RESEARCH ARTICLE

Application of infrared spectral analyses for medicinal plants containing calcium (Ca²⁺)

Ignat Ignatov[1*], Valentin Balabanski[2] & Mariana Angelcheva[3]

[1]Scientific Research Center of Medical Biophysics (SRCMB), Sofia, Bulgaria
[2]Sofia, Bulgaria
[3]Department of Kinesitherapy and Rehabilitation, National Sports Academy "V. Levski", Sofia, Bulgaria

*Email: mbioph@abv.bg; mbioph@gmail.com

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS etc.
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Abstract

A study was performed with the methods of Non-equilibrium energy spectrum (NES), Differential non-equilibrium energy spectrum (DNES), mathematical models and water cluster analysis on the changes of hydrogen bonds energy distribution in aqueous solutions due to calcium ions (Ca²⁺) from the medicinal plants: *Origanum vulgare* L., *Thymus vulgaris* L., *Mentha longifolia* L., *Petroselinum crispum* Mill., *Sideritis scardica* Griseb., *Salvia officinalis* L. All of them are known to contain more than 1000 mg/100 g of calcium ions. The local maximum at ($E = -0.1112$ eV) ($\lambda = 11.15$ µm) ($\tilde{\nu} = 897$ cm⁻¹) in all investigated solutions was associated with the presence of calcium ions by Student's t-test at $P<0,05$. In the proposed practical approach, the solutions had similar clustering patterns at $E = -0.1387$ eV, corresponding to water clusters of 16-20 water molecules. These findings from different plant species further expand the evidence about the specific influence of calcium ions on the -0.1112 eV hydrogen bonds energy level in aqueous solutions.

Keywords

Spectral analyses, NES, DNES, containing calcium ions (Ca²⁺)

Introduction

According to ethnographic data, 772 species of medicinal plants grow in Bulgaria. This makes up 6.43% of the world's herbal resources (1). The Bulgarian Law on Medicinal Plants defines those that can be used to obtain herbs, and "herbs are individual morphological plant parts or whole plants, as well as fruits and seeds thereof, which when fresh or dried, are intended for medicinal and prophylactic purposes, to produce medicinal products, food and also for cosmetic and technical purposes (2). "Herbal medicinal product" means a healing product containing medicinal substances as well as one or more herbal substances, one or more herbal preparations, or one or more herbal substances in combination with one or more herbal preparations (3). The medicinal plants are rich in biologically active substances, microelements and other chemical substances and are used in the prevention and treatment of various diseases as a complementary resource.

Calcium is a common mineral in the human body and it is present mainly in the bone apparatus and teeth. It plays an important electrolyte role during the cell physiological and biochemical processes.

Calcium also plays a role in the release of acetylcholine in the syn-

apse, which requires influx of calcium ions, followed by fusion of vesicles with the presynaptic membrane (4), calcium ions (Ca^{2+}) are cofactors of enzymes etc. (5). Calcium is essential for the normal functioning of bones, nervous, musculoskeletal, cardiovascular systems (6, 7).

Studies show that reduced calcium concentration is one of the major factors causing muscle fatigueless (4, 8).

A study carried out on a Swedish women community showed that calcium intake at doses of 1400 mg/ per day and higher is associated with a higher mortality rate from CVD (cardiovascular disease) and coronary heart disease, than an intake of 600-1000 mg/ per day (9). Calcium deficiency is observed mainly in teen-aged girls and in males, aged 9 to 18 and over the age of 51 (10). Researchers have found fourfold variation in calcium content in foliar litter among 14 ligneous species growing in a communal garden in central Poland; these variations have been associated with the soil change - pH, soil saturation, calcium substitutability, presence of parasites, etc. (11, 12).

Research has shown that water, having passed through some kinds of minerals, contains calcium ions (Ca^{+2}), the amounts depending on the content of calcium ions in these minerals. Thus, after passing through zeolite from the deposits next to the village of Beli Plast, Bulgaria, that are estimated as the largest in the world, the calcium ions concentration is 34.8 mg.L^{-1} (13). The presence of calcium ions in soils improves their quality (14). The herbs used in the present study had been grown in soils enriched with zeolite. An engineering method is applied in order to get information for accurate mass and permeability of water drops from medicinal plants, which contain calcium ions (Ca^{2+}) over 1000 mg /100 g.

The aim of the present study was to analyze the parameters of calcium ions (Ca^{2+}) and other associated parameters of 1% aqueous solutions of extracts from medicinal plants with the methods of Non-equilibrium Energy Spectrum (NES) and Differential Non-equilibrium Energy Spectrum (DNES). In Bulgaria there is an Act of Health allowing doctors, bachelors of medicine and specialists with necessary training to apply supplementary therapy with medicinal plants. The present work also covers quantitative indicators for calcium ions (Ca^{2+}) which can be used for determination of dosage in prevention and therapy of calcium deficiency in osteoporosis, muscle injuries, as well as during sports training and recovery.

