COMPARISON OF CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY FROM FRESH AND DRIED OSAGE ORANGE (MACLURA POMIFERA) FRUITS EXTRACTS

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Introduction. Given the widespread degradation of the environmental situation, declining nutrient levels in food, and the increasing negative impact of living in modern urban metropolises, there has been a growing interest in antioxidant substances. These antioxidants play a crucial role in the human body's defense mechanism, aimed at combating pathologies and diseases caused by reactive oxygen species (ROS) [1].

The benefits of antioxidants lie in their healing properties that counteract ROS, alleviate oxidative stress, and contribute to the prevention and treatment of various cancers. Moreover, they play a significant role in enhancing human health and increasing lifespan. As a result, the demand for antioxidants continues to rise steadily [2].

Phenolic compounds are one of the most potent antioxidants, which can protect cell membrane and tissues from damage caused by ROS. They are characterized by having one or more aromatic rings with one or more hydroxyl (OH) groups attached to them [3, 4].

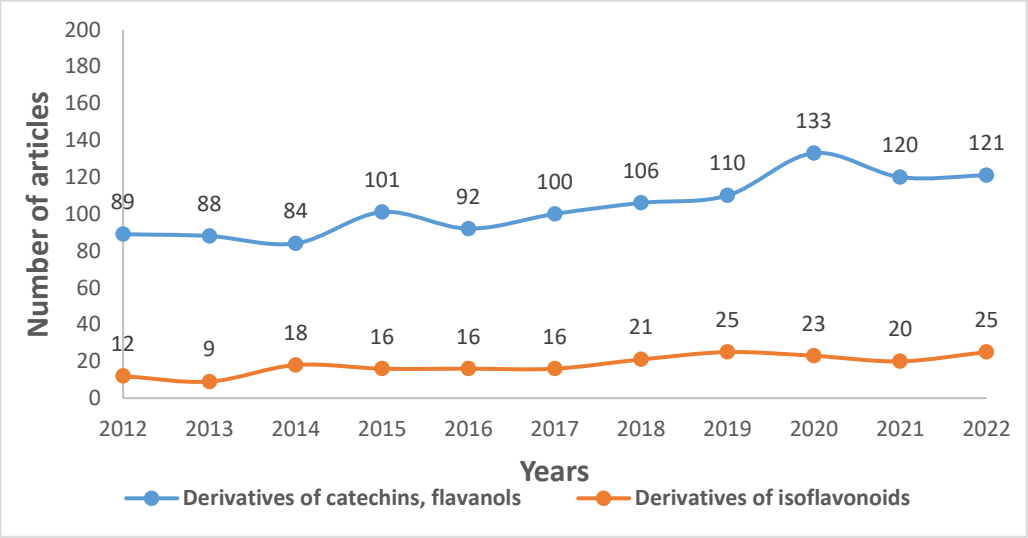

Figure 1. The number of published articles in Scopus and Pubmed databases for the period 2012 – 2022 years about developing medicines from catechins and flavanols

According to the recent literature sources that indexed in Scopus and Pubmed, today derivatives of flavan-3-ols (catechins), flavanols (kaempferol, quercetin, myricetin) and their plant sources are popular objects of research for developing and obtaining phytomedicines with antioxidant properties. But, we would like to notice that derivatives of isoflavonoids and their plant sources have not such attention in studying antioxidant activity and elaborating phytomedicines (Fig. 1). Thus, *M. pomifera* fruit was chosen as the object of our study due to presence of high amount of isoflavonoids.

Maclura pomifera (*M. pomifera*) is a tree that belong to *Moraceae* family. The fruit of osage orange has different common names related to its shape, source, traditional uses, and functions. Such names include osage orange, hedge apple, horse apple, and road apple. Fruits grow to their full size (mass of 500 g) every fall, and each fruit can bear up to 300 seeds per fruit [4]. Osage orange grows throughout the North America, East Europe, Asia and Caucasia [5]. On the territory of Ukraine, Osage orange grows in Kherson, Zaporozhye, Donetsk region and Crimea.

M. pomifera fruits contain in a chemical composition following BAS: isoflavonoids [6, 7], phenolcarboxylic acids [8], organic acids [9], xanthones [10] and triperpenoids [11]. Among all phenolic compounds, osaijn and pomiferin are the major bioactive compounds [12]. Due to the presence of osaijn and pomiferin the osage fruits extracts possess following biological activities: antimicrobial [13], antiviral [14], insect repellent [15], anticancer [16], anti-inflammatory [17], antinociceptive [17], cardioprotective [18], anti-cholinesterase activities [19], and antioxidant [20].

