

# SPECTRAL ANALYSIS OF *Sambucus nigra* L. FRUITS AND FLOWERS FOR ELUCIDATION OF THEIR ANALGESIC, DIURETIC, ANTI-INFLAMMATORY AND ANTI-TUMOR EFFECTS

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## ABSTRACT

The aim of the study was to further elucidate the feasibility of *Sambucus nigra* L. fruits in recovery from inflammations and prevention and healing as additional complementary remedy of tumors, as well as the beneficial analgetic and diuretic effects of its flowers. Non-equilibrium energy spectrum (NES) and Differential non-equilibrium energy spectrum (DNES) spectral analyses of hydrogen bonds energies in 1% water extracts were performed. The 1% water extracts of *Sambucus nigra* L. fruits and flowers were studied. The control sample was with deionized water with chemical purity – 99.99%; pH – 6,0–7,5; electric conductivity – 10  $\mu$ S/cm. Increased average energy of hydrogen bonds compared to deionized water was found in both types of extracts as follows:  $\Delta E_n = -0.0139 \pm 0.0007$  eV for fruits and  $\Delta E_n = -0.0079 \pm 0.0004$  eV for flowers. The biggest local extremums of hydrogen bond energies in both types of extracts were measured at (-0.1112 eV) ( $\lambda = 11.15 \mu\text{m}$ ) ( $\tilde{\nu} = 897 \text{ cm}^{-1}$ ), (-0.1212 eV) ( $\lambda = 10.23 \mu\text{m}$ ) ( $\tilde{\nu} = 978 \text{ cm}^{-1}$ ) and (-0.1387 eV) ( $\lambda = 8.95 \mu\text{m}$ ) ( $\tilde{\nu} = 1117 \text{ cm}^{-1}$ ). These extremums are identical with extremums observed in other studies where they have been found to correspond to analgetic and diuretic, anti-inflammatory and anti-tumor effects.

**Keywords:** *Sambucus nigra* L.; flowers; fruits; anti-inflammatory; anti tumor; spectral analyses; NES; DNES.

## INTRODUCTION

*Sambucus nigra* L. is a native plant from the family *Adoxaceae*. It is species complex of flowering plants in Europe and North America. *Sambucus nigra* L. grows in dry fertile soils, primarily in sunny locations and including both wet and dry. The flowers and the berries have a tradition of culinary use, primarily for cordial diseases and wine.

*Sambucus nigra* L. fruits are popular in Bulgaria with their anti-tumor and anti-oxidant effects [1-5]. Investigations by Georgieva, Angelov and Boyadzhiev have quantified their polyphenolic content and antioxidant capacity [5], i.e.  $TPC_{GA}=2.22$  (g/l extract),  $IC50_{extr}=10.80$  and  $IC_{AA}=1.42$ . *S. nigra* L. flowers have been applied in the form of tea and syrup with diuretic effect. In addition, patients with oncological diseases have been recommended by medical doctors to take certain doses of *S. nigra* L. [6,7].

The following amounts of potassium ( $K^+$ ) in mg per 100 g have been determined and successfully applied in Bulgaria for recovery from inflammatory and viral diseases: *Sambucus Nigra* L. fruits (391) [8] *Urtica dioica* L. (475) [9], *Moringa oleifera* Lam (460) [10], *Malva sylvestris* L. (690) [11] and *Plantago major* L. (535) [12]. However, *Urtica dioica* L. has not been used in SARS-CoV-2 cases because of its blood clotting effects [13-18].

The present study is based on the interaction of *S. nigra* L. fruits and flowers extracts with water molecules by analyzing their non-equilibrium spectra (NES) and differential-equilibrium energy spectra (DNES). Its aim is to further elucidate their anti-inflammatory and anti-tumor properties.

In Bulgaria, medical doctors and pharmacists have been extensively applying medicinal plants and herbs. This is a national custom regulated by the Act of Health of 2005. Over the last 30 years, herbs and medicinal plants from abroad have been increasingly utilized. *S. nigra* L. fruits have been applied in cases of inflammatory diseases of

internal organs and systems as well as for anti tumor therapy. Analgetic and diuretic effects of *S. nigra* L. flowers have also been utilized.

## MATERIALS AND METHODS

### Non-Equilibrium Energy Spectrum (NES) and Differential Non-Equilibrium Energy Spectrum (DNES)

Non-equilibrium energy spectrum (NES) and Differential non-equilibrium energy spectrum (DNES) analysis was performed with an optical device invented by Antonov [17,18], having a hermetic chamber for evaporation of water drops under fixed temperature of 22–24 °C. Water drops were placed on thin maylar foil on top of a glass plate. They were illuminated with monochromatic light produced by a yellow filter with  $580\pm 7$  nm wavelength. Wetting angles of evaporating water drops were measured in the range from  $72.3^0$  to  $0^0$ . DNES-spectra were calculated in the range: -0.08–-0.1387 eV (corresponding to  $\lambda=8.9\text{--}13.8$   $\mu\text{m}$ ) with dedicated software. Thus, the average energies ( $\Delta E_{H...O}$ ) of hydrogen O...H-bonds between  $H_2O$  molecules in the corresponding solutions were derived.

