RESEARCH ARTICLE

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Comparative Study of Antioxidant Capacity, Polyphenol and Flavonoid Content of Water, Ethanol and Water-Ethanol Hibiscus Extracts

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ABSTRACT

Hibiscus is a widely used plant, which has been proven to have numerous positive effects on human health, such as lowering blood pressure, maintaining optimal blood cholesterol levels, liver protection, prevention of oxidative stress, etc. In this study, the content of polyphenols, flavonoids and antioxidant capacity of aqueous, ethanolic and hydroethanolic (50/50 *v/v*) hibiscus extracts, prepared by maceration and ultrasonic extraction, was analyzed. Analysis of antioxidant activity was performed in vitro, using FRAP and DPPH methods. The results showed that the mixture of water and ethanol had a significantly higher effect of extraction of bioactive components from hibiscus than the remaining two solvents. The lowest content of polyphenols and flavonoids, and thus the weakest antioxidant activity was recorded in extracts prepared in absolute ethanol. By comparing the efficiency of the techniques used, maceration proved to be slightly more efficient in the case of aqueous and hydroethanol extracts, while higher polyphenol content and higher antioxidant activity were observed in ethanolic extracts prepared by ultrasonic extraction.

Keywords: Hibiscus, Maceration, Ultrasonic Extraction, FRAP, DPPH.

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I. Introduction

Hibiscus sabdariffa Linn., also known as roselle, is an annual shrub belonging to the family Malvaceae. It is considered a potential source of nutrients and pharmaceutical compounds. Roselle is native to Asia or Tropical Africa and can be found in tropical and subtropical regions, including China, Egypt, Indonesia, Mexico, Nigeria, Thailand, and Saudi Arabia [1], [2]. The genus Hibiscus includes more than 300 species [3]. The species H. sabdariffa comprises a large number of cultivated types which are broadly classified under two varieties, H. sabdariffa var. sabdariffa and H. sabdariffa var. altissima Wester. Former is bushy, pigmented and cultivated for the edible calyces. The latter contains inedible calyces and is cultivated for its stem fibre. Besides calyces and stem, roselle can be cultivated for its leaves, seeds and fruits, as all parts have industrial, medicinal and other applications [4]. Roselle is mainly cultivated for its calyx, which can be green, red and dark red. The red calyxes are the most used, due to their concentration of anthocyanins, which have been proven to be potent antioxidants [5].

Roselle is used in many folk medicines, but is also known for its culinary, botanical, floral and cosmetic uses. Scientific results have suggested many therapeutic properties of roselle herbal preparations, such as diuretic, choleretic, analgesic, antidiabetic, sedative, antitussive, antilipidemic, antihypertensive, antimicrobial, immunomodulatory, hepatoprotective, antioxidant, and anti-cancer effects. It has also been suggested that it lessens blood viscosity, stimulates intestinal peristalsis and reduces body temperature [6]-[8]. Some studies suggest that extracts, infusions or decoctions from roselle can be used to support the treatment of different medical problems including many cardiovascular disorders, cancer, nervous diseases, obesity and genital problems, but further research are required to clarify its exact mechanism of action. Many research reports highlighted the dried calyces as the potential source of bioactive molecules that exert potent medicinal properties. The main bioactive compounds of roselle, to which therapeutic properties have been attributed, are phenolic acids, flavonoids, polysaccharides, anthocyanins, and organic acids, such as citric, ascorbic, hydroxycitric, tartaric and malic [9].

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Roselle is also rich in minerals, amino acids, carotene and total sugar. The content of these compounds in the plant parts of roselle is at variable levels depending on the species variety and geographical area. When it comes to roselle herbal preparations, concentration of bioactive compounds depends on isolation technique and parameters that were used. For example, an extraction technique for roselle anthocyanins plays a major role in the antioxidant activity of the extract, since antioxidant activity of the roselle extract correlates strongly to its anthocyanin content. Aqueous and ethanol extracts from the calyx H. sabdariffa L. have shown significant phenols content, hence antioxidant activity. The medicinal properties of herbal preparations also depend on which plant part was used [10], [11]. Characteristic red colour of roselle calyx is attributed to the content of anthocyanins, mainly delphinidin-3-sambubioside and cyanidin-3-sambubioside [5], [12]. Its acidic taste is due to the content of organic acids such as citric, tartaric, hibiscus and malic. The flavonoids found in the dry calyces are gossypetine, hibiscetine and sabdaretine. Some studies suggest that a flavonoid myricetine exhibits potent antioxidant properties [13]. Hibiscus protocatechuic acid is a phenolic compound isolated from the dried flower of roselle. It has been found that this compound exerts antioxidant and antitumor effects [14]. Other bioactive compunds found in roselle are alkaloids, β-sitosterol, cyanidin-3-rutinose, galactose, pectin, quercetin, stearic acid and wax. Leaves contain proteins, fats, carbohydrates, fibre, ash, calcium, phosphorus, iron, β-carotene, riboflavin, niacin and ascorbic acid [1].