A dried raw material is used in the production of extract, infusion and tincture. The main task of drying process is increasing shelf life of raw material by slowing or inhibiting the growth of microorganisms and affects the intensity of the ongoing biochemical and chemical processes [21]. The simplest, low-cost, and feasible for use is natural drying in the shade, in a ventilated area or in the sunlight. Manually collected plants are dried by spreading them out in thin layers on trays or tying in bunches and hanging them with the leaves down [22]. However, during the drying carrying out some changes in the composition of bioactive components of plant material, such as flavonoids, phenylcarboxylic acids, essential oils as a result of oxidation and esterification reactions [22]. Therefore, it is necessary to find out how the drying process affects the content of phenolic compounds and antioxidant activity of *M. pomifera* fruits extracts.

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The **aim** of work was comparison of chemical composition and antioxidant activity from fresh and dried osage orange (*Maclura pomifera*) fruits extracts.

Materials and methods. *M. pomifera* fruits were collected during the fruiting period near village Kalanchak, Kherson Oblast (46.25365482071546, 33.29571661020183).

A 10.0 g of *M. pomifera* fruits were ground 1-2 mm in size. The extraction was carried out using distilled water, 20% ethanol, 40% ethanol, 60% ethanol, and 96% ethanol at the ratio raw material/solvent 1/20 (*m/v*) in a water bath at 80º C with reflux for 1 hour. After cooling, the solutions were filtrated and concentrated to 10 mL by a rotary evaporator at 40 º C under a vacuum.

2.0 mL of the extract was placed in a weighing bottle that brought to a constant mass, evaporated in a water bath and dried at a temperature from 100 to 105° C for 3 hours. The weighing bottle was cooled in a desiccator at room temperature for 30 min and weighed [23]. The dry residue w, (%) in extract samples was calculated according to equation 1:

$$w(\%) = \frac{m_{dry} \cdot 100}{V_a} \text{ (Eq.1)}$$

where, m_{dry} – mass of the dry residue after drying an aliquot of the extract sample, g; V_a – volume of extract sample aliquot, mL.

The total content of phenolic compounds was measured by the Folin-Ciocaltau assay, the absorbance was measured at 760 nm [24]. The phosphomolybdotungstic reagent was used for performing assay. The calibration curve (Y = 0.1055X + 0.1745 (R^2=0.9951)) was plotted with interval concentrations 1.0 – 5.0 μg/mL, the calibration equation. The total phenolic compounds content in extracts (X), expressed as gallic acid was calculated according to equation 2:

$$X(\%) = \frac{C_x \times K_{dil} \times 100}{V} \text{ (Eq.2)}$$

where, C_x – concentration of gallic acid according to the calibration curve, $C \times 10^{-6}$, g/mL; V – extract volume, mL; K_{dil} – coefficient of dilution, mL.

The total flavonoids were determined using the complex formation assay with $AlCl_3$; the absorbance was measured at 417 nm [25]. The concentration of standard solution of rutin was 0.02 mg/mL. The total flavonoids content in extracts (X), expressed as rutin, was calculated according to equation 3:

$$X(\%) = \frac{A \times K_{dil} \times m_{st} \times 100}{A_{st} \times V} \text{ (Eq.3)}$$

where, A – absorbance of analyzed solution; A_{st} – absorbance of standard solution of rutin; V – volume of extract, mL; K_{dil} – coefficient of dilution, mL, m_{st} – mass of rutin, g.

The total hydroxycinnamic acids derivatives content was measured by assay of complex formation with $NaNO_2$-Na_2MoO_4, the absorbance was measured at 525 nm [26]. The total content of hydroxycinnamic acids derivatives in extracts (X), expressed as chlorogenic acid was calculated according to equation 4:

$$X(\%) = \frac{A \times K_{dil}}{188 \times V} \text{ (Eq.4)}$$

where, A – absorbance of analyzed solution; 188 – specific adsorption coefficient of chlorogenic acid; V – volume of extract, mL; K_{dil} – coefficient of dilution, mL.