Materials and Methods

Measurements of calcium ions (Ca2+) content in herbs according to Bulgarian legislation

In Bulgaria, amounts of metal ions in all kinds of substances are measured in licensed laboratories withn the overall legal framework including physico-chemical, microbiological, elemental composition. The measurements comply with the Bulgarian state standard and ISO (2, 3).

Drop dosage and transparency measurement

In our research, water drops were used with average mass of 48 mg (15). The concentration of investigated herbal extracts in deionized water was 1% v/v. For statistical purposes, each experimental sample (herbal solution) was measured 10 times together with 10 measurements of the control one (deionized water).

Non-equilibrium Energy Spectrum (NES) and Differential Non-equilibrium Energy Spectrum (DNES) Spectral Analysis

Measurements with the NES and DNES were conducted with a patented optical device (16-19). The process was based on evaporation of water drops on mylar foil lying on a glass plate in a hermetic chamber. The technical characteristics of the device were:

1. $\lambda = 580 \pm 7$ nm **–** wavelength of monochromatic light;

2. 72.3^0 to 0^0 **–** angle of evaporation of water drops;

3. ($+22–24^0$C) **–** range of the temperature of the evaporation of water drops;

4. E=-0.08– -0.1387eV;($\lambda =8.9–13.8$ μm)($\tilde{v} =1124$ **–**724.5 cm^{-1}) **–** parameters of the hydrogen bonds of water molecules in the spectral range of the device).

Parameters of NES and DNES water spectra

The *Energy distribution spectrum* was defined with the function f(E), because the process of evaporation of water drops was non-equilibrium. The method is called Non-equilibrium energy spectrum (NES), and it is a method for research of non-equilibrium process of water evaporation (16-19).

Luck views the water like consisting of O-H···O groups (20). Most of them are bonded by energy of the connection (-E) and the remaining are free (E=0). It is accepted that E has a negative value

This is called model of the two states of Luck (21-22). Each water molecule has two hydroxyl groups. The number of O-H···O groups in a certain volume of water is twice as big as the number of the molecules in it.

Part of hydrogen bonds is restructuring in the vicinity of the spherical part of the drop surface and as a result one obtains the dependence between the surface tension δ and the hydrogen bond energy (23-25):

$$\delta = -kT/\ln[1+\alpha/[\exp (-\beta E)+\alpha] \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Here, k is the Boltzman constant, $\beta=1/kT$, T – the absolute temperature, E – the hydrogen bond energy, α is the ratio of 2 subvolumes of the phase space related to structuring and restructuring of hydrogen bonds, α =28±8 and *I*=5.03. 10^{18} m^{-2} is density of water molecules at the hydrophobic surface layer.

The values of E and α are fixed by a comparison with experiment. Expression (1) explains fraction C of the actual surface tension γ, i.e. δ = Cγ (25). According to (23, 25) the contribution of non-hydrogen bond interaction

amounts to 20% of the true value of γ and C=4/5.

Consider the Helmholtz free surface energy F=γΣ where Σ is the spherical part of the drop's surface (23).

At the instant of mechanical equilibrium, F should be minimal, i.e. dF=0=d(γΣ) (23)

$$0 = \gamma\Sigma - \gamma_0\Sigma_0 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

The process of evaporation of water drops is at constant temperature 20 °C in a hermetic camera [2].

The expressions for Σ_0 and Σ are as follows (23)

$$\Sigma = \pi D^2/2(1+\cos\theta); \Sigma_0 = \pi D^2/2(1+\cos\theta_0) \ldots\ldots(3)$$

$$-E/kT = C\gamma/lkT \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(4)$$

$$E = C\gamma o(1+\cos\theta)/l(1+\cos\theta_0) \ldots\ldots\ldots\ldots\ldots.(5)$$

During the process, the wetting angle changes in discrete steps and characterizes the average energy of hydrogen bonds as follows:

$$\theta = \arccos(-1+bE), \text{ where } b = l(1+\cos\theta_0)/C\gamma o \ .. \ (6)$$

where θ is the wetting angle, E is the average energy of hydrogen bonds, b is a temperature-dependent parameter (17-19, 26).

