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# Morphological and biochemical characteristics of plant parts *Mahonia aquifolium* (Pursh) Nutt. and some physical indicators of its extracts in activated water

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The research focused on determining the economic value of a selected collection of 20 shrubs from a wild-growing population of *Mahonia aquifolium* (Pursh) Nutt. from Arboretum Mlyňany and Nitra region. By morphological analysis, we determined weight of fruits 0.18–0.50 g, the height of fruits 5.57–13.22 mm, the width of fruits 0.98–11.00 mm, and the number of seeds 1.67–5.30 pcs. The content of macro- and microelements was found in the fruits and leaves. *M. aquifolium* samples are a very valuable source of potassium as the main mineral element contained in leaves (10.437 mg.kg$^{-1}$) and fruits (9.763 mg.kg$^{-1}$). Microelements such as manganese and iron prevailed in leaves (80.1 mg.kg$^{-1}$ of Mn and 35.0 mg.kg$^{-1}$ of Fe), fruits (29.7 mg.kg$^{-1}$ of Mn and 25.0 mg.kg$^{-1}$ of Fe), and heavy metals (Al, As, Cd, Ni, Pb, Hg) are present only in the small amounts with the most abundant aluminium (17.6 mg.kg$^{-1}$ of Al in leaves and 3.6 mg.kg$^{-1}$ of Al in fruits) content and can be used as indicator suggesting the environmental pollution status in the region. We determined the antioxidant activity by the Trolox method in methanol extracts (76.2 and 101.2 mg TE.g$^{-1}$ DW), in ethanol extracts (54.3 and 47.4 mg TE.g$^{-1}$ DW), in acetone extracts (63.4 and 51.9 mg TE.g$^{-1}$ DW) and water extracts (35.5 and 60.3 mg TE.g$^{-1}$ DW) for fruits and leaves, respectively. Extraction of whole fruit (A1-WF), mashed fruit (A1-MF), and fruitless clusters (A1-CT) in structured (activated) water obtained by Kalyxx for 5 days determined a significant reduction trend pH in mashed fruits (A1-MF). The electrolytic conductivity and total dissolved solids of the extracts decreased significantly from the third day of extraction in variants A1-MF and A1-WF. Significant stability of pH, electrolytic conductivity and total dissolved solids during the experimental period was determined for the fruitless clusters' extracts (A1-CT). The results show that *Mahonia aquifolium* has a multifunctional practical use even in the conditions of the Slovak Republic.

**Keywords:** *Mahonia aquifolium*, activated water, morphometry analysis, fruit, leaves, macro- and microelements, antioxidant activity

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## Introduction

*Mahonia* Nutt. is the second-largest genus included in the family Berberidaceae Juss., next to the genus *Berberis* L., mostly headquartered in moderate areas in the middle temperate zones. *Mahonia* is distributed mainly in East and Southeast Asia, also in western North America, Central America, and western South America, including 31 species in China (Ying, 2001; Wu et al., 2009).

A variety of ethnomedical usages of *Mahonia* have been recorded in ancient Chinese books and references (He and Mu, 2015). The phytochemical research of this genus has resulted in the identification of more than 150 chemical constituents, among which alkaloids are predominant in all parts (root, leaves, stem, flowers, seeds). Investigation of *Mahonia aquifolium* (Pursh) Nutt. identified berberine, jatrorrhizine, palmatine, oxyacanthine, berbamine, isotetrandrine, isocorydine, corydine, isoboldine, aromoline, obamegine and oxyberberine (Košťálová et al., 1986) in roots, and isotetrandine besides of the other above-mentioned isolated from seeds (Košťálová et al., 1986), while berberine (Košťálová et al., 1981), corytuberine, magnoflorine, isocorydine, corydine, isoboldine, and berbamine were isolated from leaves and magnoflorine like principal alkaloid of the above-ground bark together with isocorydine, berbamine, corytuberine, scoulerine and columbamine were found in other plant parts (Slavík et al., 1985). Isocorydine and berbamine were found in fresh flowers of this species.

*M. aquifolium* is a good source of phenols, flavonoids, anthocyanins, and antioxidants and other isolated compounds and crude extracts have been shown to exhibit a wide spectrum of *in vitro* and *in vivo* pharmacological effects, including antioxidant (Pyrkosz-Biardzka et al., 2014; Andreicuț et al., 2018), antimicrobial and antibacterial (Slobodníková et al., 2004), anticancer (Damjanović et al., 2020), anti-mutagenic (Čerňáková et al., 2002), antimalarial activities (Iwasa et al., 1998), antitumoral, immunomodulatory and anti-inflammatory effects (Andreicuț et al., 2018, 2019), skin disorders like psoriasis (Gieler et al., 2009) and atopic dermatitis (Donsky and Clarke, 2007).

In addition, it has been used as a medicinal plant, and as a dye and food source in North America and Europe (Abrams, 1950; Auge and Brandl, 1997). The berries of *M. aquifolium* have a long tradition of use as an edible fruit, especially in Indigenous American communities. Today, the berberine content dissuades many from using these berries as food (Baumann, 2008).

For our experiments with various plant parts of *M. aquifolium* was important water, mainly activated (structured) water. Water is a complex subject of study, and its properties depend on a great number of factors. Currently, considerable attention is being focused on the study of the structural properties of water and the possibility of data transfer through water and memory of water (Johansson, 2009). This principle is based on quantum electrodynamics (Del Giudice et al., 1988). This follows that liquid water should be a multiphase, non-equilibrium, and, therefore, the active complex system. That alone makes water a complex dynamic system with much richer behaviour than that of any homogenous matter. For example, under certain conditions, it should change its state in response to the weak resonance signals, and for a long time maintain such a condition. This property is known as “structured water” (Clark et al., 2010, Korotkov and Orlov, 2010).

Water has over 50 anomalous chemical-physical properties; no other substance behaves like this. These properties have important implications for engineering, chemistry, biology, and medicine. Yet so far water research is full of contradictory results. There are many water scientists, who have crossed the line into the science of the capacity of water as a unique molecule to hold and transfer information. Most of them have described structured water as having a unique arrangement of molecules that makes it biologically active. That is, structured water has a life-affirming effect on all living species. These findings led most of these scientists to support the long tradition of using homeopathy because structured water holds the energy of the specific ingredient (as opposed to the scientific idea that water holds only physical or chemical forms) and when a homeopathic formulation is delivered in structured water, it transmits the energy of that ingredient to cells. All living things are based on energy to function, and it is the strength of cellular energy that determines its capacity for life (Chaplin, 2000; Voeikov and Del Giudice, 2009; Pollack, 2013; Voeikov and Korotkov, 2017; Korotkov, 2019).

There is a lot of evidence that drinking structured water is beneficial for plant growth Souza et al., 2006; Abdul Qados and Hozayn, 2010), the effect on productivity (Hozayn and Abdul Qados, 2010; Kumar Gora et al., 2018), human health (Ling, 2006; Ho et al., 2019; Korotkov et al., 2019).

Our study aimed to determine some morphological characteristics of fruits and seeds, analysed the elementary profile of dried fruits and leaves, their macro- and microelements, and at last evaluation of structured (once-activated) various water extracts with fruits and clusters to observe a change in pH, electrolytic conductivity, and total dissolved solids content in examined variants.

## Material and methodology

### Biological material

For experimental purposes, we used genotypes *M. aquifolium* from Arboretum Mlyňany and Nitra region (Slovak Republic). Fruits with peduncles were taken from shrubs in September and October 2018 in the full ripening stage and analysed in the morphometric laboratory at the Institute of Plant and Environmental Sciences in Nitra (Slovak Republic).

### Morphometrical analysis

Samples were marked as MA and the appropriate number (MA-01 – MA-20). The total number of evaluated genotypes was 20. They have evaluated the following characters:

a) fruits – 30 fruits were evaluated from each genotype (n = 30), weight of fruit (g), height of fruit (mm), width of fruit (mm);

b) seeds – 30 seeds were evaluated from each genotype (n = 30) number of seeds in one berry.

The weights were determined by a digital scale (Kern ADB-A01S05, Germany; KERN DS – type D-72336, Kern and Sohn GmbH, Germany), accurate to 0.01 g. Fruits were measured by a digital calliper (METRICA 111 – 012, Czech Republic) accurate to 0.02 mm.

### Image analysis

a) fruit: the shape of the fruit, the shape of the basal part of the fruit (at the stalk), cross- and longitudinal section, basic colour of the skin at the full maturity, the colour of the pulp of ripe fruit;

b) seeds: the shape of seeds.

Images were obtained using the stereomicroscope ZEISS SteREO Discovery.V20 (MicroImaging GmbH 37081 Göttingen, Germany), and Fuji FinePix S 7000 and Panasonic DMC FZ50 digital cameras.