Antioxidant activity of extracts was evaluated by potentiometric method [27]. Antioxidant activity was calculated according to equation 5 and expressed as mmol-equiv./$m_{dry\ res.}$:

$$AOA = \frac{C_{OX} - \alpha \times C_{red}}{1 + \alpha} \times K_{dil} \times 10^3 \times \frac{m_1}{m_2} \text{ (Eq.5)}$$

where, $\alpha = C_{ox}/C_{red} \times 10^{(\Delta E - E\text{ethanol})nF/2.3RT}$; C_{ox} – concentration of $K_3[Fe(CN)_6]$, mol/L; C_{red} – concentration of $K_4[Fe(CN)_6]$, mol/L ; $E_{ethanol} - 0.0546 \cdot C_{\%} - 0.0091$; $C_{\%}$ – concentration of ethanol; ΔE – change of potential; F = 96485.33 C/mol – Faraday constant; n = 1 – number of electrons in electrode reaction; R = 8.314 J/molK – universal gas constant; T – 298 K; K_{dil}– coefficient of dilution, mL.; m_1 – mass of dry residue; m_2 – mass of dry residue in 1.0 mL of extract.

Pearson's (r) correlation coefficient was used to analyze the correlation between antioxidant activity and the amount of dry residue, phenolic compounds, flavonoid, hydroxycinnamic acids. The correlation coefficient to takes a value in the range of -1 to +1. Correlation is very high if it is within the range from 0.90 to 1.00; from 0.70 to 0.90 is a high correlation; from 0.50 to 0.70 is a moderate correlation; from 0.30 to 0.50 is a low correlation; from 0.00 to 0.30 negligible correlation [28].

Six samples were analyzed for all the experiments, and all the assays were performed 5 times. The results were expressed as mean values with confidence intervals. MS EXCEL 7.0 and STATISTIKA 6.0 were used to execute the statistical analysis.

Results and discussions. The 40% ethanolic *M. pomifera* extract had the highest value of dry residue from the fresh (11.93±0.12%) and dried fruits (8.59±0.09%), while 96% ethanolic *M. pomifera* extract demonstrated the lowest value of dry residue in the fresh (7.18±0.07%) and dried (5.74±0.06%) fruits. According to results, the dry residue of the 96, 60, 40, 20 and aqueous *M. pomifera* extracts from fresh fruits was higher in 20.2, 24.1, 27.6, 27.5 and 30.3% than *M. pomifera* extracts from dried fruits, respectively. (Table 1).

The highest amount of phenolic compounds was observed in the 60% ethanolic *M. pomifera* extract from both fresh (0.70±0.02%) and dried (0.50±0.02%) fruits, while the lowest content of phenolic compounds was in aqueous *M. pomifera* extract from both fresh (0.20±0.01%) and dried (0.14±0.01%) fruits. The results presented in Table 1 show that amount of phenolic compounds higher in the fresh than in dried *M. pomifera* fruits extracts (96% ethanolic extract – in 30.6%, 60% ethanolic extract – in 20.2%, 40% ethanolic extract – in 27.8%, 20% ethanolic extract – in 28.6% and aqueous extract – in 29.5%).

The 60% ethanolic *M. pomifera* extract had the most significant content of flavonoids from fresh and dried fruits, while in the aqueous extract was the lowest amount

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of flavonoids from fresh (0.39±0.02%) and dried (0.28±0.01%) fruits, whereas in the aqueous extract was the lowest amount of flavonoids from fresh (0.08±0.005%) and dried (0.05±0.005%) fruits. According to results, the amount of flavonoids of the 96, 60, 40, 20 and aqueous fresh fruits extracts was higher in 37.5, 28.1, 28.6, 30.2 and 38.0% than *M. pomifera* extracts from dried fruits, respectively. (Table 1)

The most significant content of hydroxycinnamic acids was observed in the 40% ethanolic *M. pomifera* extract from both fresh (0.60±0.02%) and dried (0.29±0.01%) fruits, while the aqueous extract demonstrated the lowest amount of hydroxycinnamic acids in both fresh (0.11±0.005%) and dried (0.08±0.005%) fruits. According to results, the content of hydroxycinnamic acids in extracts from fresh fruits was higher than from dried one (96% ethanolic extract – in 27.5%, 60% ethanolic extract – in 27.5%, 40% ethanolic extract – in 31.7%, 20% ethanolic extract – in 27.6% and aqueous extract – in 28.6%). (Table 1)

The antioxidant activity increases in the following extract from fresh and dried *M. pomifera* fruits order: aqueous < 20% < 96% < 40% < 60% extract. The result presented in Table 1 show that the level of antioxidant activity of the 96, 60, 40, 20 and aqueous fresh *M. pomifera* fruits extracts was higher in 39.5, 31.4, 33.4, 30.5 and 54.8% than in extracts from dried fruits, respectively. (Table 1)