Fig. 1. Illustrates water drop on mylar foil pad on glass plate for measurement of wetting angle of evaporation of water drop.
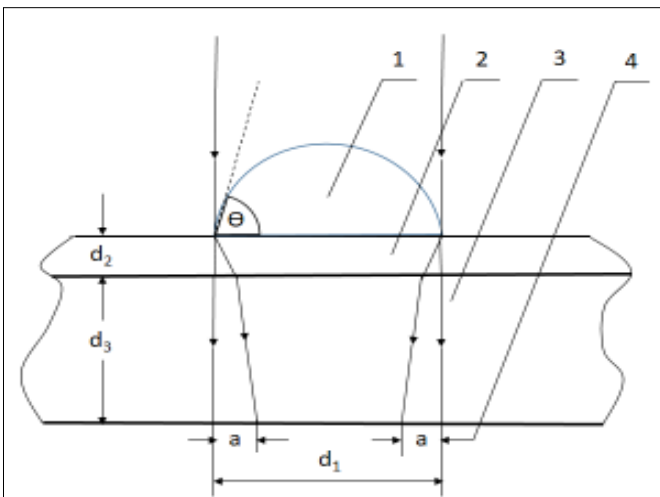

Fig. 1. Scheme of water drop on mylar foil pad on glass plate for measurement of wetting angle of evaporation of water drop. **1**-drop, **2** – thin mylar foil, **3** –glass plate, **4** – refraction ring width (**a**). The wetting angle θ is a function of **a** and **d₃**.

The relation between the *Energy distribution spectrum f(E)* in eV⁻¹ and f(θ) is:

$$f(E) = \frac{14,33\,f(\theta)}{[1-(1+bE)^2]^2} \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots(7)$$

The parameters of E are in eV, μm and cm⁻¹.

The Differential Non-equilibrium Energy Spectrum (DNES) is defined with the difference:

$$\Delta f(E) = f(experimental) - f(control) \ldots\ldots\ldots\ldots..(8)$$

DNES is a measure of modification of water structure as a result of a certain varied experimental factor. The overall effect of all other uncontrolled factors is the same for the experimental and the control samples.

The dimension of DNES is eV⁻¹.

Mathematical models and cluster analyses

Mathematical models show the number of water molecules in clusters (27, 28). The distribution is by hydrogen bond energies. Formulas are used in the calculation that show the possible number of water molecules at certain hydrogen bond energies. Each molecule has 2 hydrogen bonds from the hydrogen atoms to 2 oxygen atoms from other molecules. There are also 2 hydrogen bonds from 1 oxygen atom to hydrogen atoms from other molecules. Thus, each water molecule can bond with 4 other molecules. Hydrogen bonds are electromagnetic and display quantum probabilistic behavior. That is why, entropy as a measure of chaos and order in a given system can be applied for analysis of their energy distribution. The metric of transformational information entropy (29, 30) has been proposed for information theoretical analysis of NES and DNES spectra.

In *Haberlea rhodopensis* Friv., there has been evidence of 2-molecule water clusters.

Haberlea rhodopensis Friv. is a Balkan endemic plant found primarily in Rhodope mountains in Bulgaria (Fig. 2). It is known for its drought resistance and biosis-anabiosis-biosis cycle (31). The biosis-anabiosis-biosis cycle could reveal new properties of the water. A study has shown that, during the drying process of *H. rhodopensis,* the number of free water molecules decreases and water dimers are formed (32).

Fig. 2. Flowers of *Haberlea rhodopensis.*

Mathematical models of water molecules clustering in aqueous solutions have been developed (33). In 2021 a cluster model was proposed at (E = -0.1387 eV) (λ = 8.95 µm) ($\tilde{v}$ = 1117 cm^{-1}) with 20 water molecules arranged in a dodecahedral structure with 0.822 nm diameter of the circumscribed sphere (20, 21). In the present investigation, both of the above findings were used. The number of water molecules at (E = -0.1387 eV) (λ = 8.95 µm) ($\tilde{v}$ = 1117 cm^{-1}) of *Hypericum perforant* L. are 11 (34).

Table 1 illustrates the average distributions at different hydrogen bond energies in the *Thymus vulgaris* L. solution and the deionized water. For each energy level where at least one of the average experimental or control values is nonzero, both values are different according to Student's t-test at P<0,05.

Mathematical models, using percentage distribution in the medicinal plants, were established on the basis of Ignatov's method. The plants are as follows: *Sambucus nigra* L. (35), *Sideritis scardica* Griseb. (Mursalki tea; Pirin Tea) (36), *Moringa oleifera* Lam. (36), *Urtica dioica* L.*, Malva sylvestris* L.*, Plantago major* L. (37). Pharmacological properties are also described (38).

In the 6 herbs studied, an analysis at the local maximum of (E = -0.1112 eV) (λ = 11.15 µm)   ($\tilde{v}$ = 897 cm^{-1}) was applied and it was associated with calcium ions content (Ca^{2+}) higher than 1000 mg/100 g.

Herbs that contain Calcium

Some herbs and vegetables can cause bone mass loss if consumed excessively over a long period of time. Spinach,

Table 1. Average distributions at different hydrogen bond energies and numbers of water molecules in clusters for *Thymus vulgaris* L. (red color) and deionized water (blue color).