### Determination of dry matter, ash, and protein content

Total dry matter, ash, and protein content were determined according to the EN method (CSN EN 12145, 1997). Total lipid content was determined according to methods specified in the ISO method (ISO 659:1998).

### Determination of carotenoid

Total carotenoid content expressed as β-carotene was analyzed at a wavelength of 445 nm spectrophotometrically (VIS spectrophotometer UV Jenway Model 6405 UV/VIS). Sample (1 g) was disrupted with sea sand and extracted with acetone until complete discolouration. Petroleum-ether was added and then water, the purpose for the separation of phases. After the separation, the petroleum ether-carotenoid phase was obtained and the absorbance was measured (ČSN 560053, 1986).

### Determination of mineral contents

A sample for elemental analysis was prepared using the wet ashing method in a microwave oven (Milestone 1200, Milestone, Italy). A total of 0.25 g sample matrix was decomposed in a mixture of nitric acid (6 mL) (Analytika Praha Ltd, Czech Republic) and hydrochloric acid (2 mL) (Analytika Praha Ltd, Czech Republic). After the decomposition sample was filtered using a filter with 0.45 mm pore size and filled up to 25 mL in a volumetric flask with ultrapure water. Elemental analysis was performed using ICP-OES (Ultima 2, Horiba Scientific, France) according to the procedure described by Divis et al. (2015).

### Determination of antioxidant activity

The antioxidant activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH): the ethanol (1 mL), methanol (1 mL), acetone (1 mL), and distilled water extracts were mixed with 4 mL of DPPH solution (0.025 g of radical in 100 mL of solvent). The absorbance of the sample extract was determined using the spectrophotometer at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) 10–100 mg.L$^{-1}$ ($R^2$ = 0.983) was used as a standard and the results were expressed in mg.g$^{-1}$ Trolox equivalents (TE).

### Determination of physicochemical characteristics in plant extracts with activated water

To detect changes in pH, electrolytic conductivity (EC), and total dissolved solids (TDS) from fruit extracts and clusters, we performed a special experiment using activated water. We activated the water with a prototype of the Kalyxx equipment. Water activation is ensured by pouring water through the Kalyxx, in which the galvanic effect is realized. In the experiments, we used control variants (C) and once-activated water variants (A1).

In the experiment, we evaluated three different products of mahonia to determine the pH of the obtained extracts: (a) whole fruit, (b) mashed fruit, (c) fruitless cluster. We put the fruits and clusters into the activated water variant on Monday, December 9, 2018, and we ended the experiment on Friday, December 13, 2018, at 2:00 p.m. We ensured the pH measurement with a pH meter for 5 days at 8:00 a.m. and 2:00 p.m. every day.

The physicochemical analyses were performed by the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 1990). The parameters were measured by EUTECH instrument conductometer (set 2041138) – pH, EC – electrolytic conductivity ($\mu S.cm^{-1}$), and total dissolved solids (TDS) ($mg.L^{-1}$).

The electrolytic conductivity of extracts is defined as that of a 20% weight in volume solution in water at 20 °C, where the 20% refers to dry matter. This was the temperature at which all subsequent measurements were made, the uniform temperature being necessary since the conductivity of electrolytes varies with the temperature (Heald, 1902). The result is expressed in micro-Siemens per metre ($\mu S.m^{-1}$). The electrolytic conductivity of a solution of 20 g dry matter of plant parts in 100 mL solvent is measured using an electrolytic conductivity cell. The determination of the electrolytic conductivity is based on the measurement of the electrical resistance, of which the EC is the reciprocal (Vorwohl, 1964a, b).

### Statistical analysis

It evaluated the variability of the test files in each character using descriptive statistics. For the characteristics of the files, it was used the basic descriptors of variability: average, minimum measured value, maximum measured value, and the coefficient of variation (%). The degree of variability was determined by the coefficient of variation values. The given parameter is independent of the unit of the evaluated character. Theoretically, they can acquire different values (Stehlíková, 1998). Data were analyzed with the ANOVA test and differences between means were compared through the Tukey-Kramer test ($p < 0.05$) in the program STATISTICA 1.10. The variability of all these parameters was evaluated using descriptive statistics.

## Results and discussion

### Morphometry analyses of fruits and seeds

When evaluating wild-growing genotypes of *Mahonia aquifolium*, we determined the average weight of fruits in the range of 0.18 g (MA-16) – 0.50 g (MA-14). The coefficients of variation determined in the range of 12.16–49.35% document that the character shows a medium to a very high degree of variability.

Sorokopudov and Chlebnikov (2007) found an average fruit weight in the range of 0.15 to 0.30 g in a study of the mahonia genetic resources collection. Gunduz

**Table 1** Variability of fruit traits of selected wild-growing genotypes of *Mahonia aquifolium* (Pursh) Nutt.

| **Weight of fruits (g)** | | | | | | | **Number of seeds** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameters/ Samples** | **n** | **min** | **max** | **$\bar{x}$** | **V** | **H** | **Parameters/ Samples** | **n** | **min** | **max** | **$\bar{x}$** | **V** | **H** |
| **Genotypes with low values** | | | | | | | | | | | | | |
| **MA-20** | 30 | 0.10 | 0.26 | 0.18 | 27.50 | i | **MA-12** | 30 | 1.00 | 3.00 | 1.67 | 60.00 | h |
| **MA-18** | 30 | 0.13 | 0.37 | 0.19 | 36.19 | i | **MA-18** | 30 | 0.00 | 4.00 | 1.90 | 63.01 | h |
| **Genotypes with high values** | | | | | | | | | | | | | |
| **MA-15** | 30 | 0.38 | 0.58 | 0.47 | 15.67 | ab | **MA-07** | 30 | 3.00 | 7.00 | 4.70 | 28.46 | ab |
| **MA-14** | 30 | 0.20 | 0.62 | 0.50 | 26.40 | a | **MA-14** | 30 | 4.00 | 8.00 | 5.30 | 23.62 | a |
| **Height of fruits (mm)** | | | | | | | **Width of fruits (mm)** | | | | | | |
| **Genotypes with low values** | | | | | | | | | | | | | |
| **MA-12** | 30 | 6.12 | 9.70 | 5.57 | 12.62 | g | **MA-18** | 30 | 4.98 | 6.45 | 5.75 | 8.25 | l |
| **MA-18** | 30 | 6.90 | 8.03 | 7.75 | 4.71 | h | **MA-16** | 30 | 5.30 | 7.04 | 5.99 | 8.67 | kl |
| **Genotypes with high values** | | | | | | | | | | | | | |
| **MA-15** | 30 | 9.57 | 11.82 | 10.64 | 6.46 | b | **MA-15** | 30 | 7.23 | 9.37 | 8.51 | 7.39 | b |
| **MA-01** | 30 | 12.32 | 13.79 | 13.22 | 4.02 | a | **MA-01** | 30 | 10.33 | 11.79 | 11.00 | 4.38 | a |

Notes: n – the number of measurements; min, max – minimal and maximal measured values; $\bar{x}$ – arithmetic mean; V – coefficient of variation (%); H – LSD homogeneity test at $P_{0.05}$

(2013) studied morphological properties of *Mahonia aquifolium* from Turkey with fruit weight and fruit seeds weight between 2.9 and 7.3 g and 0.4 and 1.2 g, respectively.

The average height of fruits in the collection of wild-growing genotypes was in the range of 5.57 (MA-12) – 13.22 (MA-01) mm. The coefficients of variation confirm the low 4.02% (MA-01) to medium 14.05% (MA-04) degree of variability of the character. We determined the average width of fruits in the interval from 0.98 (MA-01) to 11.00 (MA-02) mm. The coefficients of variation determined in the range of 4.38% (MA-01) – 25.03% (MA-12) document that the character shows a medium to a high degree of variability.

Gunduz (2013) determined average fruit width and length in the range of 7.0 ±0.3–9.9 ±0.4 mm and 8.0± 0.3 and 11.9 ±0.3 mm, respectively. Fruits are berries that reached 4–6 mm in diameter at the maturity stage according to Şofletea and Curtu (2007).

The average number of seeds in the fruits was determined in the range of 1.67 (MA-12) – 5.30 (MA-14) pieces. The coefficients of variation determined in the range of 15.19% (MA-01) – 66.67 (MA-03) document that the character shows a medium to a very high degree of variability. In comparison with other authors, this trait in the interval 3.0 ±0.3–4.6 ±0.7 (Gunduz, 2013) shows a small difference.

The results from the analysis of variance (ANOVA) of the evaluated traits (Table 2) confirm the statistically significant differences between and within the evaluated genotypes.

Sorokopudov and Chlebnikov (2007) characterize the berries in the ripe state as red-blue to blue, with more or less intact grey coating. Şofletea and Curtu (2007), who studied *Mahonia aquifolium* in Romania determined fruits of black colour with a bluish tinge and abundantly pruinose.