*S. nigra* L. flowers and fruits water extracts with 1% concentration were investigated and their Differential non-equilibrium energy spectra (DNES) were calculated in comparison with deionized water [14,16].

### *S. nigra* L. Flowers and Fruits Water Extracts

The 1% water extracts of *S. nigra* L. fruits and flowers were studied. The control sample was with deionized water with chemical purity – 99.99%; pH – 6,0–7,5; electric conductivity – 10  $\mu\text{S/cm}$ .

*S. nigra* L. fruits and flowers were from syrups with registration of Bulgarian Food Safety Agency as food additive. *S. nigra* L. fruits was with code 77372070. *S. nigra* L. flowers was with code SKU5624.

## Content of *Sambucus nigra* L. Fruits

Table 1 shows the content of *S. nigra* L. fruits

**Table 1. Content of *S. nigra* L. fruits**

Composition	mg per 100 g
Potassium (K)	391.3
Calcium (Ca)	28.1
Sodium (Na)	2.2
Magnesium (Mg)	26.0
Iron (Fe)	1.9
Manganese (Mn)	0.36
Zinc (Zn)	0.27
Phosphorus (P)	54.0

## RESULTS AND DISCUSSION

### Average Energies of Hydrogen Bonds

The average energy ( $E_{H...O}$ ) of hydrogen H...O-bonds between individual H<sub>2</sub>O molecules in 1% water extract of *S. nigra* L. fruits was measured as -0.1288 eV. The average energy ( $E_{H...O}$ ) of hydrogen H...O-bonds between individual H<sub>2</sub>O molecules in 1% water extract of *S. nigra* L. flowers was measured as -0.1228 eV. The result for the control substance (deionized water) was  $E_a = -0.1149$  eV. The data obtained with the NES method were further processed with the DNES method as a difference of the NES (1% fruits/flowers *S. nigra* L. extracts) minus the NES (deionized water control), thus leading to the corresponding DNES spectra. Ten samples of each extract were measured and statistical difference was shown with Student's t-test at a confidence level  $P < 0.05$ . The difference between the average hydrogen bonds energy of the 1% *S. nigra* L. fruits water extract and deionized water was calculated as  $\Delta E_{fr} = -0.0139 \pm 0.0007$  eV, while the corresponding difference for the 1% *S. nigra* L. flowers water extract was calculated as  $\Delta E_{fl} = -0.0079 \pm 0.0004$  eV. These results show increased average energy of hydrogen bonds the *S. nigra* L. water extract compared to deionized water. They can be attributed to the complex macroelement and microelement content of *S. nigra* L. extracts and its overall influence on hydrogen bond networks. Along these lines, the macroelement and microelement content of *Sambucus nigra* L. fruits is presented in Table 1 [12].

### Normalized Distributions of Hydrogen Bonds Energies

Calculated significant differences of  $\Delta E_{H...O}$  values between individual H<sub>2</sub>O molecules in *S. nigra* L. extracts compared to deionized water point to rearrangement of water molecules in clusters with possible effects on human health on molecular and cellular level.

The local extremums ( $eV^{-1}$ ) in the normalized hydrogen bonds energy distributions of *S. nigra* L. extracts ( $-E_v/(-E_t)$ ), where  $E_v$  is a particular measured value of hydrogen bonds energy within the distribution and  $E_t$  is the total energy of hydrogen bonds in the samples, are presented in Table 2 and Fig. 1 [19-24]. These distributions are basically connected with rearrangement of H<sub>2</sub>O molecules having equal energies of hydrogen bonds as well as with their grouping into clusters. The same mathematical model has been successfully applied for analysis of extracts from other plants [10,11,16,25].

### Local extremums of hydrogen bonds energy and their correspondence to health effects

Previous studies of other aqueous solutions and physically activated water have found the connection between local extremums of hydrogen bonds energy and particular health effects [26-30]. In the case of investigated *S. nigra* L. Extracts from fruits and flowers, the following identical local extremums were detected.

The statistical significance is 0.05%. Ten samples of each extract were measured and statistical difference was shown with Student's t-test at a confidence level  $P < 0.05$ .

#### 1% *S. nigra* L. fruits water extract

-0.1212 eV corresponding to anti inflammatory effects

-0.1387 eV corresponding to anti tumor-effects

#### 1% *S. nigra* L. flowers water extract

-0.1112 eV corresponding to diuretic and analgetic effects

-0.1212 eV corresponding to anti-inflammatory effects

**Table 2. Normalized distribution of hydrogen bonds energy [%] in 1% water extracts from *Sambucus nigra* L fruits and flowers**

