PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 9(1): 41–47
https://doi.org/10.14719/pst.1429

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RESEARCH ARTICLE

Spectral analyses of fresh and dry *Hypericum perforatum* L. Effects with colloidal nano silver 30 ppm

Ignat Ignatov[1*], Teodora P. Popova[2], Ralitsa Bankova[3] & Nikolai Neshev[4]

[1] Scientific Research Center of Medical Biophysics (SRCMB), Sofia 1111, Bulgaria
[2] Department of Infectious Pathology, Hygiene, Technology and Control of Food of Animal Origin, Faculty of Veterinary Medicine,University of Forestry, 10 Kl. Ohridski Blvd., Sofia 1756, Bulgaria
[3] Department of Internal Noncommunicable Diseases, Pathhology and Pharmacology, Faculty of Veterinary Medicine, University of Forestry, 10 Kl. Ohridski Blvd., Sofia 1756, Bulgaria
[4] Faculty of Physics, Sofia University "St. Kliment Ohridski", Sofia 1000, Bulgaria

*Email: mbioph@abv.bg

OPEN ACCESS

ARTICLE HISTORY
Received: 25 September 2021
Accepted: 21 November 2021

Available online
Version 1.0 : 01 January 2022

Check for updates

Additional information

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

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

Abstract

Spectral analyses of 1% water extracts of fresh and dry *Hypericum perforatum* L. and 1% dry *H. perforatum* with colloidal nano silver (NSPs) 30 ppm were conducted. The nano silver is standardised and patented by the Swiss company Evodrop. Non-equliblrium energy spectrum (NES) and Differential non-equliblrium energy spectrum (DNES) methods were used for the spectral analysis. A comparative analysis of 1% extracts of fresh and dry *H. perforatum* was performed in order to determine the local extremums for effects of nerve tissue conductivity at (-0.1112) eV, anti-inflammatory (-0.1212) eV, anti-tumor effects (-0.1387) eV. The results showed stimulating effect on the nervous system and improvement of nerve conduction (local extremums E= -0.1112 eV)(λ=11.15 μm) ($\tilde{\nu}$=897 cm^{-1}), as well as anti-inflammatory effect (E = -0.1212 eV)(λ=10.23 μm) ($\tilde{\nu}$=978 cm^{-1}) and inhibition of development of tumor cells at a molecular level (E=-0.1387 eV) (λ=8.95 μm) ($\tilde{\nu}$=1117 cm^{-1}). It was found that clusters of 16 and 15 water molecules are formed in the water herbal extracts of fresh *H. perforatum* and of dry *H. perforatum* with AgNPs 30 ppm. The fresh plant showed better results then the dry one. The addition of colloidal nano silver 30 ppm led to better results of the drug.

Keywords

Hypericum perforatum, colloidal nano silver, anti-inflammatory action, NES, DNES

Introduction

The application of herbs by doctors and pharmacists is a widespread practice worldwide, including in Bulgaria, which is also a part of the national culture. Nowadays, in our country It is regulated by the Act of Health of 2005. In recent decades, foreign plants and products thereof have also been used more and more frequently.

Hypericum perforatum L. (St. John's wort), is a herbaceous perennial plant which grows in Europe, West Asia and North Africa. This herb is also grown in South Africa, Australia, New Zealand, India and South America and it is used as a medicinal plant there. In Bulgaria, it grows all over the country in the lowlands - on grassy places and along roads (1). In our folk medicine, St. John's wort is among the most commonly used herb. It is applied mainly as a means of treating a number of gastrointestinal diseases (gastric and duodenal ulcers with hyperacidity, acute and chronic gastritis, enterocolitis, indigestion, diarrhea, stomatitis, periodontitis, gingivitis, pharyngitis, etc.) (2). The

herb has a healing effect on wounds and is used at contaminated purulent and slow-healing wounds on the skin and mucous membranes, cuts, abrasions and burns. The flavonoids in the herb give it a capillarotonizing effect, so it is applied in diseases accompanied by bleeding, inflammation, swelling and more. such as hemorrhoids, heavy menstruation, rheumatism, inflammation of the bladder, colitis and others. The herb has a sedative effect and is also useful in patients with depression and neurological suffering (2, 3). The aboveground parts of *H. perforatum* (leaves, stem and flowers) are used, which are picked during flowering known as *Hyperici herba*. The products containing St. John's wort are in the form of tablets, capsules, teas, tinctures, alcohol and oil extracts.

