Journal of Medicinal Herbs

journal homepage:www.jhd.iaushk.ac.ir

Spectroscopic analysis of a herbal medicine, including glucosamine and chondroitin, with antioxidant and anti-inflammatory effects

Zeynep Aygun*

Bitlis Eren University, Vocational School of Technical Sciences, 13000, Bitlis, Turkey;

***Email:** zeynep.yarbasi@gmail.com*

ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *December 15th 2020*
Accepted *April 11th 2021*

Key words:
- *Glucosamine*
- *Herbal medicin*
- *Spectroscopic techniques*

ABSTRACT

Background & Aim: In our daily lives, we need to use some supplements to support our bodily functions besides our daily diet. It is essential to get some additional minerals and vitamins required to reinforce our normal diet in today's conditions. There are different food supplements that vary according to the body's need. It is important to know if these supplements have the right ingredients or not. In the present study, our aim is to investigate the ingredients and antioxidant potential of a kind of herbal drug used for protection against deterioration of joint cartilage.

Experimental: In order to examine the magnetic and structural properties of the herbal drug, several spectroscopic methods (Electron Paramagnetic Resonance, Fourier Transform Infrared Spectrophotometer, X-Ray Diffraction, Scanning Electron Microscopy, Energy Dispersive Spectroscopy) were applied at room temperature. The powdered form of the herbal medicine pill was used for analysis.

Results: We observed a broad signal with a g value of ≈2.0010 and with a line width of 185 G due to the higher Mn concentration by electron paramagnetic resonance. However, in the spectrum, we determined a weak shoulder with g value of 2.0040 which can be attributed to a carbon centered radical. Two x-ray diffraction peaks centered at ≈26.5° and 38° were assigned to turmeric which is an important component for assisting in reducing of inflammation and pain. The elemental composition results were in good agreement with the other applied techniques.

Recommended applications/industries: It will be important to carry out alternatively supportive spectroscopic studies in health-related applications.

1. Introduction

Enough amounts of main nutrients such as protein, carbohydrate and fat create a balanced diet required by the body. But besides that, some additional vitamins and minerals are also essential for various bodily functions in different periods of our lives. In order to supply normal diet and to feel healthier, herbal remedies are preferred and have great interest worldwide (Aygun, 2017).

Herbal medicine is a very important element of complementary medicines. There are many studies on natural compounds and herbal medicines but the results are various and inconsistent, due to the fact that each medicinal herb has the pharmacological effect depends on several metabolites combination and their synergistic effects (Ghasemian *et al.,* 2016). Glucosamine ($C_6H_{13}NO_5$) which can be in the form of glucosamine sulfate ($C_{12}H_{28}N_2O_{14}S$), glucosamine hydrochloride, or N-acetyl-glucosamine is widely used as a supplement in the treatment for osteoarthritis, and some kinds of pain (Benavente *et al.,* 2015). Osteoarthritis is a degenerative disease of the cartilage in the joints. Glucosamine naturally exist in many of the body's tissues and is used in the treatment of arthritis. Glucosamine for arthritis is usually formulated as the hydrochloride salt or glucosamine sulfate and frequently with chondroitin sulfate ($C_{13}H_{21}NO_{15}S$). In pharmaceutical preparations, glucosamine sulfate has the higher biological effect due to the existence of the sulfate (Foot and Mulholland, 2005).

On behalf of issues mentioned above, it will be useful to investigate the sample which may has antioxidant potential due to its ingredient. Antioxidants can react with free radicals and prevent the reaction before vital molecules are damaged (Aygun, 2017). In this paper, the magnetic properties of the herbal medicine have been analyzed by EPR (Electron Paramagnetic Resonance) spectroscopy. EPR spectroscopy is an effective and skillful tool in researches because of the ability of detecting free radicals and paramagnetic centers. Additionally, XRD (X-ray diffraction) technique has been performed to get information about the chemical composition and crystalline property of the structure. EDS (Energy Dispersive Spectroscopy) method has been used to determine the elemental composition. The functional groups and the state of the bonds in the structure have been determined by FTIR (Fourier-transform infrared spectrophotometer). Since some herbal medicines are sold commercially with wrong ingredients, it will be important to learn about the content of medicines for public health. In this study, our purpose is to examine the medicine sample whether the given contents are present or not in it. To our knowledge there is no paper reported for the sample with EPR, XRD, FTIR, SEM and EDS techniques, hence, the present study is conducted to report magnetic and structural properties of the herbal medicine.