-E(eV) x-axis	*T. vulgaris* L. y-axis (%((-E$_{value}$) */ (-E$_{total\ value}$)**	Control Sample of Deionized Water (%((-E$_{value}$)*/ (-E$_{total\ value}$)**	-E(eV) x-axis	*T. vulgaris* L. y-axis (%((-E$_{value}$) */ (-E$_{total\ value}$)**	Control Sample of Deionized Water (%((-E$_{value}$)*/ (-E$_{total\ value}$)**
0.0937	0	0	0.1187	0	11.3
0.0962	16.3	2.1	0.1212	16.3[2]	4.6
0.0987	16.3	0	0.1237	5.8	9.1[2]
0.1012	0	0	0.1262	0	11.3
0.1037	0	11.3	0.1287	0	4.6
0.1062	0	9.1	0.1312	0	0
0.1087	5.8	0	0.1337	5.8	9.1
0.1112	11.6[1]	4.6[1]	0.1362	5.8	4.6
0.1137	0	9.1	0.1387	16.3[3]	4.6[3]
0.1162	0	4.6	–	–	–

Table 2 and Fig. 3 show average numbers of H$_2$O molecules in cluster in the *T. vulgaris* L. solution and those in the deionized water control sample. For each energy level where at least one of the average experimental or control values is nonzero, both values are different according to Student's t-test at P<0,05.

sorrel, dock leaves (not root) contain oxalic acid, which binds calcium and interferes with calcium absorption (39). If bone mass loss is a problem, these herbs and vegetables should be avoided. The herbs described below contain calcium; they are widely available in Bulgarian flora and could also be used for prevention of calcium deficiency.

Table 2. Average numbers of water molecules in clusters for the *Thymus vulgaris* L. solution (red color) and the deionized water control sample (blue color).

-E(eV) x-axis	*T. vulgaris* L. y-axis Water number H$_2$O	Control sample of deionized water number H$_2$O	-E(eV) x-axis	*T. vulgaris* L. y-axis Water number H$_2$O	Control sample of deionized water number H$_2$O
0.0937	0	0	0.1187	0	12
0.0962	16	3	0.1212	15[2]	5
0.0987	16	0	0.1237	6	9[2]
0.1012	0	0	0.1262	0	12
0.1037	0	12	0.1287	0	0
0.1062	0	9	0.1312	0	0
0.1087	6	0	0.1337	6	9
0.1112	12[1]	5[1]	0.1362	6	5
0.1137	0	9	0.1387	17[3]	5[3]
0.1162	0	5	–	–	–

They are easily recognizable and anyone could collect them from nature or cultivate them at home (40-44).

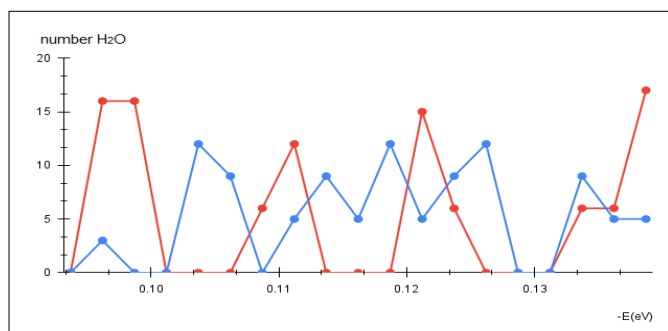**Fig. 3.** Number of H_2O molecules in clusters in *T. vulgaris* L. solution (red color) and in the deionized water control sample (blue color).

Origanum vulgare L. – Oregano

The content of calcium is 1990 mg/100 g

Oregano (*Origanum vulgare*) is a perennial herb. It grows in bushes and clearings, in stony places and in sparse forests, mainly in the foothills and mountains throughout the country up to 1600 m above the sea level.

It forms a tuft in which 80-100 stems can be counted and new stems emerge every year. It reaches a height of 80 cm and the tops of the plants branch out and form umbrella-like inflorescences, in which a large number of small flowers with pale pink, pink or almost white color with brown cups are collected

Thymus vulgaris L. – Thyme

The content of calcium is 1890 mg/100 g

In Bulgaria there are species of this genus that are difficult to be distinguished from each other. Thyme is a semi-shrub, about 30 cm high, forming tufts. Its above-ground part is used, which contains essential oil, flavonoids, tannins etc. Its main effect is expectorant, anti-inflammatory, anti-spasmodic, anti-bacterial.

Mentha longifolia L. – Mint

The content of calcium is 1488 mg/100 g

Perennial herb under cultivation. Many subspecies, varieties and forms are known from mint. Leaves rich in essential oil, tannins and bitter substances are used.