St. John's wort is among the most studied medicative plants worldwide due to its rich chemical content and curative properties. The biologically active substances (BAS) present in it can be classified into several groups as: phloroglucinols; naphtodianthrons; flavonoids, procyanidins, tannins and related substances; simple phenolic compounds (phenylpropanoids and others); xanthones and volatile substances (Table 1) as well as essential oil (3, 4).

Table 1. Bioactive compounds present in dried *Hyperici herba* [3, 4]

0	Compounds
Phloroglucinols	Hyperforin; Adhyperforin; Hyperfirin; Adhyperfirin
Naphthodianthrones	Hypericin; Protohypericin; Pseudohypericin; Protopseudohypericin; Cyclopseudohypericin
Flavonoids, procyanidins, tannins, and related compounds	Quercetin; Quercitrin; Isoquercetrin; Hyperoside; Rutin; Miquelianin; Guaijaverin; Avicurarin; Astilbin; Quercetin; 3-O-(2”-acetyl)-β-dgalactopyranoside; Kaempferol; Nicotiflorin; I3,II8-Biapigenin; Amentoflavone; Amentoflavone; Procyanidin B2; Isoorientin; Cyanidin 3-O-α-rhamnoside; Catechin; Epicatechin; Epigallocatechin
Phenylpropanoids and other simple phenolic compounds	Neochlorogenic acid; Chlorogenic acid; Cryptochlorogenic acid; Caffeic acid; p-Coumaric acid; 3-O-(Z)-P-coumaroylquinic acid; 3-O-(E)-P-coumaroylquinic acid; p-Hydroxybenzoic acid; Ferulic acid; Protocatechuic acid; Vanillic acid
Xanthones	Mangiferin, 1,3,6,7-Tetrahydroxyxanthone
Volatile compounds	Spathulenol; Caryophyllene oxide; Viridiflorol; β-Caryophyllene; 1-Tetradecanol; β-Funebrene;1-Dodecanol; γ-Muurolene;

The main compound answerable for the antidepressant effect of *H. perforatum* is hyperforin (5). It also has anti-inflammatory, antibacterial, anti-tumor and anti-angiogenic effects. An anticlastogenic effect of hyperforin on the indirect mutagen benzopyrene has also been observed (5). Hypericin exhibits antibacterial, antiviral and anti-inflammatory activity (6). It is reported to have antidepressant properties. Other minor ingredients are protohypericin, pseudohypericin (with anti-inflammatory properties), protopseudohypericin and cyclopseudohypericin. These compounds also have antiretroviral and cytotoxic properties (3). Flavonoids, bioflavonoids, anthocyanidins and catechins are the main phenolic compounds (2-4%), contained in *H. perforatum.* They have been shown to manifest antioxidant effect, the ability to remove free radicals have cardioprotective, antidiabetic, anti-inflammatory, antiallergic, anti-cancer and potential antiviral effects (7). Procyanidins exhibit antioxidant, antiviral, antimicrobial and vasoactive properties (8). The ethereal oil (0.07-0.25%) of dried flowering stems contains mainly the substances α-pinene and 2-methyloctane (4).

St. John's wort extracts have a broad spectrum antimicrobial activity against Gram-positive (*Staphylococcus aureus, S. mutans)* and Gram-negative bacteria (*Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa*), as well as yeasts (*Candida albicans*) (6). Methicillin-resistant and penicillin-resistant *S. aureus* are particularly susceptible to hyperforin (9). It was reported that hypericin exhibits *in vitro* antiviral effect against herpes simplex virus-1 (10). Hypericin has been suggested to inactivate coated viruses by altering viral proteins and inhibiting the ability of viruses to fuse with cell membranes (9).