By evaluating the collection of genotypes in our study, we confirm the findings of these authors. The fruits of all evaluated genotypes were characterized by blue colour and grey film on their surface. This is documented in the photo documentation in Figure 1.

The differences in fruit shape are also evident in Figure 1 on the left (A). In general, egg-shaped modifications predominate. Figure 1 on the right (B) presents the shape in the basal part of the fruits of the evaluated genotypes. We determined the differences between the genotypes mainly in the size of the stem hole, which is related to the thickness of the fruit stem.

The seeds of the mahonia are about 5 mm in size, according to Mikula and Vanke (1989), and the following authors present the mahonia seeds as triangular seeds. As we can see in Figure 2(A), the seeds are also triangular, but most of them are oval. Figure

**Table 2** Analysis of variance of evaluated fruit traits of genotypes of *Mahonia aquifolium* (Pursh) Nutt.

| Factors | f | S | MS | F | H | LSD | |
|---|---|---|---|---|---|---|---|
| **Weight of fruit (g)** | | | | | | | |
| **Between genotypes** | 19 | 1.96 | 0.10 | 9.71 | 0.000 | 0.05 | 0.15 |
| **Within genotypes** | 178 | 1.89 | 0.01 | | | 0.01 | 0.17 |
| **Total** | 197 | 3.86 | | | | | |
| **Number of seeds** | | | | | | | |
| **Between genotypes** | 19 | 222.68 | 11.72 | 8.79 | 0.000 | 0.05 | 1.64 |
| **Within genotypes** | 178 | 238.79 | 1.33 | | | 0.01 | 1.89 |
| **Total** | 198 | 461.46 | | | | | |
| **Height of fruits (mm)** | | | | | | | |
| **Between genotypes** | 19 | 465.12 | 24.48 | 23.96 | 0.000 | 0.05 | 1.44 |
| **Within genotypes** | 178 | 181.83 | 1.02 | | | 0.01 | 1.66 |
| **Total** | 197 | 646.95 | | | | | |
| **Width of fruit (mm)** | | | | | | | |
| **Between genotypes** | 19 | 666.51 | 35.08 | 39.73 | 0.000 | 0.05 | 1.33 |
| **Within genotypes** | 179 | 158.06 | 0.88 | | | 0.01 | 1.54 |
| **Total** | 198 | 824.56 | | | | | |

Notes: f – number of degrees of freedom; S – the sum of squares; MS – average square; F – Fischer test value; P – statistical significance by Fischer test; H – homogeneity; LSD – a least significant difference

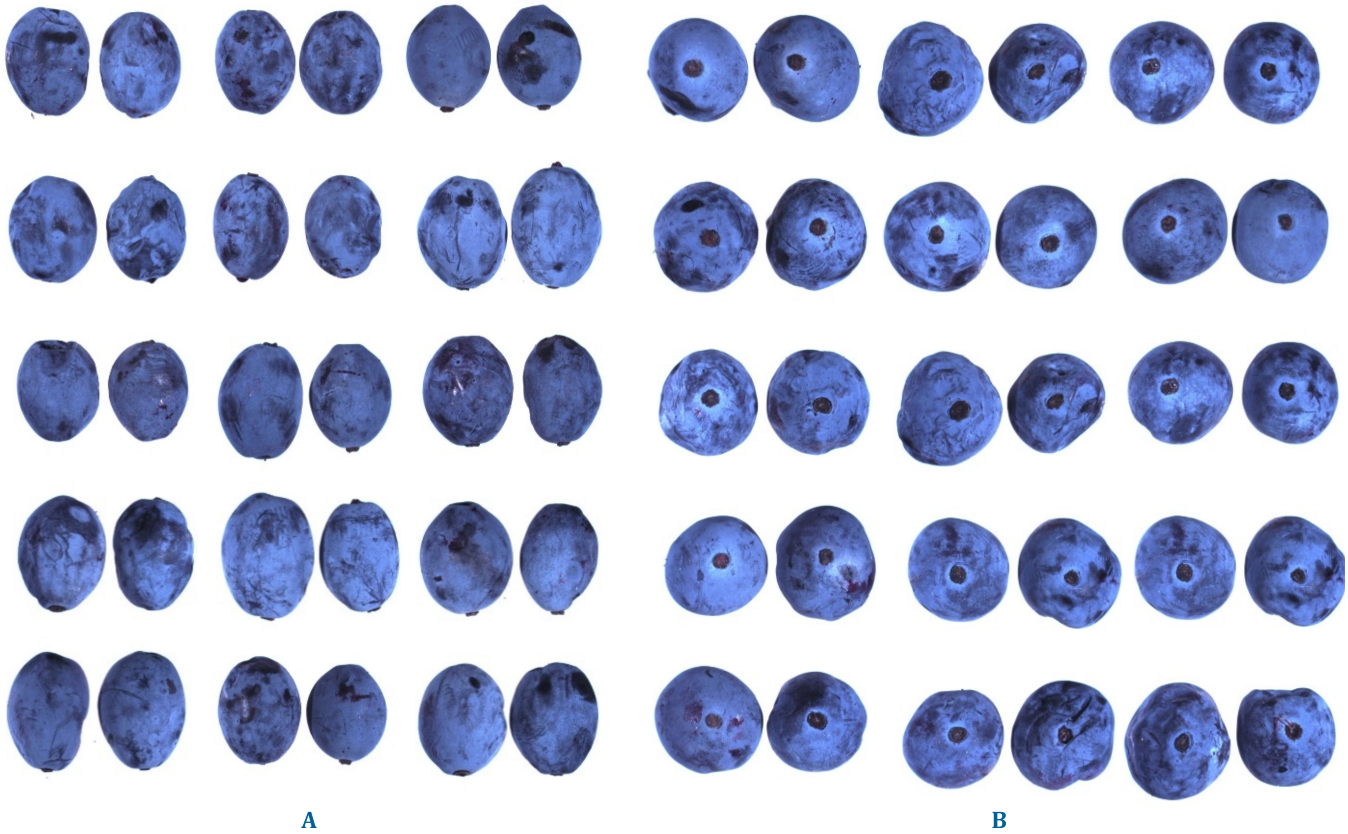


**Figure 1** Variability in the shape and the colour of fruits of evaluated genotypes of *Mahonia aquifolium* (Pursh) Nutt.

2(B) showed genotype differences in colour, size, and number of clusters on the stem.

## Elementary components

In this study, we determined elementary components of dried fruits *M. aquifolium* in comparison to other two species *Hippophae rhamnoides* L. and *Morus nigra* L. Dry matter and ash were similar in all three species. The content of proteins was following 10.89%, 15.16%, and 7.22% for *M. aquifolium*, *H. rhamnoides*, and *M. nigra*, respectively. Interesting differences were recorded in total fat, where the highest content was in *H. rhamnoides* (14.96%), followed *M. aquifolium* (9.76%), and the lowest in *M. nigra* (2.37%). Saturated

**Figure 2** Comparison of shape and colour of seeds (A) and detail of clusters (B) of *Mahonia aquifolium* (Pursh) Nutt.

**Table 3** Comparison of elementary components in evaluated dried fruits of *Mahonia aquifolium* (Pursh) Nutt. with other species

| Component | SI | *Mahonia aquifolium* (mean ±SE) | *Hippophae rhamnoides* (mean ±SE) | *Morus nigra* (mean ±SE) |
|---|---|---|---|---|
| **Dry matter** | % | 82.70 ±2.03 | 87.88 ±2.12 | 86.36 ±1.67 |
| **Proteins** | % | 10.89 ±0.12 | 15.16 ±0.18 | 7.22 ±0.08 |
| **Ash** | % | 3.20 ±0.07 | 3.68 ±0.04 | 2.70 ±0.01 |
| **Fats** | % | 9.76 ±0.10 | 14.96 ±0.17 | 2.37 ±0.07 |
| **β-carotene** | mg.kg$^{-1}$ | 14.20 ±0.12 | 55.40 ±1.18 | 2.80 ±0.04 |
| **Saturated fatty acids** | g. 100 g$^{-1}$ fat | 11.70 ±0.13 | 41.90 ±1.22 | 20.50 ±0.76 |
| **Monounsaturated fatty acids** | g. 100 g$^{-1}$ fat | 18.00 ±0.21 | 21.90 ±0.19 | 31.40 ±1.05 |
| **Polyunsaturated fatty acids** | g. 100 g$^{-1}$ fat | 59.20 ±2.37 | 33.00 ±1.78 | 43.40 ±2.08 |

fatty acids were predominant in *H. rhamnoides* in the amount of 41.90 g.100 g$^{-1}$ DW of total fat content, while polyunsaturated fatty acids were the predominant in *M. aquifolium* and *M. nigra*, accounting for 59.20 and 43.40 g.100 g$^{-1}$ DW of total fat content. Regarding the β-carotene, the most represented was in *H. rhamnoides* (55.40 mg.kg$^{-1}$), while in *M. aquifolium* and *M. nigra* samples were the following amounts 14.20 and 2.80 mg.kg$^{-1}$, respectively (Table 3).