2. Materials and Methods

The herbal medicine contains glucosamine sulfate with chondroitin, formulated in Canada, was purchased commercially. One pill was powdered in a mortar prior to spectroscopic analysis. Room temperature EPR spectrum of the sample was scanned by an X-band JEOL JESFA-300 EPR system with 100 kHz modulation field and 9.20 GHz frequency. In EPR experiment, diamagnetic tube was used for sample performing. BRUKER D8 ADVANCE was performed to detect the XRD pattern of sample operated at 40 kV and 40 mA with a scanning speed of 2.5°/min. Cu-K_{α} radiation of wavelength l = 1.54060 Å was used and data were recorded for the 2ϴ≈5°- 90°. EDS analysis was done by JEOL JSM-6610 spectrometer. Bruker VERTEX 70v system was used for the FTIR analysis.

3. Results and discussion

3.1. EPR study

The g value of the EPR line is obtained from the equation $h\upsilon = g\beta H$, where; H was the magnetic field; υ, the microwave frequency; h, the Planck constant and β, the electron Bohr magneton. Electron spin resonance (ESR) spectrum of herbal medicine recorded at room temperature is given in Figure 1.

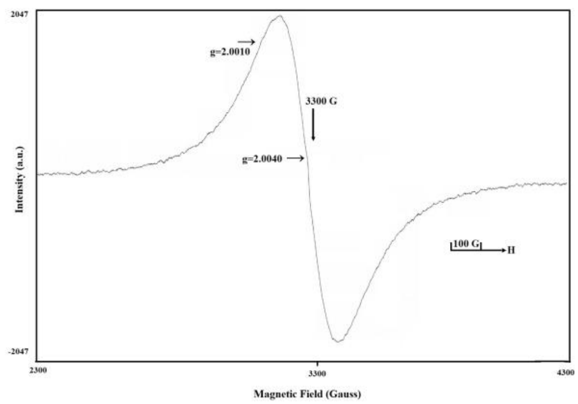

Figure 1. EPR spectrum of the sample scanned at room temperature.

Depending on the concentration, we can analyze the EPR spectrum of Mn for two cases. At lower concentrations of Mn, due to the interaction between the S=5/2 spin of the unpaired 3d electrons with the spin of the 55Mn nucleus (I= 5/2), sextet hyperfine-splitting centered at g≈2.00, with a line width of ~92 G was observed. These sextet lines are associated with the allowed transitions ($\Delta m_s=\pm1$, $\Delta m_I=0$). At higher concentrations, Mn ions cannot isolate from each other and increase the dipole-dipole interaction. In this case, the hyperfine-splitting fully disappears and causes to see the hyperfine structure as a broad resonance peak (Kaftelen *et al.*, 2013; Ming *et al.*, 2012). For the sample, we observed a broad signal with a g value of ≈2.0010 and with a line width of 185 G due to the higher Mn concentration. But also, in the EPR spectrum, we determined a weak shoulder with a g value of 2.0040, overlapped with Mn peak, which can be attributed to a carbon centered radical (Aygun, 2017; Ukai *et al.*, 2008). Due to the broad Mn peak, we were not able to see the well-resolved radical signal. It is well known that there is a correlation between free radicals and health. Antioxidants can interact with free radicals and prevent the damage caused by free radicals (Yarbasi *et al.*, 2011). Due to the presence of radical signal determined by EPR, we can conclude that the sample has antioxidant property.

3.2. XRD study

XRD pattern obtained from room temperature x-ray diffraction experiment is shown in Figure 2.

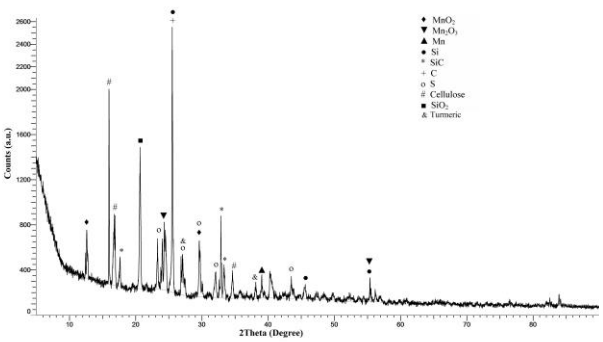

Figure 2. XRD pattern of the sample taken at room temperature.