Analyses at (-0.1212) eV of the effects with mathematical models (11) and Ignatov’s, Gluhchev’s, Neshev’s and Mehandjiev’s method for the size of water clusters (12) are performed. The authors reported that at (-0.1287) eV the size of the water cluster is 0.822 nm. The purpose of the present study was to analyse and compare the results of dry *Hypericum perforatum* L. with colloidal nano silver and fresh *H. perforatum* by spectral analysis.

Materials and Methods

Device for Non-equliblrium energy spectrum (NES) and Differential non-equliblrium energy spectrum (DNES) analysis

The apparatus for NES and DNES assays is created by A. Antonov and it is based an optical principle (13). It contains a hermetically closed camera for water drops evaporation at constant temperature (+22–24° C). The drops of water are set on a transparent waterproof pad, which consists of a glass plate and thin maylar folio. Monochromatic light with filter for yellow color with wave length at = 580±7 nm is used. The apparatus measures the angle of evaporation of water drops between 72.3° and 0°. The DNES was determined in the range between -0.08 and -0.1387 eV or = 8.9–13.8 μm by an especially created computer program. The major assessment criterion in the experiments was the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds among H_2O molecules in water specimens. It is measured in eV.

Characteristics of NES and DNES water state spectra

The *f*(θ*)* function is denoted as spectrum of energy distribution. It is featured by the non-equilibrium procedure of evaporation of water droplets.

The operating tenet of the method for determination of wetting angle of fluid drops on a solid surface, presented by (13, 14) was used.

The relation of the energy of hydrogen bonds between water molecules *f(E)* and *f*(θ is expressed as follows:

$$f(E) = \frac{14.33\, f(\theta($$
$$[1-(1+bE)^2\,]^2 \qquad (15)$$

In the formula, *b* depends on the water surface tension, on the number of water molecules at the surface layer of water per unit area, and the initial contact angle of the drop.

The water state spectrum is received from the non-equilibrium process of evaporation of drops of water and, due to that, the term NES is used (16). The evaluated measurement error for E is ±0.0011eV.

The difference:

$$E\,(\theta)\;\; E(\theta)_{sample}\;\; E(\theta)_{control\ sample} \qquad (17)$$

is denoted as DNES.

DNES is a measurement of the alteration of structure of water as a result of a given impact factor. The combined effect of all other influencing factors except the studied one is the same for the control and the specimen, therefore it is canceled out.

Mathematical models

Research was conducted of 1% extracts of the medicinal plant using mathematical models of (12) applied by (18, 19).

Colloidal nano silver 30 ppm

Colloidal silver nanoparticles (AgNPs) at concentrations of 30 ppm were used. It is standardised and patented by the Swiss company Evodrop.

Drugs

Aboveground parts of *Hypericum perforatum* L. (leaves, stem and flowers), harvested during flowering were tested. A fresh herb was picked up on the day of the experiment as well as a dried herb were examined. Each of both herb samples (fresh and dried) was soaked in deionized water (1 g herb in 0.100 l water), as well as in deionized water with AgNPs at final concentrations of 30 ppm (of dry herb) for 24 hr at room temperature in the same 1% ratio (1 g herb in 0.100 l water with AgNPs). The received water herbal extracts were subjected to spectral analyses.

T-test of Student was applied with 10 measurements to compare the effects of samples of the three experimental groups:

1) 1% extracts of fresh *H. perforatum* in deionized water (the first group).
2) 1% extracts of dry *H. perforatum* in deionized water (the second group).
3) 1% extracts of dry *H. perforatum* with colloidal nano silver 30 ppm in deionized water (the third group).

The control group was deionized water.

Results and Discussion

Results from spectral analyses with methods NES and DNES

1% water extracts of fresh and dry *H. perforatum*, and of dry *H. perforatum* with colloidal nano silver 30 ppm are presented on Table 2 and Fig. 1.