The present study, bioaccumulation, and biosorption of minerals and heavy metal concentration (K, P, Ca, Mg, Na, S, Fe, Mn, Zn, Al, Cu, Ni, Cd, As, Sn, Hg, Se, Pb) were observed in leaves and fruits of *M. aquifolium* (Table 4).

Macroelements (K, Ca, P, Mg, S) are the most represented group, dominated by potassium contained in leaves (10437 ±95 mg.kg$^{-1}$), and fruits (9763 ±79 mg.kg$^{-1}$). Potassium is the main mineral element with an average of one-third of the total. Potassium, mostly as a cation ($K^+$), together with calcium ($Ca^{2+}$) are the most abundant inorganic elements in plant cellular tissues. Many studies have reported on the role of $K^+$ in several physiological functions, including controlling cellular growth and wood formation, xylem-phloem water content and movement, nutrient and metabolite transport, and stress response (Sardans and Peñuelas, 2021).

Calcium (Ca) is third in abundance and very close to P in abundance in plant tissue. The highest amounts of Ca are found in mitochondria. It is involved in cell division and cell elongation (Helper, 1994). It is a messenger in several developmental and environmental changes (Heintz, 1960; Sanders et al., 2002). It is responsible for cell integrity (Zhang et al., 2018) and therefore plant survival.

In our experiment calcium was the prominent element in the leaf's samples in the amount of 5152 ±111 mg.kg$^{-1}$. A high amount of phosphorus was found in leaves (2306 ±68 mg.kg$^{-1}$) and fruit samples (2389 ±77 mg.kg$^{-1}$). We can compare the percentages of individual components, which show that potassium was the most represented element in the leaves (48%), as well as fruits (63%), and phosphorus was the second most abundant element in the leaves (24%) of the total minerals, while calcium was represented in the fruits (15%) like the second abundant element.

Microelements (Mn, Fe, Zn, Cu, Al, Se, and Ni) are the second represented group of biogenic elements, where the content of manganese, iron, and zinc prevailed in leaves and fruit samples. The content of manganese and iron predominate in leaves (80.1 ±3.12 mg.kg$^{-1}$ of Mn and 35.0 ±2.65 mg.kg$^{-1}$ of Fe) and fruits (29.7 ±mg.kg$^{-1}$ of Mn and 25.0 ±1.56 mg.kg$^{-1}$ of Fe) following by zinc in the both samples (27.0 ±1.38 mg.kg$^{-1}$ in leaves and 18.0 ±0.8 mg.kg$^{-1}$ in fruits). Microelements have a specific function in the plant tissues. Fe and Zn are essential for the synthesis of chlorophyll, Fe and Mn in photosynthesis, Fe, Mn, and Zn as electron transport mechanisms, and other several enzymes' systems (Voss, 1998). Heavy metals (Hg, As, Cd, Cr, Pb) are present with the most abundant Cr (0.91 ±0.08 mg.kg$^{-1}$ in leaves) and Pb (0.28 ±0.02 mg.kg$^{-1}$ in leaves) content and others only in the trace level (<0.20 mg.kg$^{-1}$).

Sorokopudov et al. (2017) determined a variety of mineral composition of fruits of *Mahonia aquifolium* collected in the Belgorod (Russia) region between years 2009–2011 (Pb 0.40–0.63 mg.kg$^{-1}$, Zn 2.00–3.44 mg.kg$^{-1}$, Cu 0.12–1.45 mg.kg$^{-1}$, Ca 0.034–0.068%, P 0.040–0.056%, K 0.24–0.41%, Fe 5.3–12.8 mg.kg$^{-1}$, Mn 4.36–8.36 mg.kg$^{-1}$).

Samecka-Cymerman and Kempers (1999) reported a study on the concentration of macroelements (N, P, K,

**Table 4** Composition of macro- and microelements of leaves and fruits of selected wild-growing genotypes of *Mahonia aquifolium* (Pursh) Nutt.

| Element | Leaves (mean ±SE) | Fruits (mean ±SE) |
|---|---|---|
| | **Macroelements (mg.kg$^{-1}$)** | |
| **K** | 10437 ±95 | 9763 ±79 |
| **P** | 2306 ±68 | 2389 ±77 |
| **Ca** | 5152 ±111 | 1126 ±65 |
| **S** | 1465 ±99 | 1290 ±69 |
| **Mg** | 2126 ±110 | 1015 ±65 |
| **Na** | 6.0 ±0.7 | 15.0 ±0.8 |
| | **Microelements (mg.kg$^{-1}$)** | |
| **Zn** | 27.0 ±1.12 | 18.0 ±0.8 |
| **Fe** | 35.0 ±2.65 | 25.0 ±1.56 |
| **Cu** | 10.0 ±0.9 | 10.0 ±0.9 |
| **Mn** | 80.1 ±3.12 | 29.7 ±1.38 |
| **Cr** | 0.91 ±0.08 | <0.2 |
| **Se** | 0.33 ±0.02 | <0.2 |
| | **Metals (mg.kg$^{-1}$)** | |
| **Al** | 17.6 ±0.4 | 3.6 ±0.2 |
| **As** | <0.3 | <0.3 |
| **Cd** | <0.01 | 0.013 ±0.001 |
| **Ni** | <0.2 | 0.98 ±0.08 |
| **Hg** | 0.013 ±0.001 | 0.007 ±0.0001 |
| **Pb** | 0.28 ±0.02 | 0.16 ±0.01 |

Ca, Mg, S, and Fe) and heavy metals (Ni, Cr, Co, V, Zn, Mn, Pb, Cd, Cu, Hg, Ba, and Sr) in the soil and three species of the evergreen plant *Ilex aquifolium*, *Mahonia aquifolium* and *Rhododendron catawbiense* collected in various places in Poland and Netherland after urban pollution (one place is exposed to atmospheric exhausts of heavy traffic, chemical factories, metal smelters and a heat and power plant partly alimented with lignite and coals, two others are unpolluted). Especially pollution with Hg via soil is supported by a significant positive correlation between Hg content in soil and in all the examined species of which *Ilex aquifolium* seemed to be the best monitor of soil pollution with this element.

Heavy metals such as Cd, Pb, Cr, and Ni in plant tissues can be used as indicators suggesting the environmental pollution status in the region (Porrini et al., 2003; Wang and Li, 2011).

## Antioxidant activity

The highest antioxidant activity is shown in methanol extracts by the DPPH method in fruits at 76.2 mg TE.g$^{-1}$ DW and leaves at 101.2 mg TE.g$^{-1}$ DW of *M. aquifolium* samples (Figure 3). The lowest antioxidant activity was determined in water extract for fruits at 35.5 mg TE.g$^{-1}$ DW and leaves in ethanol extract at 47.4 mg TE.g$^{-1}$ DW. The analysis showed the presence and comparable values of antioxidants not only in the fruits but also in the leaves of *M. aquifolium* species. Various solvents demonstrated different values of antioxidant activity for fruits and leaves in our experiments.

Other study (Coklar and Akbulut, 2017) demonstrated antioxidant activity of fresh berries *M. aquifolium* determined by DPPH method in various solvents with following values: methanol (35.26 ±1.88 mmol TE.kg$^{-1}$ FW), ethanol (27.36 ±1.47 mmol TE.kg$^{-1}$ FW), acetone (14.41 ±0.68 mmol TE.kg$^{-1}$ FW), water (13.03 ±0.25 mmol TE.kg$^{-1}$ FW), ethyl acetate (4.36 ±0.17 mmol TE.kg$^{-1}$ FW), chloroform (0.36 ±0.04 mmol TE.kg$^{-1}$ FW). According to Gunduz (2013), fruits of *M. aquifolium* showed antioxidant activity by Trolox equivalent antioxidant capacity (TEAC) assay in the range from 4.1 ±0.1 to 21.1 ±0.1 µmol TE.g$^{-1}$ FW, total phenolics ranged from 5009.3 ±176.3 to 6646.8 ±332.1 µg GAE.g$^{-1}$ FW and total monomeric anthocyanins capacities ranged from 52.8 ±4.6 to 361.0 ±15.8 µg cy-3-glu.g$^{-1}$ FW.