II. Material and Methods

The dried sample of hibiscus was bought in a market in Tuzla. The sample was ground into a fine powder using an electric mill. Demineralized water and absolute ethanol were used for extraction and preparation of solutions for analysis. Spectroscopic measurements were performed on a Perkin Elmer Lambda 25 spectrophotometer.

A. Preparation of extracts

Hibiscus extracts were prepared by maceration and ultrasonic extraction. 1 gram of the crushed sample was transferred to a 100 mL Erlenmayer flask and poured with 50 mL of solvent. The mixtures were mixed on a vibromix at 300 rpm for 30 minutes. In parallel, ultrasonic extraction was performed on an Elmasonic S ultrasonic bath, without heating. After 30 minutes, the samples were filtered and analyzed immediately.

B. Determination of total polyphenol content (TPC)

The content of polyphenols was determined using the Folin–Ciocalteu method [15]. After 1 hour of incubation, the measurement was performed at 765 nm. The results are expressed in mg GAE/1 g of sample).

C. Determination of total flavonoid content (TFC)

The content of flavonoids in hibiscus extracts was determined using the previously described method [16]. The measurement was performed on a spectrophotometer at a wavelength of 510 nm. The results were derived from the calibration curve of quercetin (QE) and expressed in quercetin equivalents per gram.

D. Determination of the reducing ability of extracts (FRAP method)

The reduction efficiency of hibiscus extracts was determined spectrophotometrically, using a previously published procedure [17]. After mixing 3 mL of FRAP reagent and 100 µL of extract and incubation at 37°C, measurement was performed at 593 nm.

E. Determination of DPPH radical inhibition efficiency

The DPPH radical inhibition test was carried out by mixing a methanolic solution of DPPH radicals and extracts, in the appropriate volume ratio. After 30 minutes of incubation, the measurement was performed at 517 nm [18].

III. Results and Discussion

Table I shows the results of the content of bioactive components and antioxidant potential in in vitro conditions. In general, the most effective technique for the extraction of polyphenols and flavonoids in this research is maceration. Maceration proved to be more effective in the case of using water and a water-ethanol mixture. Ultrasonic extraction proved to be more efficient in the case of using absolute ethanol as an extraction agent. When filtering the mixture after the extraction, a rapid change in the color of the extracts obtained using absolute ethanol (from pink to orange) was observed. These extracts were prepared again and analyzed immediately after filtration. No color change was noted in other extracts. The content of polyphenols ranges from 7.12 for the ethanol extract obtained by maceration to 60.46 mg GAE/g sample

for the water-ethanol extract obtained by maceration. The content of flavonoids is generally low and ranges from 0.85×10^{-3} for the ethanol extract prepared by maceration to 4.62×10^{-3} mg QE/1g of sample for the water-ethanol extract prepared by maceration. The reduction potential of the extracts and their ability to inhibit DPPH radicals are correlated with the content of polyphenols and flavonoids. Ascorbic acid was used as a standard for comparing the antioxidant capacity of the prepared extracts. It was found that the standard has a higher reduction potential (FRAP value is 14 250 µmol/g) and a higher DPPH radical inhibition efficiency (IC_{50} value is 0,03 mg/mL).

Ethanol was used as a solvent for the extraction of phenolic compounds in a wide range of concentrations, i.e. from 20 to 100 %. During the extraction of a dry plant material, a better effect is achieved with a higher proportion of the water phase in the organic phase. Altiok *et al.* [20] confirmed the importance of the presence of water in the organic solvent, which increases the diffusion process, thus facilitating the extraction of phenolic compounds from plant tissue [19]-[22].

TABLE I: RESULTS OF THE CONTENT OF BIOACTIVE COMPONENTS AND ANTIOXIDANT ACTIVITY OF HIBISCUS EXTRACTS

Extract	TPC [mg GAE/g]	TFC [mg QE/g]	FRAP [µmol/g]	IC_{50} value [mg/mL]
M-1	50.27	3.32×10^{-3}	166.39	0.62
M-2	7.12	0.85×10^{-3}	27.33	3.08
M-3	60.46	4.62×10^{-3}	192.07	0.59
USE-1	46.88	3.15×10^{-3}	160.54	0.65
USE-2	10.45	1.07×10^{-3}	33.00	2.26
USE-3	56.81	4.25×10^{-3}	184.26	0.60