Petroselinum crispum (Mill.) Fuss– Parsley

The content of calcium is 1465 mg/100 g

Fuss is a biennial herb under cultivation plant with a spindle-shaped root, which is mainly used as a spice. Its fruits and roots are used for medical purposes. All parts of the plant contain essential oil, flavonoids. Action - diuretic and antispasmodic. Parsley juice increases stomach acidity. Two types of parsley are used as a spice - curly and Italian, also known as plain or smooth. Dried parsley contains 1468 mg/100 gms and fresh parsley - 203 mg/100 g calcium.

Sideritis scardica Griseb.– Mursalski (Pirinski) Tea

Sideritis scardica Griseb. (Mursalski tea; Pirinski tea) is a herb growing in the Pirin mountains and the Mursal area of the Rhodopes mountains (Fig. 4). The herb grows also in

Northern Macedonia and northern region of Greece.

The content of calcium is 1465 mg/100 g

Pirin tea *(Sideritis scardica)* is a perennial herb with a height of 50 cm. The flowers are yellowish, located vertebrally in the axils of lemon-yellow, membranous bracts in spike-like inflorescences. It is also known as Mursalski or Pirin tea. The specific epithet *scardica* comes from *Scardus*, the Latin name of Shar Mountain, North Macedonia, where it has been found

Fig. 4. Pirin (Mursalski) tea (*Sideritis scardica* Griseb.) collected in Bulgaria.

Two Bulgarian researchers, Yaneva and Balabanski as well, have studied their healing properties. The outcomes of their research work have been cited by the European Medicines Agency, document EMA/HMP /39455/2015 Committee on Herbal Medicinal Products (HMPC).

Salvia officinalis L. – sage

The content of calcium is 1010 mg/100 g

Salvia officinalis L. is a small evergreen shrub, thatis cultivated in Bulgaria. The leaves are used, as they are rich in essential oils, tannins, alcohols, vitamins (B_1 and C), carotene. The main effect is anti-inflammatory. The content of sodium, potassium, magnesium, calcium, iron, manganese, zinc and copper is determined in dried sage leaves.

Results and Discussion

Table 3 shows the content of calcium (Ca^{2+}) as well as the average NES values and numbers of water molecules in clusters at the energy level of (-E = 0.1112 eV) (λ = 11.15 μm) ($\tilde{v}$ = 897 cm^{-1}) in the investigated solutions of the medical plants - *Origanum vulgare* L. *Thymus vulgaris* L. *Mentha longifolia* L. *Petroselinum crispum* Mill. *Sideritis scardica* Griseb. *Salvia officinalis* L.

Taking into account the experimental error of ± 1% for the NES and DNES methods, the NES values and numbers of water molecules in clusters at the above-mentioned energy level are significantly lower in solutions of other plants with lower calcium content. The following medicinal plants have the following content of calcium (Ca^{2+}) – *Debregeasia longifolia* (117.77±1.92), followed by *Cissus adnata* (77.78±1.92), *Oenanthe javanica* (66.66±0.01

Table 3. Content of calcium (Ca^{2+}), average NES values and numbers of water molecules in clusters at the energy level of (-E = 0.1112eV) (λ = 11.15μm) (ṽ = 897 cm^{-1}) in the investigated solutions.

Sl. No.	Herb	Content of Calcium (Ca^{2+})(mg/100 g)	(E=0.1112eV) (λ=11.15μm) (ṽ=897 cm^{-1}) (%((-E$_{value}$) */ (-E$_{totalvalue}$)**	(E=0.1112eV) (λ=11.15μm) (ṽ=897 cm^{-1}) Number of H$_2$O molecules
1	*Origanum vulgare* L.	1990	14.4	15
2	*Thymus vulgaris* L.	1890	11.6	12
3	*Mentha longifolia* L.	1488	9.5	10
4	*Petroselinum crispum* Mill.	1468	9.2	9
5	*Siderritis scardica* Griseb.	1465	10.5	11
6	*Salvia officinalis* L.	1010	11.5	12

* The result (-E$_{value}$) is the result of hydrogen bonds energy for one parameter of (-E), ** The result (-E$_{total\ value}$) is the total result of hydrogen bonds energy

mg/100g) (44, 45). The results with spectral analysis with method NES are – *Debregeasia longifolia* (0.87), followed by *Cissus adnata* (0.62), *Oenanthe javanica* (0.52).

It should also be pointed out that the above findings are in line with earlier works on information theoretical characteristics of plant extracts (29, 30, 46) where calcium content was shown as a distinct characteristic feature.