According to the XRD of the sample, peak sharpness clearly showed the crystalline property. Peak positions for the samples were given with corresponding compounds in XRD figure. Among the peaks, most intense peak centered at ≈25.5° was attributed to C and Si (Aygun, 2017; Chen *et al.*, 2014). Other less intense and sharp peaks corresponded to Mn, MnO_2, C, SiO_2, SiC, S, Mn_2O_3, cellulose as given in Figure 2. Two peaks centered at ≈26.5° and 38° were assigned to turmeric which is an important component for assisting in reducing of inflammation and pain, and is used with other ingredients to treat osteoarthritis pain for a long time (Kiyani *et al.*, 2019).

3.3. FTIR study

FTIR spectrum of the sample taken at room temperature is seen in Figure 3. We observed a peak at around ≈3283 cm^{-1}, an intense peak at ≈1030 cm^{-1} and ≈598 cm^{-1}. In a previous study, FTIR spectrum of glucosamine hydrochloride was reported with an intense band at around 3300 cm^{-1} and band at ≈1094 cm^{-1} (Benavente *et al.*, 2015). The peak at 3283 cm^{-1} might be attributed to ≡C–H (Ma *et al.*, 2014).

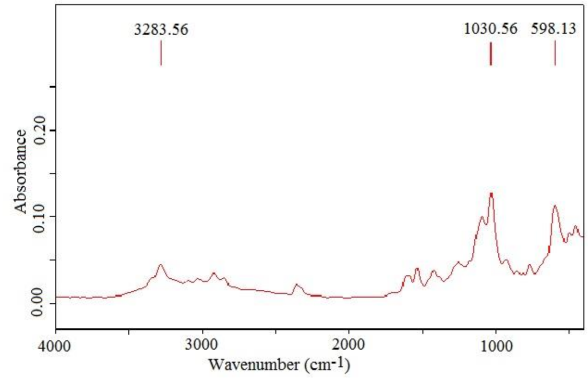

Figure 3. FTIR spectrum of the sample taken at room temperature.

According to a previous report, peak centered at 3283 cm^{-1} was attributed to stretching vibrations of N-H of amines, amine salts, sulfonamides, C-H of alkenes and alkanes, or C=O of carboxylic acids (Chekuri *et al.*, 2015). The Si−O−Si linear bonding vibration seen at ≈1030 cm^{-1} has been reported in a previous study (Obata *et al.*, 2017). The band at ≈1030 cm^{-1} was also given as C-OH stretching mode of turmeric sample (Wulandari *et al.*, 2018). The peak located at 598 cm^{-1} was attributed to ^{15}NO bonding (Collman *et al.*, 2008).

3.4. SEM-EDS study

EDS spectrum and obtained elemental composition results of the sample are given in Figure 4.

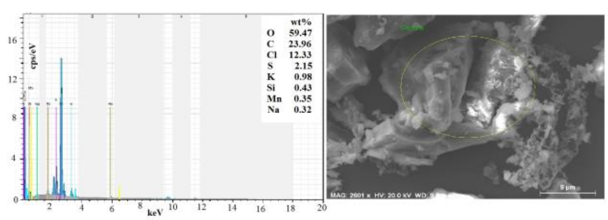

Figure 4. EDS spectrum of the sample taken at room temperature.

As can be seen in the figure 4, the presence of elements C, Si, O, S, Na, K, Cl and Mn was supported by XRD and FTIR techniques, and Mn was also seen in EPR experiment. SEM images recorded at room temperature are seen in Figure 5. The micrographs of the sample were given in 1000X and 5000 X magnifications. From the SEM images, the presence of microcrystalline structure in the sample can be concluded.

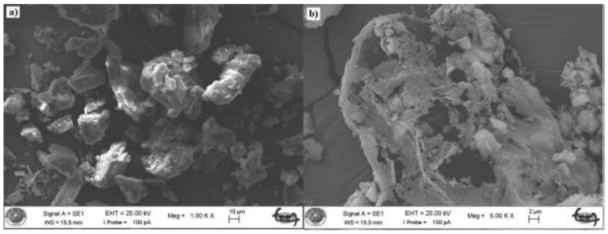

Figure 5. SEM images of the sample taken at room temperature.