The mean energy ($E_{H...O}$) of hydrogen H…O-bonds between individual H_2O molecules in NES in 1% extracts of fresh *H. perforatum* was measured at E= -0.1243 eV. The mean energy ($E_{H...O}$) of hydrogen H…O-bonds between individual H_2O molecules in NES in 1% extracts of dry *H. perforatum* was measured at E= -0.1210 eV. The mean energy ($E_{H...O}$) of hydrogen H…O-bonds between individual H_2O molecules in 1% extracts of dry *H. perforatum* with colloidal nano silver was determined at E= -0.1232 eV. The result of control sample with deionized water was E= -0.1188 eV.

The results received by DNES method were the next:

For the fresh *H. perforatum* L.: ΔE= (-0.1243)-(-0.1188)= -0.0055±0.0003 eV

For the dry *H. perforatum*: ΔE= (-0.1210)-(-0.1188)= -0.0022±0.0001 eV.

For the dry *H. perforatum* with colloidal nano silver:

ΔE= (-0.1234)-(-0.1188)= -0.0046±0.0002 eV.

For the three experimental groups the results were at level P<0,05 according to the t-test of Student. These results show a restructuring of $\Delta E_{H...O}$ values between individual H_2O molecules with a statistically significant raise of local extremums in DNES. The local extremums (eV^{-1}) were in the function of spreading of energies of hydrogen bonds.

The results of dry *H. perforatum* with colloidal nano silver were higher in comparison with these of dry *H. perforatum* The difference was ΔE= (-0.0055) – (-0.0046) = (-0.0009) eV.

The alterations of energies of hydrogen bonds of dry *H. perforatum* with colloidal nano silver *30* ppm were closer to energies of hydrogen bonds of fresh *H. perforatum.*

Mathematical models

Fresh and dry *H. perforatum* in water and dry *H. perforatum* with colloidal nano silver 30 ppm (12).

The studies of water drops was with the NES method conducted using 1% extracts of fresh and dry *H. perforatum*, as well as with dry *H. perforatum* with colloidal nano silver 30 ppm 1% in water solution. The mathematical models of 1% extracts for fresh and dry *H. perforatum* and dry *H. perforatum* with colloidal nano silver 30 ppm give important information about the likely number of hydrogen bonds as percent of H_2O molecules with different values of distribution of energies (Table 2; Fig. 1). These distributions are in connection mainly with the restructuring of H_2O molecules possessing the same energies. There is application of the method for mathematical models with different plants.

The distribution (%, $(-E_{value})/(-E_{total\ value})$ of H_2O molecules in 1% of water extracts of fresh (green line) and dry *H. perforatum* (red line) and dry *H. perforatum* with colloidal nano silver 30 ppm (brown line) as well as control sample deionized water (blue line) are shown of Fig. 1.

The results of the studies show a significant potential for stimulating effect of St. John's wort and espetially of the extracts of dried and fresh herb, on the nervous system of the body and to improve nerve conduction (the extre-

Table 2. The spreading (%, $(-E_{value})/(-E_{total\ value})$ of H_2O molecules in water samples from fresh (green color) and dry *H. perforatum* (red color) and dry *H. perforatum* with colloidal nano silver 30 ppm (brown color). The control sample is with blue color

-E(eV) x-axis	1% Water Solution of *Dry Hypericum perforatum* L. y-axis (%$((-E_{value})$ */ $(-E_{total\ value})$**)	1% Water Solution of *Fresh Hypericum perforatum* L. y-axis (%$((-E_{value})$ */ $(-E_{total\ value})$**)	1% Water Solution of *Dry Hypericum perforatum* L. with AgNP 30 ppm y-axis (%$((-E_{value})$ */ $(-E_{total\ value})$**)	Control Deionized Water (%$((-E_{value})$*/ $(-E_{total\ value})$**)	-E(eV) x-axis	1% Water Solution of *Dry Hypericum perforatum* L. y-axis (%$((-E_{value})$ */ $(-E_{total\ value})$**)	1% Water Solution of *Fresh Hypericum perforatum* L. y-axis (%$((-E_{value})$ */ $(-E_{total\ value})$**)	1% Water Solution of Dry *Hypericum perforatum* L. y-axis AgNP 30 ppm (%$((-E_{value})$ */ $(-E_{total\ value})$*)	Control Deionized Water (%$((-E_{value})$*/ $(-E_{total\ value})$**)
0.0937	0	0	0	0	0.1187	0	8.2	4.3	15.8
0.0962	0	0	0	10.5	0.1212	10.6[2]	16.4[2]	15.1[2]	0[2]
0.0987	0	0	0	0	0.1237	0	0	0	5.3
0.1012	10.5	0	4.3	0	0.1262	10.5	0	8.6	5.3
0.1037	0	4.1	4.3	10.5	0.1287	0	8.2	6.7	0
0.1062	0	4.1	0	0	0.1312	0	8.2	6.7	10.5
0.1087	15.8	0	0	10.5	0.1337	15.8	13.1	8.6	5.3
0.1112	15.8[1]	16.4[1]	11.1[1]	0[1]	0.1362	10.5	13.1	13.1	10.5
0.1137	0	0	4.3	10.5	0.1387	10.5[3]	8.2[3]	8.6[3]	5.3[3]
0.1162	0	0	4.3	0	–	–	–	–	