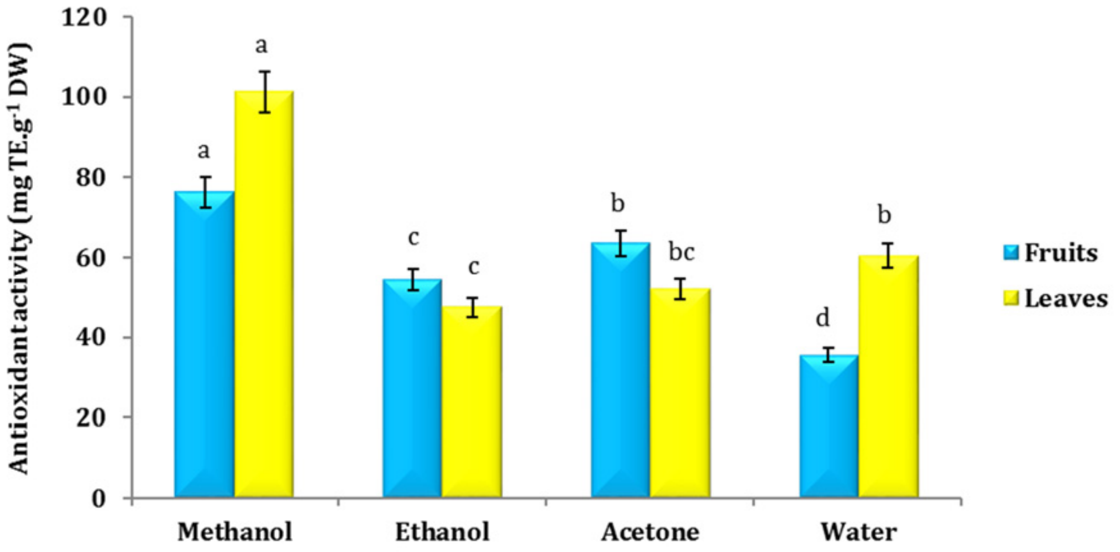


**Figure 3** Antioxidant activity of plant parts of *Mahonia aquifolium* (Pursh) Nutt. in various extracts. Different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

## Physicochemical parameters

Table 5 show that the pH in the water was determined in the range of 6.97–7.52 (C-TW) during the tested period, after the addition of whole fruits to the water the pH decreased from 7.74 to 3.41 (C-WF) and after adding the mashed fruits to the water, the pH decreased from 5.83 to 3.48 (C-MF). After adding cluster to water (C-CT), the pH stabilized in the range of 7.03 to 7.17.

In the control variant (C-TW), the dynamics of EC increased from 851 to 2430 µS.cm$^{-1}$ during the time period. After adding the fruits to the water (C-WF), the dynamics of EC decreased significantly from 634 to 881 µS.cm$^{-1}$. After adding the mashed fruits to the water (C-MF), the dynamics of EC decreased significantly from 640 to 887 µS.cm$^{-1}$. After adding clusters (C-CT) to the water, the EC dynamics stabilized in the range of 637 to 672 µS.cm$^{-1}$.

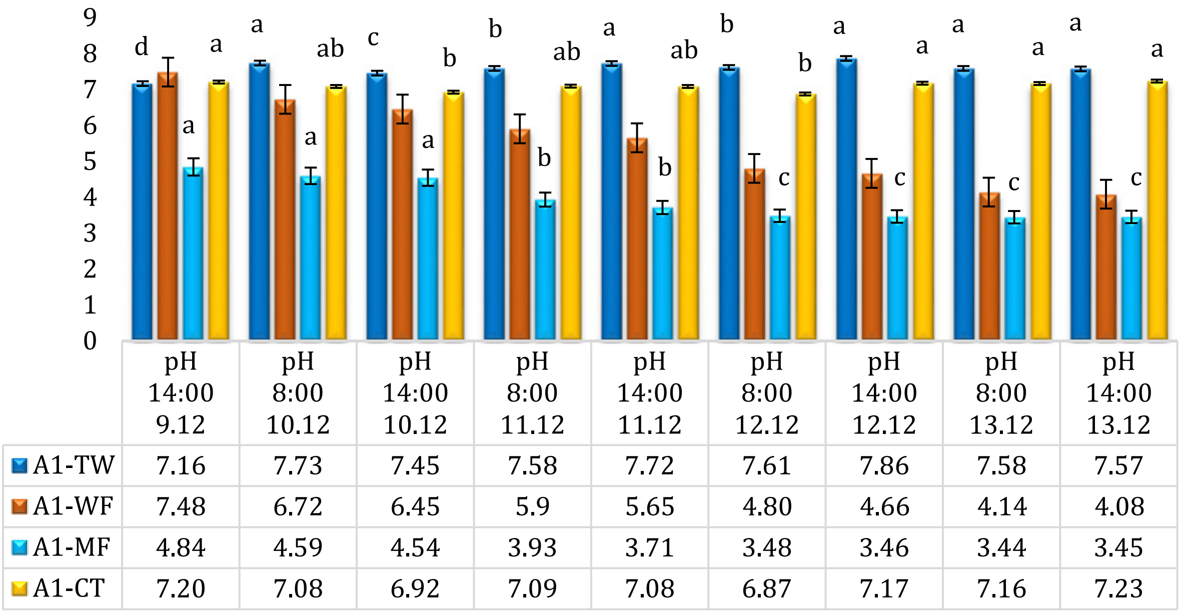
The investigation of TDS presented the following results. The dynamics in the control variant increased from 425 to 1220 mg.L$^{-1}$ during the time period. After adding the whole fruits to the water (C-WF), the dynamics of TDS decreased significantly from 317 to 439 mg.L$^{-1}$. After adding the mashed fruits to the water (C-MF), the dynamics of TDS decreased significantly from 320 to 443 mg.L$^{-1}$. After the addition of clusters (C-CT) to the water, the dynamics of TDS stabilized significantly in the range 318 to 335 mg.L$^{-1}$.

Structured (once-activated) water with plant samples changed properties in all variants and evaluated traits as follows. Figure 4 presents a comparison of variants in once-activated tap water (A1) in pH changes

**Table 5** Comparison of control variants of water and extracts (without activation) in the evaluated parameters (ph, EC and TDS) during the tested time period

| Variants | C-TW | C-WF | C-MF | C-CT | C-TW | C-WF | C-MF | C-CT | C-TW | C-WF | C-MF | C-CT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Date/Parameters** | pH | | | | electrolytic conductivity (µS.cm$^{-1}$) | | | | total dissolved solids (mg.L$^{-1}$) | | | |
| **09.12.2:00 PM** | 6.97 | 7.74 | 5.83 | 7.03 | 851 | 634 | 640 | 637 | 425 | 317 | 320 | 318 |
| **10.12.8:00 AM** | 7.28 | 6.78 | 5.18 | 6.91 | 1577 | 647 | 672 | 642 | 792 | 323 | 335 | 320 |
| **10.12.2:00 PM** | 7.03 | 6.44 | 5.12 | 6.76 | 1319 | 676 | 669 | 639 | 662 | 338 | 335 | 319 |
| **11.12.8:00 AM** | 7.46 | 5.10 | 3.99 | 6.94 | 1536 | 709 | 719 | 647 | 770 | 353 | 360 | 323 |
| **11.12.2:00 PM** | 7.40 | 4.68 | 3.81 | 6.96 | 1751 | 702 | 749 | 641 | 879 | 351 | 374 | 320 |
| **12.12.8:00 AM** | 7.04 | 3.79 | 3.47 | 6.78 | 1986 | 777 | 825 | 655 | 993 | 388 | 412 | 328 |
| **12.12.2:00 PM** | 7.52 | 3.74 | 3.45 | 6.52 | 2380 | 803 | 857 | 698 | 1190 | 401 | 428 | 336 |
| **13.12.8:00 AM** | 7.19 | 3.43 | 3.43 | 6.95 | 2350 | 879 | 867 | 666 | 1180 | 439 | 434 | 333 |
| **13.12.2:00 PM** | 6.98 | 3.41 | 3.48 | 7.17 | 2430 | 881 | 887 | 672 | 1220 | 439 | 443 | 335 |

Notes: C-TW – sample of tap water; C-WF – extract of whole fruit in water; C-MF – extract of mashed fruit in water; C-CT – extract of cluster in water