Table 1. The quantitative content of phenolic compounds, flavonoids, hydroxycinnamic acids, and antioxidant activity, calculated from the extraction of fresh and dried *M. pomifera* fruits extracts

Raw material	Extractant	Dry residue, %	Total phenolic compounds content, %	Total flavonoid content, %	Total hydroxycinnamic acids, %	Antioxidant activity, mmol-equiv./$m_{dry\ weight}$
Fresh	**96% ethanol**	7.18±0.07	0.59±0.02	0.29±0.01	0.32±0.02	60.10±1.20
	60% ethanol	8.93±0.09	0.70±0.02	0.39±0.02	0.40±0.02	70.10±1.40
	40% ethanol	11.93±0.12	0.65±0.02	0.14±0.01	0.60±0.02	68.88±1.38
	20% ethanol	9.54±0.10	0.41±0.02	0.10±0.005	0.29±0.01	43.19±0.86
	aqueous	10.84±0.11	0.20±0.01	0.08±0.005	0.11±0.005	22.11±0.44
Dried	**96% ethanol**	5.74±0.06	0.41±0.02	0.18±0.005	0.23±0.005	43.13±0.86
	60% ethanol	6.79±0.07	0.50±0.02	0.28±0.01	0.29±0.01	48.11±0.96
	40% ethanol	8.59±0.09	0.47±0.02	0.10±0.005	0.41±0.02	45.86±0.92
	20% ethanol	6.87±0.07	0.29±0.01	0.07±0.005	0.21±0.01	30.90±0.62
	aqueous	7.59±0.08	0.14±0.01	0.05±0.005	0.06±0.005	10.00±0.20

Pearson`s (r) coefficients between antioxidant activity and value of dry residue, content of phenolic compounds, flavonoids, and hydroxycinnamic acids in fresh *M. pomifera* fruits extracts were 0.1985, 0.9250, 0.6090 and 0.8726, respectively, whereas in the case of dried *M. pomifera* fruits extracts were 0.1546, 0.9875, 0.7168 and 0.8599, respectively. (Table 2, 3)

Table 2. Pearson`s (r) correlation coefficient between antioxidant activity and biologically active compounds content in *M. pomifera* fruits extracts from fresh raw material

	Dry residue	Total phenolic compounds content	Total flavonoid content	Total hydroxycinnamic acids content
Antioxidant activity	0.1985	0.9825	0.6994	0.8726
Correlation Level	Negligible	Very high	Moderate	High

Table 3. Pearson`s (r) correlation coefficient between antioxidant activity and biologically active compounds content in *M. pomifera* fruits extracts from dried raw material

	Dry residue	Total phenolic compounds content	Total flavonoid content	Total hydroxycinnamic acids content
Antioxidant activity	0.1546	0.9875	0.7168	0.8599
Correlation Level	Negligible	Very high	High	High

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Isoflavonoids are phenolic substances, which are known as plant constituents responsible for various noteworthy biological activities such as antioxidant, anticancer, and against gynecological problems [29]. Previous studies evidently demonstrated that prenylated isoflavonoids are major phenolic compounds in *M. pomifera* fruits. Osajin and pomiferin are highly similar prenylated isoflavonoids that only differ with one hydroxyl group [30]. In a recent study of Barak *et al.* [31], the total content of phenolic compounds was 0.14±0.02% expressed as gallic acid in 80% methanolic extract. Compared to our results, in our study, the content of phenolic compounds is higher in 5.36 and 3.72 times than in 96% ethanolic extract from fresh and dried raw material, respectively. Difference may be due to use of a different type of solvent and geographical differences.

Ethanol, methanol, and acetone are commonly used for extraction of phenolic compounds. In a research of Gajic I. *et al*. [7], the high content of phenolic compounds in extract was obtained by ethanol (0.50±0.02%) than by acetone (0.35±0.02%). In our study, the 60% ethanolic *M. pomifera* extracts had the significant amount of phenolic compounds than extracts obtained by other solvents. In our view, the high polar solvents can promote solubility of phenolic compounds and thus boost their extraction. However, if glycosides of flavonoids or glycosides of hydroxycinnamic acids are dominated among phenolic compounds they will be extracted better by 70 – 50% ethanol solutions due to decreasing polar property of aglycone by glycoside fragment. Therefore, isoflavonoids and hydroxycinnamic acids have glycoside form in *M. pomifera* fruits.