For (E = -0.1112 eV) (λ = 11.15 μm) (ṽ = 897 cm-1) is the local extremum for stimulating effect on the nervous system and improving nerve conduction.
For (E = -0.1212 eV)(λ=10.23 μm)(ṽ=978 cm^{-1}) is the local extremum for anti inflammatory activity.
For (E=-0.1387 eV)(λ=8.95 μm)(ṽ=1117 cm^{-1}) is the local extremum for suppressive effect on multiplication of neoplasm cells at the molecular level.

Notes:

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

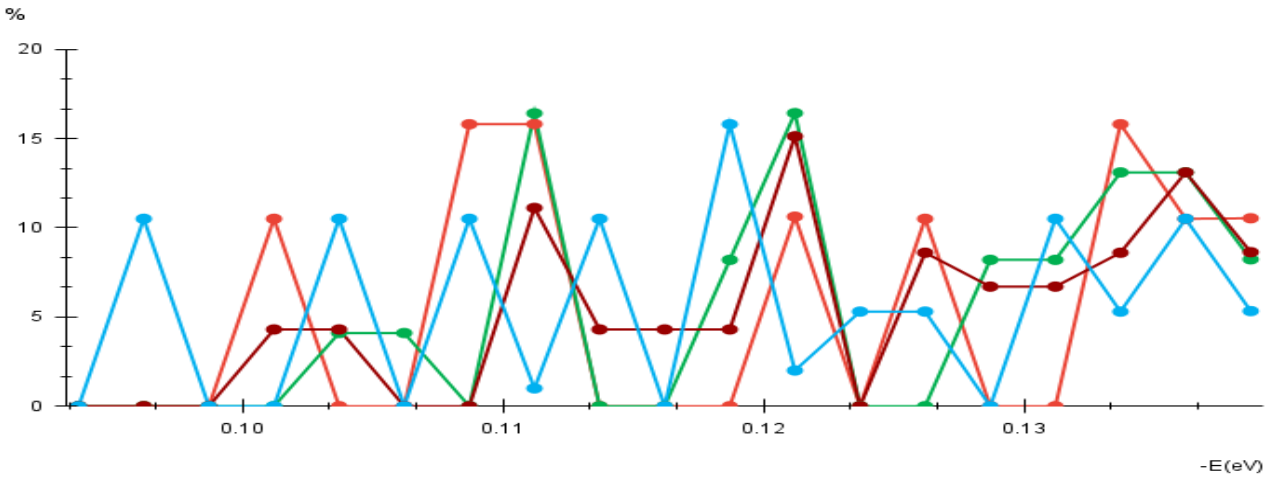

Fig. 1. Mathematical model, made by the method of Ignatov and Mosin (2013) of 1% water solutions of fresh (green color) and dry *H. perforatum* (red color), and dry *H. perforatum* with colloidal nano silver 30 ppm (brown color). The control sample is with blue color.