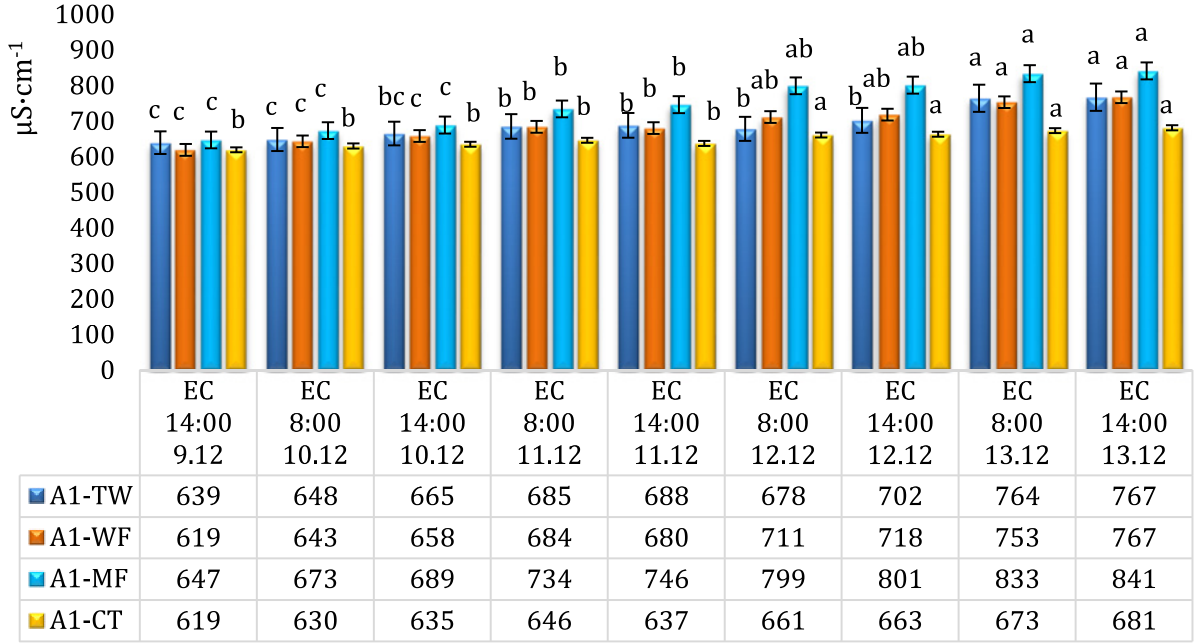


| | pH 14:00 9.12 | pH 8:00 10.12 | pH 14:00 10.12 | pH 8:00 11.12 | pH 14:00 11.12 | pH 8:00 12.12 | pH 14:00 12.12 | pH 8:00 13.12 | pH 14:00 13.12 |
|---|---|---|---|---|---|---|---|---|---|
| A1-TW | 7.16 | 7.73 | 7.45 | 7.58 | 7.72 | 7.61 | 7.86 | 7.58 | 7.57 |
| A1-WF | 7.48 | 6.72 | 6.45 | 5.9 | 5.65 | 4.80 | 4.66 | 4.14 | 4.08 |
| A1-MF | 4.84 | 4.59 | 4.54 | 3.93 | 3.71 | 3.48 | 3.46 | 3.44 | 3.45 |
| A1-CT | 7.20 | 7.08 | 6.92 | 7.09 | 7.08 | 6.87 | 7.17 | 7.16 | 7.23 |

**Figure 4** Comparison of variants with once-activated water (A1) at pH during the experimental period
A1-TW – sample of activated tap water; A1-WF – extract of whole fruit in once-activated water; A1-MF – extract of mashed fruit in once-activated water; A1-CT – extract of the cluster in once-activated water. Different superscripts in each column indicate the significant differences in the mean at p <0.05

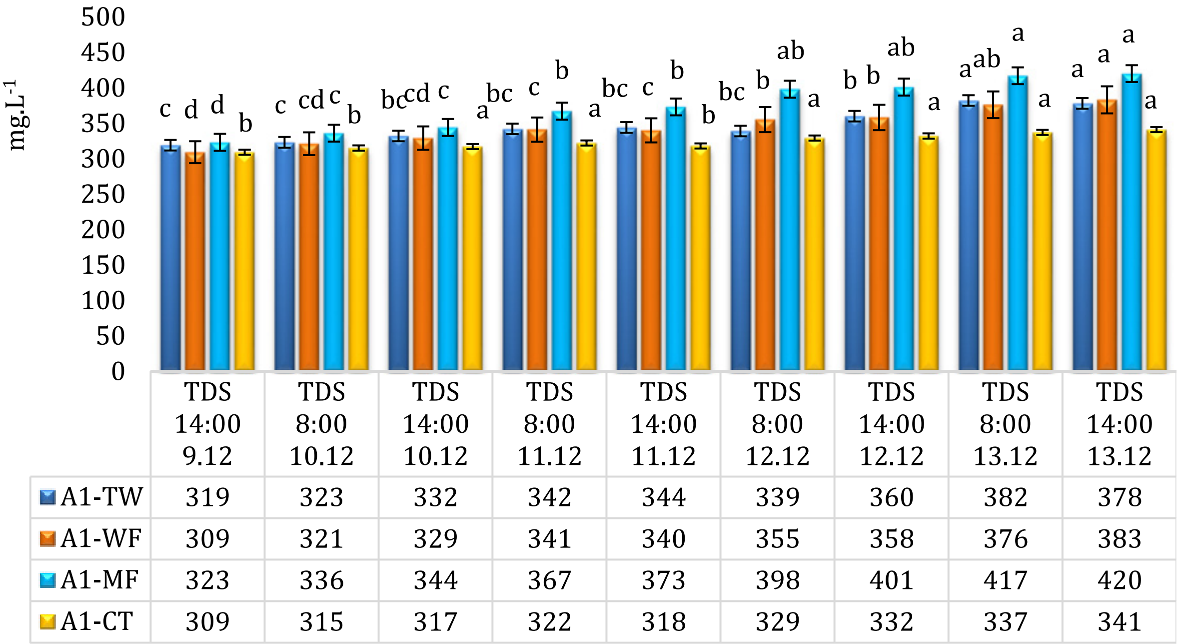
throughout the experiment. The trend of decreasing pH was evident in all variants. Activation of tap water (A1-TW) conditioned minimal pH changes. We also determined similar changes in the extraction of the cluster (A1-CT) in once-activated water. During the extraction of whole fruits (A1-WF) and mashed fruits (A1-MF), we recorded a decrease in pH from the beginning of the experiment, while a more significant decrease was determined for the variants mashed fruits (A1-MF).

On the first day of the experiment, there were no differences in electrolytic conductivity (EC) between



| | EC 14:00 9.12 | EC 8:00 10.12 | EC 14:00 10.12 | EC 8:00 11.12 | EC 14:00 11.12 | EC 8:00 12.12 | EC 14:00 12.12 | EC 8:00 13.12 | EC 14:00 13.12 |
|---|---|---|---|---|---|---|---|---|---|
| A1-TW | 639 | 648 | 665 | 685 | 688 | 678 | 702 | 764 | 767 |
| A1-WF | 619 | 643 | 658 | 684 | 680 | 711 | 718 | 753 | 767 |
| A1-MF | 647 | 673 | 689 | 734 | 746 | 799 | 801 | 833 | 841 |
| A1-CT | 619 | 630 | 635 | 646 | 637 | 661 | 663 | 673 | 681 |

**Figure 5** Comparison of variants with once-activated water (A1) at electrolytic conductivity (EC) during the experimental period ($\mu S.cm^{-1}$)
A1-TW – sample of activated tap water; A1-WF – extract of whole fruit in once-activated water; A1-MF – extract of mashed fruit in once-activated water; A1-CT – extract of the cluster in once-activated water. Different superscripts in each column indicate the significant differences in the mean at p <0.05



| | TDS 14:00 9.12 | TDS 8:00 10.12 | TDS 14:00 10.12 | TDS 8:00 11.12 | TDS 14:00 11.12 | TDS 8:00 12.12 | TDS 14:00 12.12 | TDS 8:00 13.12 | TDS 14:00 13.12 |
|---|---|---|---|---|---|---|---|---|---|
| A1-TW | 319 | 323 | 332 | 342 | 344 | 339 | 360 | 382 | 378 |
| A1-WF | 309 | 321 | 329 | 341 | 340 | 355 | 358 | 376 | 383 |
| A1-MF | 323 | 336 | 344 | 367 | 373 | 398 | 401 | 417 | 420 |
| A1-CT | 309 | 315 | 317 | 322 | 318 | 329 | 332 | 337 | 341 |

**Figure 6** Comparison of variants with once-activated water (A1) at total dissolved solids (TDS) during the experimental period (mg.L$^{-1}$)
A1-TW – sample of activated tap water; A1-WF – extract of whole fruit in once-activated water; A1-MF – extract of mashed fruit in once-activated water; A1-CT – extract of the cluster in once-activated water. Different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

the variants. The change was seen on the third day, where the highest conductivity was achieved by the mashed fruits variant (A1-MF), which continued until day 5 (Figure 5) in the interval from 647 (1st day) to 841 µS.cm$^{-1}$ (5th day). The lowest EC can see in the clusters variant (A1-CT) in the interval from 619 (1st day) to 681 µS.cm$^{-1}$ (5th day).

Activation of water (A1-TW) and addition of whole fruits (A1-WF), mashed fruits (A1-MF) and clusters (A1-CT) to activated water in generally resulted in a reduction and stabilization of EC values during the experiment. It follows that water activation significantly improves the quality of water for human use.