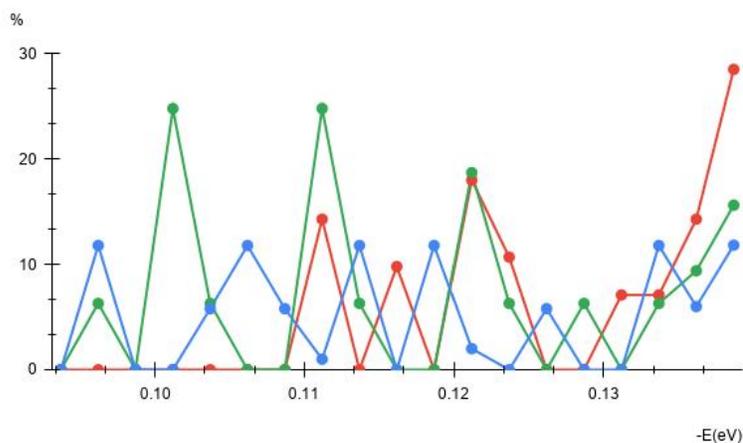
-E(eV)	1% <i>Sambucus nigra</i> L. fruits water extract [%]	1% <i>Sambucus nigra</i> L. flowers water extract [%]	Deionized Water Control [%]	-E(eV)	1% <i>Sambucus nigra</i> L. fruits water extract [%]	1% <i>Sambucus nigra</i> L. flowers water extract [%]	Deionized Water Control [%]
0.0937	0	0	0	0.1187	0	0	11.8±0.6
0.0962	0	6.3±0.3	11.8±0.6	0.1212	<b>18.0±0.9<sup>(2)</sup></b>	<b>18.7±0.9<sup>(2)</sup></b>	<b>0</b>
0.0987	0	0	0	0.1237	10.7±0.5	6.3±0.3	0
0.1012	0	24.8±1.2	0	0.1262	0.	0	5.8±0.3
0.1037	0	6.3±0.3	5.8±0.3	0.1287	0	6.3±0.3	0
0.1062	0	0	11.8±0.6	0.1312	7.1±0.4	0	0
0.1087	0	0	5.8±0.3	0.1337	7.1±0.4	6.3±0.3	11.8±0.6
0.1112	<b>14.3±0.7<sup>(1)</sup></b>	<b>24.8±1.2<sup>(1)</sup></b>	<b>0</b>	0.1362	14.3±0.7	9.4±0.3	6±0.3
0.1137	0	6.3±0.3	11.8±0.6	0.1387	<b>28.5±1.4<sup>(3)</sup></b>	15.6±0.8 <sup>(3)</sup>	<b>11.8±0.6</b>
0.1162	9.8±0.5	0	0	-	-	-	-

Note: For (E=-0.1112 eV) ( $\lambda=11.15 \mu\text{m}$ ) ( $\tilde{\nu}=897 \text{ cm}^{-1}$ ) is the local extremum for stimulating effect on the nervous system and improvement of nerve conductivity;

For (E = -0.1212 eV) ( $\lambda=10.23 \mu\text{m}$ ) ( $\tilde{\nu}=978 \text{ cm}^{-1}$ ) is the local extremum for anti-inflammatory effect;

For (E=-0.1387 eV) ( $\lambda=8.95 \mu\text{m}$ ) ( $\tilde{\nu}=1117 \text{ cm}^{-1}$ ) is the local extremum for inhibition of development of tumor cells at the molecular level;

Note: (1), (2), (3) are the biggest local extremums of the corresponding distribution in ascending order of hydrogen bonds energy values



**Fig. 1. Graphic representation of normalized distribution of hydrogen bonds energy [%] in and 1% water extracts of *Sambucus nigra* L. fruits (red line), *Sambucus nigra* L. flowers (green line) and deionized water control (blue line)**

The normalized distributions of hydrogen bonds energies in *S. nigra* L. fruits and flowers compared to control deionized water were different. Their difference may be connected with the different numbers of hydrogen bonds in the corresponding samples, as well as with their physical characteristics and composition. In particular, statistically significant difference in restructuring of H<sub>2</sub>O molecules in the samples

according to their energies was observed. These experimental findings imply that rearrangements of hydrogen bond networks in aqueous solutions may stimulate the immune system, thus inducing anti-inflammatory effects.

In addition, previous studies have verified the diuretic effects of *Sambucus nigra* L. flowers [26]. They also exert positive influence on the cardio

vascular system in hypertonia, dyslipidemias and produce analgetic effects [31-36]. Berries and flowers of this plant also show antiviral properties and ability to mitigate symptoms of upper respiratory disorders [37].

Recommended preventive daily dose of *Sambucus nigra* L. is one teaspoon syrup for two weeks. The same dose is medically recommended as complementary treatment for cancer patients [38].

## CONCLUSIONS

Spectral analysis of hydrogen bonds energy distributions of 1% *S. nigra* L. fruit and flower extracts has shown significant rearrangement of their hydrogen bond networks compared to deionized water control. In addition to the beneficial chemical composition of the plant, such structural changes in the solutions are identical with those observed in other studies of favorable health effects of solutes and physical activation of water. Consequently, *S. nigra* L. fruits and flowers have been reasonably applied in medical treatment and prevention so far and their useful properties should be investigated even further.

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## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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