In addition, dependence of activation of the mitochondrial calcium uniporter on natural plant flavonoids had been found. Cell stimulation often triggers an increase in Ca^{2+}]$_c$ (cytosolic [Ca^{2+}]), either through activation of plasma membrane Ca^{2+} channels or through release of Ca^{2+} from intracellular stores, mainly the ER (endoplasmic reticulum) (47). In the synthesis of flavonoids the starting compound is phenylalanine. Phenilalanine and tyrosine are derivates of lignan. The enzyme phenylalanine ammonia-lyase converts phenylalanine to cinnamic acid (36). The quality of water influences on the effects of phenylalanine. The model system was Microbial synthesis of deuterium labelled L-phenylalanine with different levels of isotopic enrichment by facultative methylotrophic bacterium *Brevibacterium methylicum* (48-50).

All the above findings and considerations point to possible fundamental interplay of hydrogen bond networks in water and ions dissolved in it during biochemical processes.

Conclusion

The present extended study of 6 medicinal plants with high calcium content has confirmed the findings of previous pilot studies concerning the distinct effect of calcium ions on hydrogen bonds energy distribution in aqueous solutions. Thus, the investigation of 1% aqueous solutions of extracts from different plant species containing various solutes apart from calcium ions, has identified a particular hydrogen bonds energy level of (-E = 0.1112 eV) (λ = 11.15 μm) (ṽ = 897 cm^{-1}) that is directly influenced by calcium ion content. This result could be used for further elucidation of

the possible interplay of hydrogen bond networks in water and the ions dissolved in it during biochemical processes as well as for development of new *in vivo* spectral methods for neurological diagnostics.

Authors contributions

All authors contributed equally.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

References

1. Sustainable Herbal Harvest in Bulgaria, Public Awareness Campaign, The project is implemented by "Information and Nature Conservation" Foundation, Bulgarian National Radio and "Eco-Logic consultancy". Co-funded by The European Commision's financial instrument for the environment – Life+. 2021.

2. Law on medicinal plants. Prom. DV. No. 29. April 7. 2000. [in Bulgarian].

3. Law on medicinal products in human medicine, Prom. SG No. 31. April 13, 2007.

4. Dolphin AC, Lee A. Presynaptic calcium channels: specialized control of synaptic neurotransmitter release. Nature Review Neuroscience. 2020;21:213-25. https://doi.org/10.1038/s41583-020-0278-2

5. Committee to review dietary reference intakes for vitamin D and calcium, food and nutrition board, institute of medicine. Dietary reference Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press. 2010.

6. Dalley DK, Marie SB. Chapter One - Extra-skeletal effects of dietary calcium: Impact on the cardiovascular system, obesity and cancer. Advances in Food and Nutrition. 2021; 96:1-25.

https://doi.org/10.1016/bs.afnr.2021.02.012

7. Liu X, Pan Z, Store-operated Calcium entry in the cardiovascular system. Ion Channels in Biophysics and Physiology. 2022; 303-30. https://doi.org/10.1007/978-981-16-4254-8_14

8. Ignatov I, Angelcheva M, Angushev I. Doses of Ca^{2+}, Mg^{2+}, K^+, Na^+, Mn^{2+} and Zn^{2+} in mountain spring and mineral waters in hydration reaction during sport training and recovery; combination with vitamins. Journal of Pharmaceutical Research International. 2021; 33(16):76-85. https://doi.org/10.9734/jpri/2021/v33i1631299

9. Michalsson K, Melhus H, Lemming E, Walk A, Byberg L. Long term calcium intake and rates of all cause and cardiovascular mortality: community based prospective longitudinal cohort study. BMJ. 2013; 346:f228. https://doi.org/10.1136/bmj.f228

10. Cashman KD. Calcium intake, calcium bioavailability and bone health. British Journal of Nutrition. 2002; 87(2):S169-S177. https://doi.org/10.1079/BJN/2002534

11. Dauer JM, Chorover EJ, Oliver E, Chadwick A, Oleksyn EJ, Mark JE et al. Controls over leaf and litter calcium concentrations among temperate trees. Biogeochemistry. 2007; 86:175-87. https://doi.org/10.1007/s10533-007-9153-8

12. Raczuk JE, Biardzka B, Daruk J. The content of Ca, Mg, Fe and Cu in selected species of herbs and herb infusions. Rocz Panstw Zakl Hig. 2008; 59(1):33-40.

13. Popova TP, Ignatov I, Valcheva N, Ignatov AI. Research of zeolite and zeolite water from Rhodope mountains, Bulgaria. Journal of the Turkish Chemical Society Section A: Chemistry. 2022; 9 (3):901-08. https://doi.org/10.18596/jotcsa.1058556

14. Bhattacharyya T, Chandran P, Ray SK, Pal DK, Mandal C, Mandal DK. Distribution of zeolitic soils in India. Current Science. 2015; 189(7):1305-13. https://doi.org/10.18520/v109/i7/1305-1313

15. Mehandjiev D, Ignatov I, Neshev N, Huether F, Gluhchev G, Drossinakis CH. Formation of clusters in water and their distribution according to the number of water molecules. Bulgarian Chemical Communications. 2022; 54(3).

