Bioperformances of honey-phytonanosilver in silica materials

M. E. BARBINTA-PATRASCU[a,*], C. UNGUREANU[b,*], N. BADEA[b,*], M. CONSTANTIN[a], V. PURCAR[c,*], A. ISPAS[d,*]
[a]*University of Bucharest, Faculty of Physics, Department of Electricity, Solid-State Physics and Biophysics, 405 Atomistilor Street, PO Box MG-11, Bucharest-Magurele, 077125, Romania*
[b]*University "Politehnica" of Bucharest, Faculty of Applied Chemistry and Material Science 1-7, Polizu Str., 011061, Bucharest, Romania*
[c]*National Research&Development Institute for Chemistry and Petrochemistry ICECHIM, 202 Splaiul Independentei, 6th district, P.O. 35-174, 060021, Bucharest, Romania*
[d]*Department of Prosthodontics, "Iuliu Hatieganu" University of Medicine and Pharmacy, 8 Babeş Street, Cluj-Napoca, 400012 Romania*

Innovative silica-based bioactive materials containing mandarin extract, "green" silver nanoparticles and honey, were originally designed for biomedical purposes. Silver nanoparticles were phytosynthesized from aqueous extract of mandarin peels. The samples were spectral analyzed by UV-Vis absorption and FT-IR ATR spectroscopy. The biomaterials prepared in this study exhibited high antimicrobial activity against *Escherichia coli*, and also good antioxidant properties evaluated *in vitro* by chemiluminescence technique. These organic/inorganic materials could be valuable candidates for many biophotonic and biomedical applications.

(Received March 19, 2020; accepted June 16, 2020)

Keywords: Biocomposite materials, Silica, honey, Mandarin, "Green" silver nanoparticles

1. Introduction

Bacterial resistance to the antibacterial agents poses a very serious threat to public health and for all types of antibiotics [1]; this situation has led to a reconsideration of the therapeutic use of ancient remedies, such as plant-based products, including honey [2]. Honey – this miraculous product of bees – possesses good wound-healing and antimicrobial activity due to the enzymatic production of hydrogen peroxide [3] and contains numerous bioactive compounds such as flavonoids and other polyphenols which may function as antioxidants [4].

Beside honey, the *Citrus* fruits (like oranges, grapefruits, mandarins) are also important sources of biological active compounds including phenolic compounds, flavonoids, ascorbic acid, and pectin that are recognized antimicrobial and antioxidant agents, important to human nutrition. Recent papers have demonstrated the important role of *Citrus* fruits in human health, and their consumption has been increased in the last years [5, 6]. Recent literature reveals the use of peels extract from various *Citrus* fruits such as mandarin as sources for synthesis of silver nanoparticles [7-10].

Silver nanoparticles are recognized for their optical properties, being used to design nanostructured nanocomposite films with ohmic electrical behaviour [11], dye-sensitized solar cell with enhanced performance [12], or in biomedical field [7].

The association of an antimicrobial agent in inorganic or organic matrix to be used in novel materials with high bio-performances is an interesting way to achieve the control and prevent microbial contamination [13, 14].

The organic-inorganic silica-based hybrid (nano)materials have attracted prominent attention for biomedical or optical and electronics applications [15, 16].

By combining 3D silica frameworks with vegetal extracts, new hybrid materials with specific optical properties can be achieved [17]. The use of a silica matrix presents many advantages, such as optical transparency, thermal stability, chemical inertness, and biocompatibility.

The main objective of the present study is to design and to evaluate the bio-properties of innovative silica-based bioactive materials containing mandarin (*Citrus reticulata*) extract, green silver nanoparticles, and honey. Previous synthesis of tangerine-generated silver - silica bioactive materials was realized in our laboratory [18]. The honey was introduced in the synthesis in order to obtain new materials that have not yet reported. Bio-based materials prepared in our work, were characterized by spectral (UV-Vis absorption and FT-IR ATR spectroscopy) methods. Their bio-performances were assessed by evaluating the antioxidant activity (by chemiluminescence technique) and antibacterial properties against *Escherichia coli* bacterium (by agar disc diffusion method).