Changes in the content of BAS in herbs may be caused by the technological processes applied, such as drying and freezing. Herb drying inhibits microbial growth and leads to a stable, easily moveable product that is available throughout the year, but it may also change the content of phenolic compouds [32]. Izli N. *et al.* [33] reported that fresh kiwi fruits had higher content of phenolic compounds than in dried raw material in 40%. In our study, the content of BAS and antioxidant activity was significant higher in extracts obtained from fresh raw material compared to their dried counterparts. Moreover, we confirmed mentioned above fact by studying antioxidant activity of obtained extracts from fresh and dried raw material. Thus, drying of raw material cause loss of phenolic compounds and reduce antioxidant activity of their extracts.

In our research, we conducted study of correlation between antioxidant activity and content of BAS. We found the very high correlation between antioxidant activity and phenolic compounds, while the high correlation was between antioxidant activity and content of hydroxycinnamic acids, and flavonoids, whereas the lowest value of correlation coefficient was between antioxidant activity and dry residue. In our view, it may be relates with fact that extracts contain a lot of BAS, which are not have antioxidant activity, for instance, amino acids, polysaccharides and organic acids. So, phenolic compounds are the main contribute to the antioxidant activity of *M. pomifera* fruits extracts.

Conclusions. The presented study of research demonstrated the high content and potent antioxidant activity of osage orange fruits extracts. The 60% ethanol is the most appropriate solvent to extract the highest amount of phenolic compounds from osage orange fruits. The phenolic compounds are the main contribute to the antioxidant activity of osage orange fruits extracts. The extracts from fresh raw material had high content of BAS and antioxidant activity from 27.5 – 54.8% than from dried raw material. Thus, the fresh osage orange fruits should be use in the developing and creating phytomedicines with antioxidant property.

Comparison of chemical composition and antioxidant activity from fresh and dried Osage orange (*Maclura pomifera*) fruits extracts

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Introduction. Osage orange fruit is perspective raw material for developing new medicines with antioxidant and antibacterial properties. In pharmacy for obtaining tincture, infusion and extracts use only dried raw material, but there is question about influence fresh raw material on content of phenolic compounds and pharmacological properties of tincture, infusion and extracts. **The aim of work** was comparison of chemical composition and antioxidant activity from fresh and dried osage orange (*Maclura pomifera*) fruits extracts. **Materials & methods.** The object of the study osage orange fresh and dried fruits. The extracts were prepared using 96, 60, 40, 20% (*v/v*) ethanol and distilled water. The number of phenolic compounds, flavonoids, and hydroxycinnamic acids was determined by a spectrophotometric analysis method, whereas the antioxidant activity of obtained extracts was evaluated by potentiometric method. **Results & discussions.** The highest content of phenolic compounds (0.70±0.02% and 0.50±0.02%) and flavonoids (0.39±0.02% and 0.29±0.02%) was in 60% ethanolic *M. pomifera* fresh and dried fruits extracts, respectively, while amount of hydroxycinnamic acids (0.60±0.02% and 0.41±0.02%) and value of dry residue (11.93±0.12% and 8.59±0.09%) were dominated in 40% *M. pomifera* fresh and dried fruits ethanolic extracts, respectively. The 60% *M. pomifera* fresh (70.10±1.40 mmol-equiv./$m_{dry\ weight}$) and dried (48.11±0.96 mmol-equiv./$m_{dry\ weight}$) fruits extracts had the highest level of antioxidant activity among other extracts. The content of biologically active substances and antioxidant were higher in the *M. pomifera* fresh fruits extracts than in dried ones. The correlation analysis showed that there was a very high positive correlation of antioxidant activity and total phenolic compounds content, high correlation – total flavonoid, hydroxycinnamic acids content and there was no correlation in case of dry residue. **Conclusions.** The 60% ethanol is the most appropriate solvent to extract the highest amount of phenolic compounds from osage orange fruits. The extracts from fresh raw material has high content of biologically active substances and antioxidant activity than from dried raw material. The

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phenolic compounds are the main contribute to the antioxidant activity of osage orange fruits extracts. Thus, the fresh osage orange fruits should be use in the developing and creating phytomedicines with antioxidant property.

Key words: Osage orange, fresh and dried fruits, extracts, phenolic compounds, antioxidant activity, correlation

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