4. Conclusion

In this paper, we investigated the herbal medicine widely used for pain related with osteoarthritis. We performed several spectroscopic techniques to determine structural, magnetic and morphological properties of the sample. It can be concluded that the herbal drug can be evaluated as suitable for its stated purpose in line with its content (S, N, Si, C, Mn, turmeric). With the help of coherent results obtained by EPR, XRD, EDS and FTIR spectroscopic methods, the usefulness of the sample can be appreciated positively in point of used as supplement especially for the treatment of arthritis or osteoarthritis due to the ingredients and antioxidant property.

5. References

Aygun, Z. 2017. Application of spectroscopic techniques for antioxidant property analysis of various food supplements and Ganoderma lucidum coffee. *Pakistan Journal of Scientific and Industrial Research Series B: Biological Sciences*, 60(3): 145-153.

Benavente, M., Arias, S., Moreno, L., Martínez, J. 2015. Production of glucosamine hydrochloride from crustacean Shell. *Journal of Pharmacy and Pharmacology*, 3: 20-26.

Chekuri, M., Gangarudraiah, S., Roopavatharam, L.B., Kaphle, A., Chalimeswamy, A. 2015. Green synthesıs of stable sılver nanopartıcles usıng flower extract of *Rosa damascena*: characterızatıon, antımıcrobıal and antıoxıdant actıvıty study. *European Chemical Bulletin*, 4: 454-459.

Chen, Y., Zhang, X., Tian, Y., Zhao, X. 2014. Synthesis and characterization of silicon nanoparticles inserted into graphene sheets as high performance anode material for lithium ion batteries. *Journal of Nanomaterials*, 8: 1-6.

Collman, J.P., Yang, Y., Dey, A., Decre´au, R.A., Ghosh, S., Ohta, T., Solomon, E.I. 2008. A functional nitric oxide reductase model. *Proceedings of the National Academy of Sciences of the USA*, 105: 15660-15665.

Foot, M., Mulholland, M. 2005. Classification of chondroitin sulfate A, chondroitin sulfate C, glucosamine hydrochloride and glucosamine 6 sulfate using chemometric techniques. *Journal of Pharmaceutical and Biomedical Analysis*, 38: 397–407.

Ghasemian, M., Owlia, S., Owlia, M.B. 2016. Review of anti-inflammatory herbal medicines. *Advances in Pharmacological Sciences*, 1-11.

Kaftelen, H., Tuncer, M., Tu, S., Repp, S., Goçmez, H., Thomann, R., Weber, S., Erdem, E. 2013. Mn-substituted spinel Li4Ti5O12 materials studied by multifrequency EPR spectroscopy. *Journal of Materials Chemistry*, A, 1: 9973-9982.

Kiyani, M.M., Sohail, M.F., Shahnaz, G., Rehman, H., Akhtar, M.F., Nawaz, I., Mahmood, T., Manzoor, M., Bokhari, S.A.I. 2019. Evaluation of turmeric nanoparticles as anti-gout agent: modernization of a traditional drug. *Medicina*, 55: 10.

Ma, H., Ren, H., Zou, X., Meng, S., Suna, F., Zhu, G. 2014. Post-metalation of porous aromatic frameworks for highly efficient carbon capture from CO2 + N2 and CH4 + N2 mixtures. *Polymer Chemistry*, 5: 144.

Ming, Z., Shun, T., GongPu, L., ShuYuan, Z. 2012. Structure characterization, magnetic and photoluminescence properties of Mn doped ZnS nanocrystalline. *Science China Physics, Mechanics & Astronomy*, 55(2): 219–223.

Obata, A., Shimada, M., Iguchi, M., Iwanaga, N., Mizuno, T., Kasuga, T. 2017. Electrospun poly (γ-glutamate)/silica hybrids for tissue regeneration: influences of silane coupling agents on chemistry, degradation and florescent dye release. *International Journal of Metallurgical & Materials Engineering*, 3: 134.

Ukai, M., Kameya, H., Nakamura, H., Shimoyam, Y. 2008. An electron spin resonance study of dry vegetables before and after irradiation. *Spectrochimica Acta A*, 69: 1417-1422.

Wulandari, R., Martono, S., Rohman, A. 2018. Liquid chromatography and fourier transform infrared spectroscopy for quantitative analysis of individual and total curcuminoid in *Curcuma longa* extract.

Journal of Applied Pharmaceutical Science, 8: 107-113.

Yarbasi, Z., Karabulut, B., Karabulut, A. 2011. An EPR study of gamma irradiated medicinal plants: cress seeds and mistletoe. *Gazi University Journal of Science*, 24: 203-207.