2. Experimental part

2.1. Materials

The reagents used in the preparation of silica-based materials are: hexadecylcetyltrimethylammonium bromide (96% CTAB, MW = 364.46 g/mol, Fluka, Germany), tetraethylorthosilicate (99% TEOS, $C_8H_{20}O_4Si$, Aldrich, Germany), silver nitrate ($AgNO_3$, Gatt Koller–GmbH, Austria), and Romanian polyflower honey (ProApis). The materials needed for chemiluminescence studies: tris (hydroxymethylaminomethane base) (99.8% $C_4H_{11}NO_3$), luminol (97% $C_8H_7N_3O_2$, 5-amino-2,3-dihydro-phthalazine-1,4-dione), ethanol (99.9%, C_2H_5OH) and hydrogen peroxide (30%, H_2O_2), were purchased from Merck (Germany). The reagents required for estimation of antibacterial activity, were the following: peptone (Merck, Germany), sodium chloride (99.5% NaCl, Sigma Aldrich, Germany), yeast extract (Biolife), and agar (Fluka). Mandarin fruits were purchased from a local market.

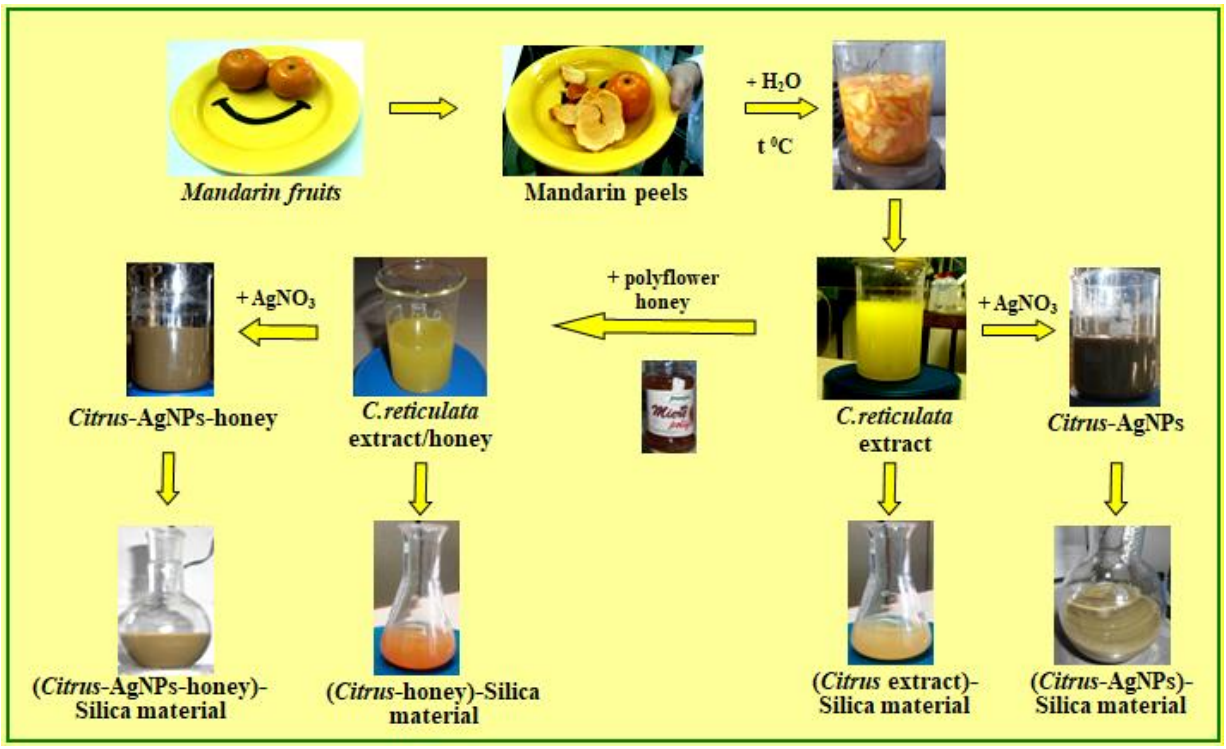

Fig. 1. Generation of silica – polyflower honey – silver phyto-based bioactive materials (color online)

2.2. Preparation procedures

Preparation of Honey@Mandarin (*Citrus reticulata*) Peel Extract (Ch). The peels of mandarin fruits were carefully washed with distilled water and then chopped in small pieces. An appropriate amount of these pieces was mixed with hot distilled water in a ratio of 1:3, and further processed as previously described [18]. To the ready-made pure extract was added 5 mL of polyflower honey, to 100 mL of pure extract, under magnetic stirring (700 rpm), resulting in honey@mandarin peel extract (Ch).