Wei Mak *et al.* [23] examined the antioxidant activity of hibiscus flowers (*Hibiscus rosa - sinensis* L.). Water and ethanol extracts were prepared by mixing on an orbital shaker at 160 rpm for 24 hours. The results obtained through this study confirm that water is a more efficient solvent compared to ethanol. The content of polyphenols, flavonoids, and the reduction potential of the extracts approximately corresponds to the values obtained through our study. Hamrita *et al.* [24] confirmed through their research that water is a more effective solvent for the isolation of polyphenols, flavonoids and tannins from *Hibiscus sabdariffa* L. calyx, compared to methanol. Garg *et al.* [25] investigated the antioxidant activity and phytochemical composition of aqueous and methanol extracts of stem and leaves of *Hibiscus rosa - sinensis*. The water extract contained more polyphenols, flavonoids, tannins and sugars than the methanol extract.

In the last few years, numerous studies have been conducted in which the antioxidant activity and chemical composition of hibiscus extracts prepared using different techniques have been examined. Although some of the results are not comparable to those from our study, through most research it has been confirmed that water has a higher extraction potential than alcohol (methanol and ethanol). Fig. 1 shows the graphical correlation of the results obtained using different extraction techniques.

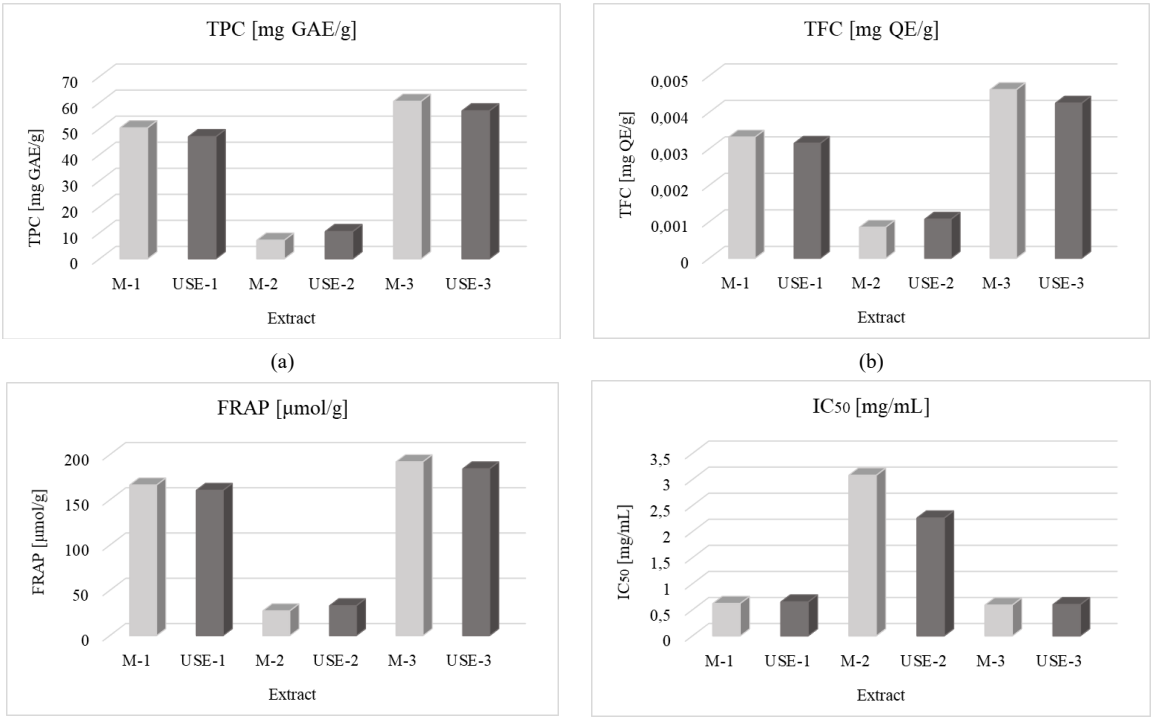

Fig. 1. Graphical comparison of: a) polyphenol content (TPC); b) flavonoid content (TFC); c) FRAP values; d) DPPH radical neutralization for hibiscus extracts.

IV. Conclusion

Water-ethanol extracts of hibiscus showed a high content of bioactive components (polyphenols and flavonoids), and the highest antioxidant capacity in *in vitro* conditions. These results also confirm that the mixture of water and lower alcohols has a higher extraction potential compared to pure alcohol and water. Maceration as a non-invasive, conventional extraction technique proved to be more effective in the isolation of bioactive components in cases where water and water-ethanol mixture were used as solvents. Ultrasonic extraction proved to be more effective in isolation using absolute ethanol.

Conflict of Interest

Authors declare that they do not have any conflict of interest.

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