16. Antonov A. An optical method version for determination of the welling angle of liquids. Comptes Rendus de l'Académie Bulgare des Sciences. 1984;37:1199. https://doi.org/10.1136/jcp.37.10.1199-b

17. Jelev J, Antonov A, Galabova T. Method and device for evaluation of bio-psycho-physical influence of radio, television and Media Products Upon Humans. US20070276192A1; 2003.

18. Antonov A. Research of the non-equilibrium processes in the area in allocated systems. Dissertation thesis for DSc, Blagoevgrad, Sofia. 1995;1-254.

19. Todorov S, Damianova A, Sivriev I, Antonov A, Galabova T. Water energy spectrum method and investigation of the variations of the H-bond structure of natural waters. Comptes Rendus de l 'Academie Bulgare des Sciences. 2008; 61(7):857-62.

20. Luck W. American Chemical Society. In Water in Polymers, ed. By S. Rowland. 1980; 50.

21. Aparicio-Martínez S, Hal KR.. Phase equilibria in water containing binary systems from molecular based equations of state. 2007; 112-25. https://doi.org/10.1016/j.fluid.2007.02.030

22. Clark G, Haslam A J, Galindo A, Jackson G., Developing optimal Wertheim-like models of water for use in statistical associating fluid theory (SAFT) and related approaches Mol Phys. 2006; 104 (22–24):3561-81. https://doi.org/10.1080/00268970601081475

23. Todorova L, Antonov A. Note on the drop evaporation method for study of water bond distribution I. An application to filtration. Comptes Rendus de l 'Academie Bulgare des Sciences.2000; 53(7):43-46.

24. Zhang BJ, Kim KJ, Lee CT. Experimental study of subcooled flow boiling heat transfer on micro-pin-finned surfaces in short-term microgravity. Experimental Thermal and Fluid Science. 2018; 97:417-30. https://doi.org/10.1016/j.expthermflusci.2018.05.003

25. Luzar A, Svetina S, Žekš S B. The contribution of hydrogen bonds to the surface tension of water. Chemical Physics Letters. 1983; 96(4):485-90. https://doi.org/10.1016/0009-2614(83)80737-4

26. Kumbharkhane AC, Joshi YS, Mehrotra SC, Yagihara SH, Sudo S. Study of hydrogen bonding and thermodynamic behavior in water–1,4-dioxane mixture using time domain reflectometry. Physica B: Condensed Matter. 2013; 421:1-7. https://doi.org/10.1016/j.physb.2013.03.040

27. Ignatov I, Mosin OV. Structural mathematical models describing water clusters. Journal of Mathematical Theory and Modeling. 2013; 3(11):72-87.

28. Keutsch FN, Saykally RJ. Water clusters: Untanding the mysteries of the liquid, one molecule a time. PNAS. 2001; 98 (19):10533-540. https://doi.org/10.1073/pnas.191266498

29. Neshev N, Ignatov I, Drossinakis CH. Measurement of hydrogen bond energies in some selected plants with medicinal properties and their information theoretical analysis, Plant Cell Biotechnology and Molecular Biology. 2021; 22(45-46):79-94.

30. Ignatov I, Neshev N, Popova TP, Kiselova-Kaneva Y, Drossinakis CH, Bankova R et al. Theoretical analysis of hydrogen bonds energy distribution and information in a 1 % Rosa damascena Mill Oil Solution, Plant Science Today. 2022; 9(3):760-65. https://doi.org/10.14719/pst.1645

31. Markovska YK, Tsonev TSD, Kimenov GP, Tutekova AT. Physiological changes in higher poikilohydric plants – *Haberlea rhodopensis* Friv. and *Ramonda serbica* Panc. during drought and rewatering at different light regimes. Journal of Plant Physiology. 1994; 144:100-08. https://doi.org/10.1016/S0176-1617(11)81000-X

32. Kuroki SH, Tsenkova R, Moyankova D, Munkan J, Morita H, Atanassova, Djilianov D. Water molecular structure underpins extreme desiccation tolerance of the resurrection plant *Haberlea rhodopensis*. Scientific Reports. 2019; 9:3049. https://doi.org/10.1038/s41598-019-39443-4

33. Ignatov I, Gluhchev G, Neshev N, Mehandjiev D. Structuring of water clusters depending on the energy of hydrogen bonds in electrochemically activated waters Anolyte and Catholyte. Bulgarian Chemical Communications. 2021;53(2):234-39.