Phytosynthesis of Silver Nanoparticles (Ch-AgNPs) was achieved at room temperature, by addition, under vigorously stirring (700 rpm), an amount of 0.34 g $AgNO_3$ to 500 mL Ch. The color change of this mixture from orange to grey brown confirmed the AgNP synthesis.

Preparation of Silica Material Ch-SiO_2. Under continuous stirring, an amount of 50 g of CTAB, as template, was introduced into an Erlenmeyer flask containing 50 mL Ch, and then gentle warming in order to disperse the cationic surfactant. The quaternary ammonium salt, CTAB, was selected as template since it is highly soluble in water, safe and with no genotoxic effects [19]. Afterwards, the silane precursor – TEOS (500 µL) was rapidly added in the as-prepared mixture, and then kept at 50 °C under magnetic stirring (500 rpm), for 3h, and further left for 1h in static conditions. The template CTAB was then removed by a succession of ultrasound-assisted washing steps followed by centrifugation, as described in [18].

Silica Material containing Ch-AgNPs (Ch-AgNPs-SiO_2) was prepared by adding an amount of 50 g of CTAB into a flask containing 50 mL Ch-AgNPs suspension, under continuous stirring (500 rpm), and gentle warming, followed then by the above steps described for Ch-SiO_2 preparation.

The schematic procedure illustrating the preparation of the honey-based hybrids is displayed in Fig. 1.

2.3. Characterization methods

UV-Vis absorption spectroscopy analysis of samples was performed on a Lambda 2S Perkin Elmer

double beam spectrophotometer in the wavelength range of (400-800) nm.

The Fourier transform infrared (FT-IR) spectra of the developed materials were recorded on FT-IR Tensor 37 spectrophotometer (from Bruker), in *Attenuated Total Reflectance mode* (ATR, Golden Gate diamond unite), in the wavelength range (4,000÷400) cm^{-1} at 64 scans per spectrum, with a resolution of 4 cm^{-1}.

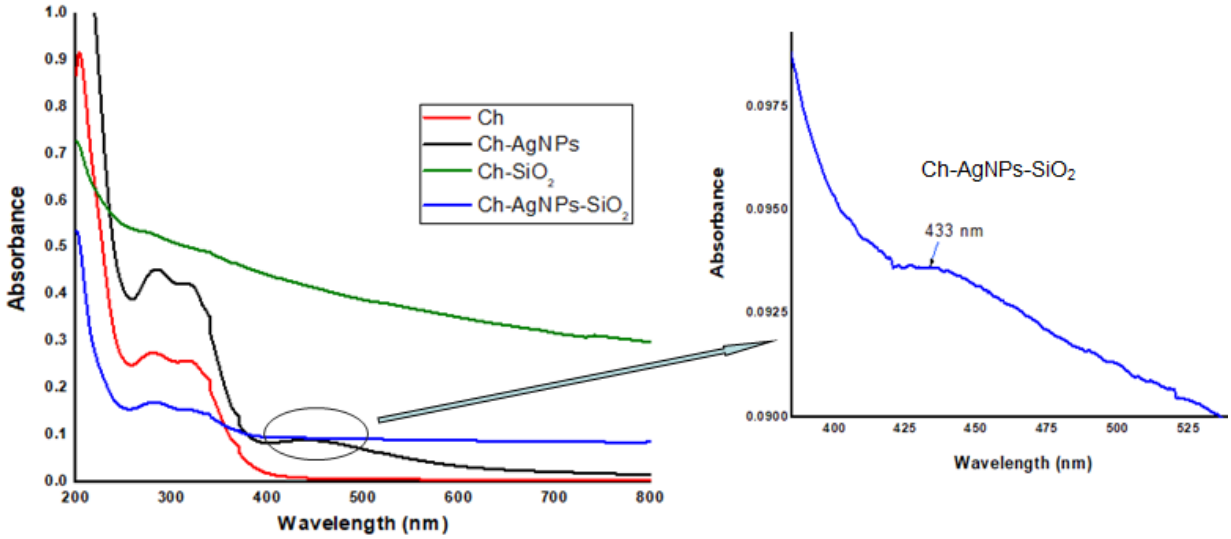

Fig. 2. UV-Vis absorption spectra of the phyto-based materials.
Inset: The spectral fingerprint of AgNPs (at 433 nm) in Ch-AgNPs-SiO_2 biohybrids (color online)