Notes:

For (E=-0.1112 eV)(λ=11.15 μm)(ṽ=897 cm^{-1}) is the local extremum for stimulating action on the nervous system and betterment of nerve conductivity.
For (E = -0.1212 eV)(λ=10.23 μm)(ṽ=978 cm^{-1}) is the local extremum for anti-inflammatory activity.
For (E=-0.1387 eV)(λ=8.95 μm)(ṽ=1117 cm^{-1}) is the local extremum for suppression of development of neoplasm cells at the molecular level

mums at E=-0.1112 eV). The tested extracts, especially this from the fresh herb, as well as that from the dry one with the addition of nanosilver, have and anti-inflammatory activity (determined at E = -0.1212 eV). The studied extracts have also the potential to inhibit the development of neoplasm cells at the molecular level, especially that of the dry herb (at E=-0.1387 eV).

In practice, colloidal silver is derived from medicinal plants with antioxidant, antimicrobial effects. Medicinal plants with such practical application are *Catharanthus roseus* (20), *Aerva lanata* (21), *Indigofera aspalathoides* (22), *Croton bonplandianum* (23), *Azadirachta indica* (24), *Aloe vera* (25), *Justicia glauca* (26), *Arnebia nobilis* (27), *Alysicarpus monilifer* (28), *Lansium domesticum* (29), *Streptomyces atrovirens* (30), *Cucumis sativus* L. (31). Results show that colloidal nano silver with bio effects is released through green synthesis. The colloidal nano silver stimulates the plant growth. Such researches were conducted using tomatoes (32). The colloidal nano silver 30 ppm of the Swiss company Evodrop that we added to dry *H. perforatum* extract in present research was studied and by other authors for biological effects (33-35).

The biochemical composition of the plants is mainly determined by the soil and water. In our opinion, the re-

sults obtained are in connection and with the content of biologically active substances in the studied herb. In studies of Bulgarian St. John's wort (36) has been found that the total content of phenols as well as the antioxidant effect of the wild herb is higher than that of the cultivated plant. The higher content of phenolic compounds in the wild herb can be explained by differences in climatic conditions, the place of collection, as well as in the pretreatment processes.

It was reported that the maximum extraction of biologically active substances such as hyperforin, adhyperforin and I3, II8-biapigenin is obtained using fresh St. John's wort exposed to sunlight during maceration (37). Aqueous extracts of *H. perforatum* obtained with boiling water have a higher content of active components than those received at room temperature (38). Hypericin is known to be completely insoluble in water. Therefore, methods for overcoming this insolubility are being developed. Hyperforin is a lipophilic compound that decomposes rapidly upon exposure to heat or light (38). Other authors (39) tested the main compounds in an infusion of dried St. John's wort in deionized water at 95–100° C for 5 min. They found that the infusion contained all bioactive components, although in lower concentrations, but lacked rutin and hyperforin. Hypericin and pseudohypericin are practically water insoluble at room temperature, but this can be overcome at higher temperatures. Therefore, St. John's wort infusion may contain almost half of the amount of these two compounds (8).

Structuring of water clusters

In 1% of water solutions of fresh and dry *H. perforatum* and dry *H. perforatum* with colloidal nano silver 30 ppm and control sample deionized water.

In the tested extracts there are organic compounds of *H. perforatum* The number of water molecules for fresh *H. perforatum* was 16 molecules at (-0.1212 eV). The number of water molecules for dry *H. perforatum* was 15 molecules at (-0.1212 eV). For (E = -0.1212 eV) (λ=10.23 μm) ($\tilde{\nu}$=978 cm^{-1}) it is the local extremum showing anti-inflammatory action (12). With these results we prove the high anti-inflammatory effect of the fresh and dried herb *H. perforatum*.

According to the model of Ignatov, Gluhchev, Neshev, Mehandjiev (12) the sizes of water clusters are: Dodeacader D_{4h} with 16 and 15 water molecules.

