Application of infrared spectral analyses for medicinal plants containing calcium (Ca\(^{2+}\))

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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 (Ca\(^{2+}\)) 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) (\(\lambda = 11.15 \, \mu\)m) (\(\nu = 897 \, \text{cm}^{-1}\)) 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 (Ca\(^{2+}\))

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
apese, 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/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/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 herbs, bachelors of medicine and specialists with necessary training to apply supplementary therapy with medicinal plants. The present work also covers quantification of dosage in prevention and therapy 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.

Materials and Methods

Measurements of calcium ions (Ca\(^{2+}\)) content in herbs according to Bulgarian legislation

In Bulgaria, amounts of metal ions in all kinds of substances are measured in licensed laboratories within 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\(^\circ\) to 0\(^\circ\) – angle of evaporation of water drops;
3. \((+22-24^\circ C)\) – range of the temperature of the evaporation of water drops;
4. \(E= - 0.08 - 0.1387 eV; (\lambda = 8.9-13.8 \mu m); (\nu = 1124-724.5 \ cm^{-1})\) – 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 \(\delta\) and the hydrogen bond energy (23-25):

\[
\delta = \kappa T \ln [1 + \alpha / (\exp(-\beta E) + \alpha)] \quad \text{.................................. (1)}
\]

Here, \(k\) is the Boltzman constant, \(\beta=1/kT\), \(T\) – the absolute temperature, \(E\) – the hydrogen bond energy, \(\alpha\) is the ratio of 2 subvolumes of the phase space related to restructuring and restructuring of hydrogen bonds, \(\alpha=28\times8\) and \(I=5.03.10^{18}\) m\(^2\) is density of water molecules at the hydrophobic surface layer.

The values of \(E\) and \(\alpha\) are fixed by a comparison with experiment. Expression (1) explains fraction C of the actual surface tension \(\gamma\), i.e. \(\delta = C \gamma\) (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 $F=\gamma \Sigma$ where $\Sigma$ is the spherical part of the drop’s surface (23).

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

\[ 0=\gamma \Sigma - \gamma_0 \Sigma_0 \]  \hspace{1cm} (2)

The process of evaporation of water drops is at constant temperature 20 °C in a hermetic camera [2]. The expressions for $\Sigma_0$ and $\Sigma$ are as follows (23)

\[ \Sigma = \pi D^2/2(1+\cos \theta) \]  \hspace{1cm} (3)

\[ -E/kT= C\gamma/kT \]  \hspace{1cm} (4)

\[ E=C\gamma_0(1+\cos \theta)/l(1+\cos \theta_0) \]  \hspace{1cm} (5)

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

\[ \theta=\arccos(-1+bE), \text{ where } b= l(1+\cos \theta_0)/C\gamma_0 \]  \hspace{1cm} (6)

where $\theta$ 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.

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

\[ \Delta f(E)=f(\text{experimental}) - f(\text{control}) \]  \hspace{1cm} (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 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).

The relation between the Energy distribution spectrum $f(E)$ in $eV$ and $f(\theta)$ is:

\[ f(E) = \frac{14.33 f(\theta)}{[1-(1+bE)^2]^2} \]  \hspace{1cm} (7)

The parameters of $E$ are in $eV$, $\mu$m and $cm^{-1}$.

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 \((\lambda = 8.95\) μm\) \((\bar{v} = 1117\) 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 \((\lambda = 8.95\) μm\) \((\bar{v} = 1117\) cm\(^{-1}\)) of Hypericum perforan 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 \(P<0.05\).

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).

| \(-E(\text{eV})\) x-axis | \(T.\ vulgaris\ L.\ y-axis\) | Control Sample of Deionized Water | \(-E(\text{eV})\) x-axis | Control Sample of Deionized Water |
|-------------------------|-----------------|-----------------|-----------------|-----------------|
|                         | \((\%(-E_{\text{total}})/(-E_{\text{cluster}}))^\times100\%\) | \((\%(-E_{\text{total}})/(-E_{\text{cluster}}))^\times100\%\) | \((\%(-E_{\text{total}})/(-E_{\text{cluster}}))^\times100\%\) | \((\%(-E_{\text{total}})/(-E_{\text{cluster}}))^\times100\%\) |
| 0.0937                  | 0               | 0               | 0.1187          | 0               |
| 0.0962                  | 16.3            | 2.1             | 0.1212          | 16.3\(^1\)      | 4.6
| 0.0987                  | 16.3            | 0               | 0.1237          | 5.8
| 0.1012                  | 0               | 0               | 0.1262          | 0               |
| 0.1037                  | 0               | 11.3            | 0.1287          | 0               |
| 0.1062                  | 0               | 9.1             | 0.1312          | 0               |
| 0.1087                  | 5.8             | 0               | 0.1337          | 5.8             |
| 0.1112                  | 11.6\(^1\)     | 4.6\(^1\)      | 0.1362          | 5.8             |
| 0.1137                  | 0               | 9.1             | 0.1387          | 16.3\(^1\)     |
| 0.1162                  | 0               | 4.6             | –               | –               |

Table 2 and Fig. 3 show average numbers of \(\text{H}_2\text{O}\) 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 \(P<0.05\).

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).

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

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. \(25\), 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 \((\lambda = 11.15\) μm\) \((\bar{v} = 897\) cm\(^{-1}\)) was applied and it was associated with calcium ions content \((\text{Ca}^{2+})\) 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, 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.

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

**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 anti-spasmodic. 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 axis 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.

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, that, is cultivated in Bulgaria. The leaves are used, as they are rich in essential oils, tannins, alcohols, vitamins (B1 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 = 11.15μm) (σ = 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 (117.7±1.92), followed by Cissus adnata (77.78±1.92), Oenanthe javanica (66.66±0.01

![Fig. 3. Number of H\(_2\)O molecules in clusters in T. vulgaris L. solution (red color) and in the deionized water control sample (blue color).](image)

![Fig. 4. Pirin (Mursalski) tea (Sideritis scardica Griseb.) collected in Bulgaria.](image)
mg/100g) (44, 45). The results with spectral analysis with method NES are – *Debregeasia longifolia* (0.87), followed by *Cissus adnata* (0.62), *Denanthe 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$^{2+}$; (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. Phenylalanine 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.

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### 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.

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