Chemiluminescence (CL) assay was used to investigate, in triplicate, the *in vitro* antioxidant activity of the as-prepared materials, on a Chemiluminometer Turner Design TD 20/20, USA, by using a free radicals' generating system consisting of: luminol (10^{-5} M), H_2O_2 (10^{-5} M), TRIS (0.2 M) –HCl (0.2 M) buffer solution (pH 8.6). For each sample, the value of antioxidant activity (AA%) was calculated as percentage of free radical scavenging, using the mathematical expression:

$$AA = \frac{I_0 - I}{I_0} \cdot 100\% \quad (1)$$

where I_0 is the maximum CL intensity for standard (the reaction mixture without the sample) at t = 5 s, and I is the maximum CL intensity for the tested sample at t = 5 s, as previously described [20].

The antibacterial activity was evaluated against *Escherichia coli* ATCC 8738 bacterium, by Kirby-Bauer test, known as agar disc diffusion method [21]. A volume of 1 mL of *Escherichia coli* suspension was spread on a solid agar medium in Petri dishes, containing a nutrient mixture with following composition: agar (20 g/L), peptone (10 g/L); NaCl (5 g/L), and yeast extract (5 g/L). The stock culture was maintained at 4 °C. The wells were made using a sterile Durham tube of 6 mm diameter and loaded with 50 µL of sample. The antibacterial activity was estimated by measuring the inhibition zone (ZOI) diameter (in mm) against test bacterium. The mean ZOI was calculated from three experiments with standard deviation procedure. The data were presented as mean ± standard deviation (SD). SD was calculated as the square root of variance using STDEV function in Excel 2010.

3. Results and discussion

3.1. Optical characterization of silica-honey-phytonanosilver materials

UV-Vis absorption spectra of samples, comparatively displayed in Fig. 2, revealed maximum absorption bands characteristic for flavonoid compounds which appear in native mandarin extract at 279 nm & 318 nm, in "green" AgNPs at 283 nm & 317 nm, in Ch-SiO_2 at 275 nm, 322 nm & 335 nm, and in Ch-AgNPs-SiO_2 at 281.5 nm & 318 nm [15].

The presence of a SPR band at 437 nm in the UV-Vis absorption spectrum of Ch-AgNPs is an indicative of AgNPs development [22]. This spectral fingerprint of AgNPs was slightly blue-shifted to 433 nm in Ch-AgNPs-SiO_2 biohybrids.

FT-IR ATR spectra of tested samples (Fig. 3) show peaks corresponding to the stretching vibrations of O–H and bending vibrations of adsorbed water molecules around 3,330 cm^{-1} and 1,640 cm^{-1}, respectively, the major contributors to these bands being polyphenolic compounds (e.g. flavonoids arising from mandarin extract) & polysaccharides (e.g. pectin derived from mandarin peels) [18]. The bands in the 2,930 and 2,880 cm^{-1} regions are

associated with the asymmetric and symmetric –CH_2– modes of the alkyl chains.

The Si-O-Si groups were observed in spectrum of Ch-SiO_2 and give two absorption peaks: 1,045 cm^{-1} (linear component) and 1,087 cm^{-1} (cyclic component), as the consequence of the inorganic network formation. The peak at ~800 cm^{-1} was assigned to asymmetric stretching vibrations of Si-O(Si) and Si-O(C) networks [23, 24].

For Ch-AgNPs-SiO_2, the siloxane (Si-O-Si) bond was found at around 1,070 cm^{-1}. This shift toward lower wavenumbers can indicate an overall decrease of the Si-O force constant characteristic of alkylsilane incorporation [25].

Peak at (1,630-1,600) cm^{-1} can be due to stretching of the carboxylic group (COO−) [26], and the absorption band at 1650 cm^{-1} might be due to the C=C [27].

The characteristic peak, at 1,604 cm^{-1}, can be assigned to the benzene stretching vibration, and the peak at 1,460 cm^{-1} might be due to the in-plane symmetric flexural vibrations of the CH_3 [28]. Peak at ~1,740 cm^{-1} can be attributed to the C=O stretching vibration [29].

Based on these spectral results, the prepared hybrid materials have resulted by assembling of organic and inorganic components through weak interactions involving hydrogen bonds, electrostatic forces, van der Waals or hydrophobic-hydrophilic interactions.