Various types of water have specific conductivity. According to Bendlin (1995) in Gordalla's study (Gordalla et al., 2007) we known ultrapure water (0.055 µS.cm$^{-1}$), demineralized (0.1–1.0 µS.cm$^{-1}$), drinking (30–2000 µS.cm$^{-1}$), brackish (20000–1000000 µS.cm$^{-1}$), lake constance water (322 µS.cm$^{-1}$), groundwater, Munich (537 µS.cm$^{-1}$), bank filtrate, river Rhine nearby Düsseldorf (702 µS.cm$^{-1}$).

On the first day of the experiment, there were no differences in total dissolved solids (TDS) between the variants. The change was seen on the third day, where the highest content of TDS was achieved by the mashed fruits variant (A1-MF), which continued until day 5 (Figure 6) in the interval from 323 (1st day) to 420 mg.L$^{-1}$ (5th day). The lowest content of TDS can see in clusters variant (A1-CT) in the interval from 309 (1st day) to 341 mg.L$^{-1}$ (5th day).

Activation of water (A1-TW) and addition of whole fruits (A1-WF), mashed fruits (A1-MF) and clusters (A1-CT) to activated water in generally resulted in a reduction and stabilization of TDS values during the experiment. Water activation significantly improves water quality.

Differences in the correlation coefficient values are significant between the tested samples of variants with once-activated water. A very strong relationship is maintained between electrolytic conductivity and total dissolved solids (r = 0.999). This trend was described in our previous studies (Horčinová Sedláčková et al., 2019a, b). Other values of the correlation coefficients between pH and electrolytic conductivity samples and pH and the total dissolved solids of the tested samples are very low (r = 0.195–0.359). A very strong negative correlation was determined between pH and electrolytic conductivity (r = -0.961), pH, and total dissolved solids (r = -0.962) in the investigated variant of mashed fruits (A1-MF).

## Conclusions

Our study was the evaluation of genotypes of *Mahonia aquifolium* based on the morphological analysis of leaves, fruits, and seeds, determination of macro- and microelements of leaves and fruits, and new original experiments with activated water of various combinations of fruits and clusters. Results confirmed variability of morphological traits such as weight of fruits, the height of fruits, and the width of fruits. When comparing the determined variation ranges for all evaluated traits, we found a significant degree of agreement. Based on micronutrient content leaves and fruits may be regarded as an important source, especially of potassium, calcium, and other minerals such as K, P, S and Mg. The highest antioxidant activity was determined in methanol extracts by the DPPH method in the dried fruits and leaves. Experiments with activated water most changed the character of pH, electrolytic conductivity, and content of total dissolved solids mashed fruits variants until the 5th day of the experiment. Results document that the study of the extraction of plant parts in activated water opens up new possibilities for recognizing the influence of water activation on the change in the properties of the extracts. Knowledge of the field can significantly affect water quality improvement, technological processes in the production of beverages and the food industry. Activation of water, and thus also the activation of extracts of plant parts does not have to result in a change in the chemical composition of the products, but at the same time, it can have a significant impact on the improvement of water or beverages quality, and thereby also improving the health of consumers.

### Conflict of Interest

The authors declare no conflict of interest.

### Ethical Statement

This article does not contain any studies that would require an ethical statement.

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## References

Abdul Qados, A.M.S., & Hozayn, M. 2010. Magnetic water technology, a novel tool to increase growth, yield and chemical constituents of lentil under greenhouse condition. In *American-Eurasian Journal of Agriculture and Environmental Sciences*, *7*(4), 457–462.

Abrams, L. 1950. Ellustrated flora of the Pacific States. 2nd ed. Stanford University Press: Stanford, CA.

Andreicuţ, A.D., Fischer-Fodor, E., Pârvu, A.E., Ţigu, A.B., Cenariu, M., Pârvu, M., Cătoi, F.A., & Irimie, A. 2019. Antitumoral and immunomodulatory effect of *Mahonia aquifolium* extracts. In *Oxidative Medicine and Cellular Longevity,* Hindawi, ID 6439021, 13 p. https://doi.org/10.1155/2019/6439021

Andreicuţ, A.-D., Pârvu, A.E., Moţ, A.C., Pârvu, M., Fischer-Fodor, E., Feldrihan, V., Cătoi, A.F., Cecan, M., & Irimie, A. 2018. Anti-inflammatory and antioxidant effects of *Mahonia aquifolium* leaves and bark extracts. In *Farmacia*, *66*(1), 49–58.

Association of Official Analytical Chemists (AOAC). 1990. Official Methods of Analysis, (15th ed.), AOAC: Arlington, TX, USA.

Auge, H., & Brandl, R. 1997. Seedling recruitment in the invasive clonal shrub, *Mahonia aquifolium* (Pursh) Nutt. In *Oecologia Berlin*, *110*, 205–211.

Baumann, L.S. 2008. Cosmeceutical critique. Aesthetic dermatology. *Mahonia*. In *Skin & Allergy News,* May 2008.

Chaplin, M.F. 2000. A proposal for structuring of water. In *Biophysical Chemistry*, *83*, 211–221.

Clark, G.N.I., Cappa, C.D., Smith, J.D., & Saykally, R.J., Heda-Gordon, T. 2010. The structure of ambient water. In *Molecular Physics*, *108*(11), 1415–1433. https://doi.org/10.1080/00268971003762134

Coklar, H., & Akbulut, M. 2017. Anthocyanins and phenolic compounds of *Mahonia aquifolium* berries and their contributions to antioxidant activity. In *Journal of Functional Food*, *35*, 166–174. http://dx.doi.org/10.1016/j.jff.2017.05.037

Čerňáková, M., Košťalová, D., Kettmann, V., Plodová, M., Tóth, J., & Dřímal, J. 2002. Potential antimutagenic activity of berberine, a constituent of *Mahonia aquifolium*. In *BMC Complementary and Alternative Medicine*, *2*(2). https://doi.org/10.1186/1472-6882-2-2

Damjanović, A., Kolundžija, B., Matić, I.Z., Krivokuća, A., Zdunić, G., Šavikin, K., Janković, R., Antić Stanković, J., & Stanojković, T.P. 2020. *Mahonia aquifolium* extracts promote Doxorubicin effects against lung adenocarcinoma cells *in vitro*. In *Molecules*, *25*(5233), https://doi.org/10.3390/molecules25225233

De Souza, A., Garci, D., Sueiro, L., Gilart, F., Porras, E., & Licea, L. 2006. Pre-sowing magnetic treatments of tomato seeds increase the growth and yield of plants. In *Bioelectromagnetics*, *27*(4), 247–257. https://doi.org/10.1002/bem.20206

Del Giudice, E., Preparata, G., & Vitiello, G. 1988. Water as a free electric dipole laser. In *Physical Review Letters*, *61*(9), 1085–1088. https://doi.org/10.1103/PhysRevLett.61.1085

Divis, P., Porizka, J., Vespalcova, M., Matejicek, A., & Kaplan, J. 2015. Elemental composition of fruits from different black elder (*Sambucus nigra* L.) cultivars grown in the Czech Republic. In *Journal of Elementology*, *20*(3), 549–557. https://doi.org/10.5601/jelem.2015.20.1.758

Donsky, H, & Clarke, D. 2007. Reliéva, a *Mahonia aquifolium* extract for the treatment of adult patients with atopic dermatitis. In *American Journal of Therapeutics*, *14*(5), 442–446. https://doi.org/10.1097/MJT.0b013e31814002c1

Gieler, U., von der Weth, A., & Heger, M. 2009. *Mahonia aquifolium*: A new type of topical treatment for psoriasis. In *Journal of Dermatological Treatment*, *6*(1), 31–34. https://doi.org/10.3109/09546639509080587

Gordalla, B., Müller, M.B., & Frimmel, F.H. 2007. The physicochemical properties of water and their relevance for life. In: Lozán, J. L., Grassl, H., Hupfer, P., Menzel L., & Schönwiese C.-D. *Global Change: Enough water for all?* Wissenschaftliche Auswertungen: Hamburg.

Gunduz, K. 2013. Morphological and phytochemical properties of *Mahonia aquifolium* from Turkey. In *Pakistan Journal of Agricultural Research*, *50*(3), 439–443.

He, J.-M., & Mu, Q. 2015. The medicinal uses of the genus *Mahonia* in traditional Chinese medicine: An ethnopharmacological, phytochemical and pharmacological review. In *Journal of Ethnopharmacology*, *175*, 668–683. http://dx.doi.org/10.1016/j.jep.2015.09.013

Heald, F.D. 1902. The electrical conductivity of plant juices. In *Botanical Gazette*, *34*(2), 81–92. http://www.jstor.com/stable/2465072

Heintz, S.G., 1960. Studies on cation-exchange capacities of roots. In *Plant and Soil*, *13*, 365–383.

Helper, P.K., 1994. Effect of calcium in cell division. In *Cell Calcium*, *16*(4), 322–330.

