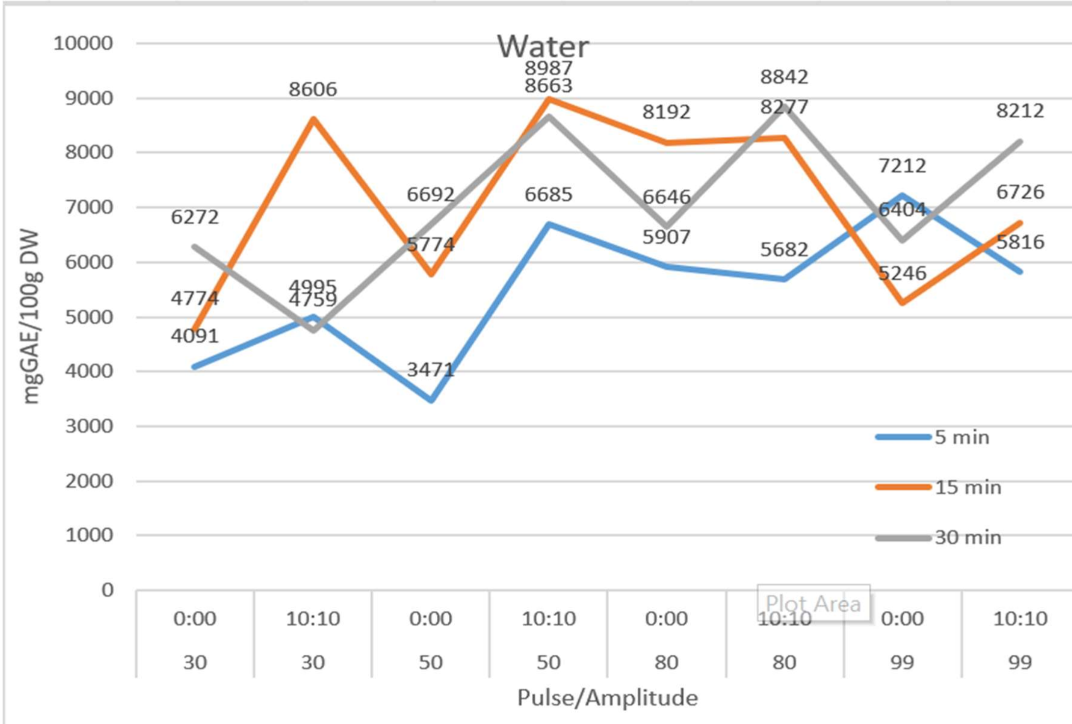

Fig. 3. Total polyphenolic content (mg GAE/100g DW)/Time/Pulse/Amplitude-solvent distilled water

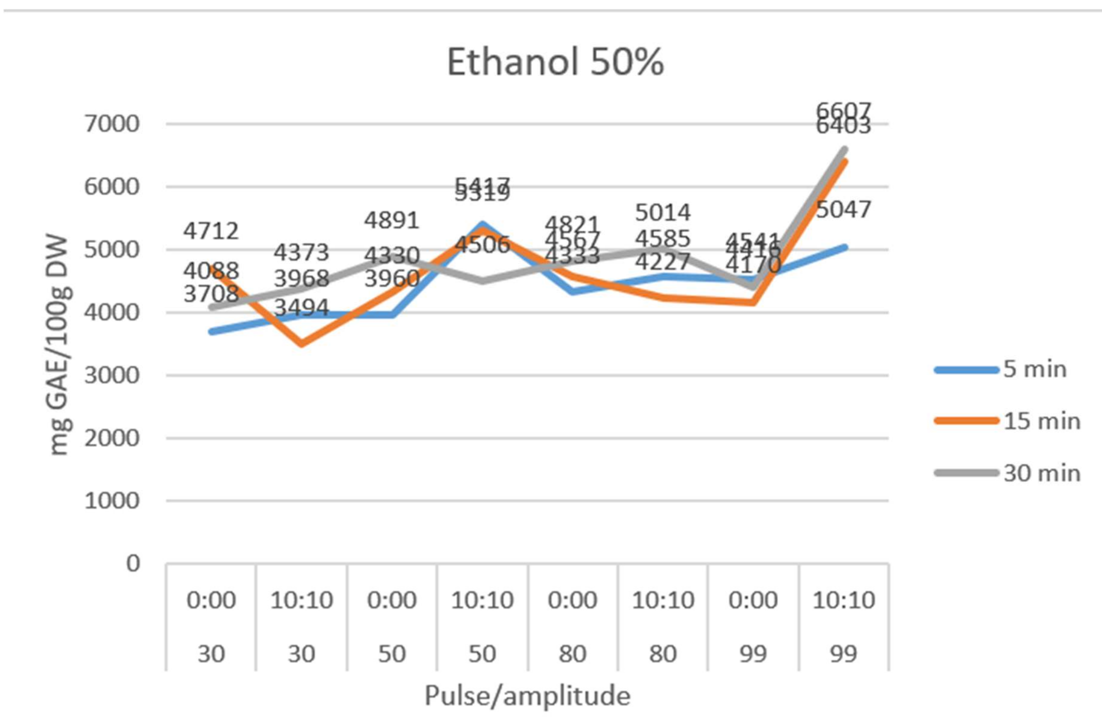

Fig. 4. Total polyphenolic content (mg GAE/100g DW)/Time/Pulse/Amplitude-solvent ethanol 50%

b. Ethanol (50%) extract

In the second series of extracts, those in which ethanol 50% was used as a solvent, can be observed a direct dependence between the ultrasonication time and the total concentration of polyphenols. As a trend, a longer ultrasonication time leads to a higher total polyphenol content.

However, the 15 min and 30 min curves are almost identical, showing ~~us~~ that the optimal the optimal ultrasonication time to obtain a high content of polyphenols is 15 min.

Regardless of the time or amplitude, the highest content of polyphenols was obtained at pulses 10:10 for the samples extracted in 50% ethanol. The results are similar to the samples extracted with distilled water.For samples extracted with ethanol, the amplitude at which the highest content of polyphenols was obtained was 99, compared to 50 in the case of those extracted with water ,with 10:10 pulses.

The highest content of polyphenols for samples extracted in 50% ethanol was obtained when the ultrasonication time was 30 min, at 99 amplitude and 10:10 pulse. The total content of polyphenols obtained was 6607 mg GAE/100g extract DW."

4. Conclusions

In the current context, when there is an increased emphasis on the valorization of natural resources, especially to obtain remedies that improve health, elderberries are a precious resource of raw material. Being rich in polyphenols, they can provide extracts with a high phytotherapeutic role.

Extraction solvents used in this study: distilled water and ethanol are easy-to-use, cheap, and non-toxic for the body. The extraction method used - ultrasonication, is also a "green" method, which manages to "remove" a very large content of polyphenols from the structure of the elderberry.

The ultrasonication of the extract, with distilled water as a solvent, for 15 min, at an amplitude of 50 and pulses 10:10, was the method that led to obtaining the highest content of polyphenols (8987 mg GAE/100 g extract DW). The highest content of polyphenols for samples extracted in 50% ethanol was obtained when the ultrasound time was 30 min, at 99 amplitude and 10:10 pulse (6607 mg GAE/100g extract DW).

In conclusion, the highest content of polyphenols was obtained with distilled water used as a solvent.

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