The possible structure of clusters consisting of 15 and 16 water molecules could be supposed on the basis of the findings of (40) that asymmetric electron density of these molecules in a condensed phase makes possible only two hydrogen bonds per molecule on average. Thus, stable clusters of water molecules should be expected to have at least three hydrogen bonds per molecule. Along these lines, our results could be interpreted within the framework of a recent theoretical investigation of lowest-energy structures of water clusters consisting of arranged or single rings or multiple rings of tetramer, pentamer and hexamer (41). The authors have shown that, when single-ring stacking structures have more than 3 layers, the number of weak hydrogen bonds among the layers increases, thus reducing the stability of the stacked structure. Consequently, a two-layer multi-ring stacking pattern is energetically more favorable than a single-ring four-layer stacking pattern. The corresponding structures for our results for water herbal extracts of fresh *H. perforatum* and of dry *H. perforatum* with AgNPs 30 ppm are shown in Fig. 2.

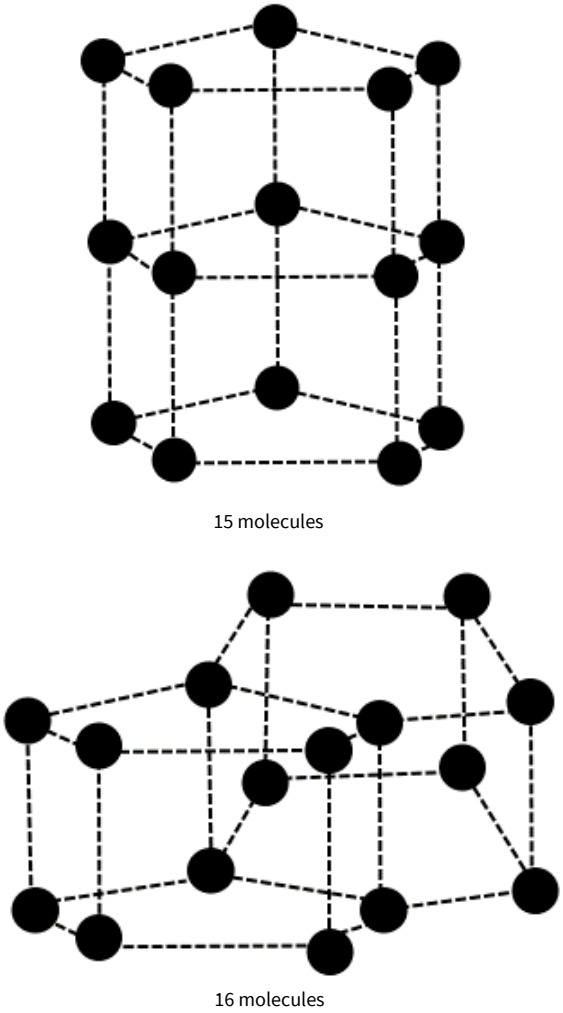

Fig. 2. Possible cluster structures in water herbal extracts of fresh *H. perforatum* and of dry *H. perforatum* with AgNPs 30 ppm.

Clusters of 16 and 15 water molecules are formed in these extracts. Here the circles depict the positions of the water molecules as a whole, without showing the exact directions of the hydrogen atoms and the dotted lines depict the hydrogen bonds.

Conclusion

For the first time, a spectral analysis of St. John's wort extracts was performed. In the current study of *H. perforatum*, AgNPs 30 ppm was added to the dry herb. Comparative analysis was performed with fresh *H. perforatum* The methods NES, DNES, mathematical models and a method for analysis of water clusters were applied.

The determined local extremums were in the zones showing stimulating action on the nervous system and betterment of nerve conduction (E=-0.1112 eV)(λ=11.15 μm) ($\tilde{\nu}$=897 cm^{-1}), also anti-inflammatory effect (E = -0.1212 eV) (λ=10.23 μm)($\tilde{\nu}$=978 cm^{-1}) and inhibition of development of

tumor cells at a molecular level (E=-0.1387 eV)(λ=8.95 μm) ($\tilde{\nu}$=1117 cm^{-1}).

The analysis revealed that in water herbal extracts of fresh *H. perforatum* and of dry *H. perforatum* with AgNPs 30 ppm are formed clusters of 16 and 15 water molecules.

The results of the fresh plant were better then these of the dry one. The effects of plant was increased with addition of colloidal nano silver (AgNPs).

Compliance with ethical standards

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

Ethical issues: None.

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