Ho, L., Thas, O., Echelpoel, W.V., & Goethals, P. 2019. A Practical protocol for the experimental design of comparative studies on water treatment. In *Water*, *11*(1), 162.

Horčinová Sedláčková, V., Šimková, J., Brindza, J., Grygorieva, O., Vergun, O. 2019a. Honey influence on the physicochemical characteristics of water and teas. In *Plodovodstvo i jagodovodstvo Rossii Moskva : Federaľnoe gosudarstvennoje bjudžetnoje obrazovateľnoje učreždenije,* 140–155. https://dx.doi.org/10.31676/2073-4948-2019-57-140-155

Horčinová Sedláčková, V., Šimková, J., Brindza, J., Grygorieva, O., Vergun, O. 2019b. Honey influence on the physicochemical characteristics of water and teas. In *Roľ fiziologii i biochimii v introdukcii i selekcii celʹskochozjajstvennych rastenij*. 1st eds. Moskva : Rosijskij universitet družby narodov, 138–141.

Hozayn, M., & Abdul Qados, A.M.S. 2010. Magnetic water application for improving wheat (*Triticum aestivum* L.) crop production. In *Agriculture and Biology Journal of North America*, *1*(4), 677–682.

Iwasa, K., Kim, H.-S., Wataya, Y., Lee, & D.-U. 1998. Antimalarial activity and structure-activity relationships of protoberberine alkaloids. In *European Journal of Medicinal Chemistry*, *33*(1), 65–69. https://doi.org/10.1016/S0223-5234(99)80077-4

Johansson, B. 2009. Effects of functional water on heart rate, heart rate variability, and salivary immunoglobulin A in healthy humans: A pilot study. In *Journal of Alternative and Complementary Medicine*, *15*(8), 871–877. https://doi.org/10.1089/acm.2008.0336

Korotkov, K. 2019. Study of structured water and its biological effects. In *International Journal of Complementary & Alternative Medicine*, *12*(5), 168–172. https://doi.org/10.15406/ijcam.2019.12.00468

Korotkov, K., & Orlov, D. 2010. Analysis of Stimulated Electrophotonic Glow of Liquids. In *Water*, *2*, 29–43.

Korotkov, K.G., Churganov, O.A., Gavrilova, E.A., Belodedova, M.A., & Korotkova, A.K. 2019. Influence of drinking structured water to human psychophysiology. In *Journal of Applied Biotechnology & Bioengineering*, *6*(4), 171–177. https://www.researchgate.net/publication/34

Košťálová, D., Brázdovičová, B., & Tomko, J. 1981. Isolation of quaternary alkaloids from *Mahonia* aquifotium (PURSH) Nutt. I. In *Chemické zvesti*, *35*(2), 279–283.

Košťálová, D., Hrochová, V., & Tomko, J. 1986. Tertiary alkaloids of *Mahonia aquifolium* (Pursh) Nutt. I l l. In *Chemical Papers*, *40*(3), p. 389–394.

Kumar Gora, M., Chand Jakhar, K., Jat, H., Kumar, P. 2018. A review: structured water technology: its effect on productivity of agricultural crops. In *International Journal of Chemical Studies*, *6*(4), 3248–3253.

Ling, N.G. 2006. The Physical state of water in living cell and model systems. In *Annals of the New York Academy of Sciences*, *125*(2), 401–417. https://doi.org/10.1111/j.1749-6632.1965.tb45406.x

Mikula, A., & Vanke, P. 1989. *Plody planých a parkových rostlin* [Fruits of wild and park plants]. Praha: SZN, 287 p. [In Slovak]

Pollack, G.H. 2013. *The fourth phase of water.* EDGESCIENC. Seattle: Ebner & Sons.

Porrini, C., Sabatini, A.G., Girotti, S., Fini, F., Monaco, L., Celli, G., Bortolotti, L., & Ghini, S. 2003. The death of honey bees and environmental pollution by pesticides: the honey bees as biological indicators. In *Bulletin of Insectology*, *56*(1), 147–152.

Pyrkosz-Biardzka, K., Kucharska, A.Z., Sokół-Łętowska, A., Strugała, P., & Gabrielska J. 2014. A comprehensive study on antioxidant properties of crude extracts from fruits of *Berberis vulgaris* L., *Cornus mas* L. and *Mahonia aquifolimum* Nutt. In *Polish Journal of Food and Nutrition Sciences*, *64*(2), 91–99. https://doi.org/10.2478/v10222-012-0097-x

Samecka-Cymerman, A., & Kempers, A.J. 1999. Bioindication of heavy metals in the town Wrocław (Poland) with evergreen plants. In *Atmospheric Environment*, *33*(3), 419–430. https://doi.org/10.1016/S1352-2310(98)00202-7

Sanders, J., Pelloux, J., Brownlee, C., & Harper, J.F., 2002. Calcium dependent sucrose uptake links sugar signaling to anthocyanin biosynthesis. In *Plant Cell*, *14*, S401–S417.

Sardans, J., & Peñuelas, J. 2021. Potassium control of plant functions: ecological and agricultural implications. In *Plants*, *10*(419). https://doi.org/10.3390/plants1002041

Slavík, J., Bochořáková, J., Košťálová, D., & Hrochová, V. 1985. Alkaloids of *Mahonia aquifolium* (Pursh) Nutt. II. In *Chemical Papers*, *39*(4), 537–542.

Slobodníková, L., Košťálová, D., Labudová, D., & Kotulová, D. 2004. Antimicrobial activity of *Mahonia aquifolium* crude extracts and its major isolated alkaloids. In *Phytotherapy Research*, 18(8), 674–676. https://doi.org/10.1002/ptr.1517

Şofletea, N., & Curtu, L., 2007. *Dendrologie*. Editura Universităţii Transilvania din Braşov, 540 p.

Sorokopudov, V.N., Deyneka, V.I., Khlebnikov, V.A. 2007. Biokhimicheskiye osobennosti nekotorykh vidov semeystva Berberidaceae pri introduktsii v Belgorodskoy oblasti [Biochemical features of some species of Berberidaeae family during introduction in the Belgorod region]. In *Sovremennyye problemy introduktsii i sokhraneniya bioraznoobraziya rasteniy.* Voronezh: IPTS VGU, 94–96. [In Russian]

Sorokopudov, V.N., Myachikova, N.I., & Georgescu, C. 2017. *Mahonia aquifolium* as a promising raw material for the food industry. In *Scientific Study & Research. Chemistry & Chemical Engineering, Biotechnology, Food Industry*, *18*(4), 427–434.

Stehlíková, B. 1998. *Základy bioštatistiky* [Fundamentals of biostatistics]. Učebné texty pre dištančné štúdium. Nitra: Ochrana biodiverzity. [In Slovak]

Voeikov, V.L., & Del Giudice, E. 2009. Water respiration – The basis of the living state. In *Water*, *1*(1), 52–75.

Voeikov, V.L., & Korotkov, K.G. 2017. *The emerging science of water*. USA: Amazon.

Vorwohl, G. 1964a. Die Beziehung zwischen der elektrischen Leitfähigkeit der Honige und ihrer trachmässiger Herkunft [The relationship between the electrical conductivity of honey and its traditional origin]. In *Ann. de Abeille*, *7*(4), 301–309. [In German]

Vorwohl, G. 1964b. Messung der elektrischen Leitfähigkeit des Honigs und der Verwendung der Messwerte zur Sortendiagnose und zum Nachweis von Verfälschungen mit Zuckerfütterungshonig [Measuring the electrical conductivity of the honey and using the readings to diagnose the variety and detection of sugar cane adulteration]. In *Zeitschrift für Bienenforschung*, *7,* 37–47. [In German]

Voss, R. 1998. *Micronutrients*. Iowa State University: Ames, IA 50011, 69 p.

Wang, J., & Li, Q. X. 2011. Chemical Composition, Characterization, and Differentiation of Honey Botanical and Geographical Origins. In *Advances in Food and Nutrition Research*, *62*, 89–137. https://doi.org/10.1016/b978-0-12-385989-1.00003-x

Wu, J.-Y., Ogisu, M., Qin, H.-N., & Lu, S.-N. 2009. A new species of *Mahonia* Nutt. (Berberidaceae) from China. In *Botanical Studies*, *50*, 487–492.

Ying, T.S. 2001. Berberidaceae. In *Delectis florae reipublicae popularis sinicae agendae academiae sinicae edita.* Flora Reipublicae Popularis Sinicae, Tomus. 29. Beijing: Science Press, 214–249.

Zhang, H.U., Xu, Q., Xiong, Y., Peng, S.L., Kumari, K.M., & Wei, D.Q. 2018. Membrane defect and water leakage caused by passive calcium permeation. In *Journal of Molecular Liquids*, *270*, 227–233. https://doi.org/10.1016/j.molliq.2017.12.127