34. Ignatov I, Popova TP, Bankova R, Neshev N. Spectral analyses of fresh and dry *Hypericum perforant* L. Effects with colloidal nano silver 30 ppm. Plant Science Today. 2022; 9(1):41-47. https://doi.org/10.14719/pst.1429

35. Ignatov I, Popova T, Yaneva I, Balabanski V, Baiti S, Angelcheva M, Angushev I. Spectral analysis of *Sambucus nigra* L. fruits and flowers for elucidation of their analgesic, diuretic, anti-inflammatory and anti-tumor effects. Plant Cell Biotechnol Mol Biol J. 2021;22(29-30):134-40.

36. Ignatov I. Sideritis scardica Griseb. (Mursalski tea; Pirinski Tea) from Bulgaria, Which is growing in zones with high percent of long living people. Plant Cell Biotechnology and Molecular Biology. 2021; 22(35-36):141-53.

37. Ignatov I, Popova T. Applications of *Moringa oleifera* Lam., *Urtica dioica* L., *Malva sylvestris* L. and *Plantago major* L. Containing potassium for recovery. Plant Cell Biotechnol Mol Biol J. 2021; 22(7-8):93-103.

38. Yaneva I, Balabanski V, Karanesheva T, Ignatov I. Some endangered healings plants in Bulgaria – legislative regulation, protection, characteristic description, application, agricultural cultivation. Bulgarian Journal of Agricultural Science. 2020; 26 (4):847-57.

39. Fasinu PS, Bello OE, Ogbesejana AB, Adetunji CO, Dada AO, Bello OM et al. Wild vegetable *Rumex acetosa* Linn.: Its ethnobotany, pharmacology and phytochemistry – A review. South African

Journal of Botany. 2019; 125:149-60. https://doi.org/10.1016/j.sajb.2019.04.018

40. Moazedi AA, Mirzaie DN, Seyyednejad SM, Zadkarami MR, Amirzargar A. Spasmolytic effect of *Petroselinum crispum* (Parsley) on rat's ileum at different calcium chloride concentrations. Pakistan Journal of Biological Sciences. 2007;10(22):4036-42. https://doi.org/10.3923/pjbs.2007.4036.4042

41. HMPC monograph: Overview of recommendation European Medicines Agency. 24th July 2018EMA/HMPC/228356/2012, Committee on Herbal Medicinal Products (HMPC); Last adaptation. 2018.

42. European Medicines Agency, Assessment report on *Sideritis scardica* Griseb., *Sideritis clandestina* (Bory & Chaub.) Hayek; *Sideritis raeseri* Boiss. & Heldr.; *Sideritis syriaca* L., herba, *EMA/HMPC/39455/2015* Committee on Herbal Medicinal Products (HMPC).

43. European Medicines Agency, London, 12 of November 2009.

44. Elbahnasawy AS, Valeeva ER, El-Sayed EM, Rakhimov II. The impact of Thyme and Rosemary on prevention of osteoporosis in rats. Journal of Nutrition and Metabolism. 2019. Article ID 1431384 https://doi.org/10.1155/2019/1431384

45. Devi OA, Das M, Saikia A, Das P, Sharma D. Evaluation of total mineral, calcium, selenium, iron content of ten medicinal plants extracts of Manipur having anti-inflammatory properties. Journal of Medicinal Plants Studies. 2016; 4(3):189-94.

46. Ignatov I, Entropy and time in living organisms. Archiv Euromedica, 1st & 2nd Edition. 2012; 74.

47. Rizzuto R, Marchi S, Bonora M, Aguiri P, Bononi A, De Stefani D et al. Ca^{2+} transfer from the ER to mitochondria: When, how and why. Biochimica and Biophisica Acta (BBA)-Bioenergetics. 2009; 1787(11):1342-51. https://doi.org/10.1016/j.bbabio.2009.03.015

48. Zhang F, Ren J, Zhan J. Identification and characterization of an efficient Phenylalanine ammonia-lyase from *Photorhabdus luminescens.* Applied Biochemistry and Biotechnology. 2021;193:1099-115. https://doi.org/10.1007/s12010-020-03477-6

49. Mosin OV, Shvets VI, Skladnev DA, Ignatov I. Microbial synthesis of deuterium labelled L-phenylalanine with different levels of isotopic enrichment by facultative methylotrophic bacterium *Brevibacterium methylicum* with RMP assimilation of carbon. Biomeditsinskaia Khimiia. 2014; 60(4):448-61. https://doi.org/10.18097/pbmc20146004448

50. Mosin OV, Shvets VI, Skladnev DA, Ignatov I. Microbial synthesis of ^2H-labelled *L*-phenylalanine with different levels of isotopic enrichment by a facultative methylotrophic bacterium *Brevibacterium methylicum* with RuMP assimilation of carbon. Supplement Series B: Biomedical Chemistry. 2013; 7(3):247-58. https://doi.org/10.1134/S1990750813030098

§§§