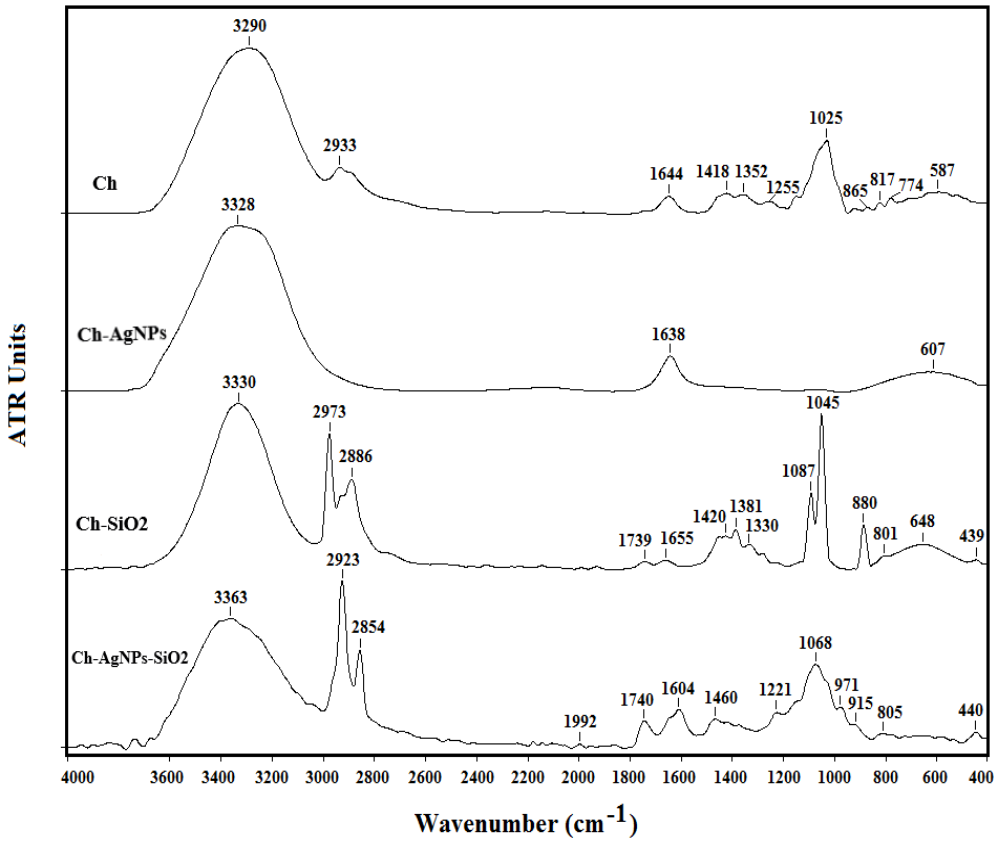

Fig. 3. FT-IR ATR spectra of tested honey-based samples

3.2. Bioperformance efficiency of silica-based materials

Antibacterial activity of samples against *Escherichia coli* ATCC 8738 is presented in Fig. 4. As seen, the "green" silver nanoparticles and the silica-based materials exhibited impressive antibacterial potency.

The strong antibacterial activity of Ch-AgNPs-SiO_2 [ZOI = (70 ± 0.54) mm] may be attributed to the presence of Ch-AgNPs. The high antibacterial activity is probably due to the cumulative effect given by the components of the innovative silica-based bioactive materials (mandarin extract, "green" silver nanoparticles, and honey) [30-32].

However, it was observed that the antibacterial activity of silica-based materials without Ch-AgNPs is low (41.00 ± 0.68 mm) comparatively with silica-based materials with Ch-AgNPs. Antibacterial activity of Ch-AgNPs (43.00 ± 0.23) mm is comparable with antibacterial activity of silica-based materials without Ch-AgNPs.

In the human and animal health field, the *Citrus* peel extract can be used in bandages (e.g. with silicon dioxide) [33] or as a nanobiofungicides [34].

The antioxidant activity of the samples was tested by simulating an oxidative stress *in vitro*, through chemiluminescence method. All the samples showed good antioxidative properties (see Fig. 5), due to the presence of mandarin peels' extract and honey that both possess antioxidant biocompounds as shown in FT-IR ATR analysis.

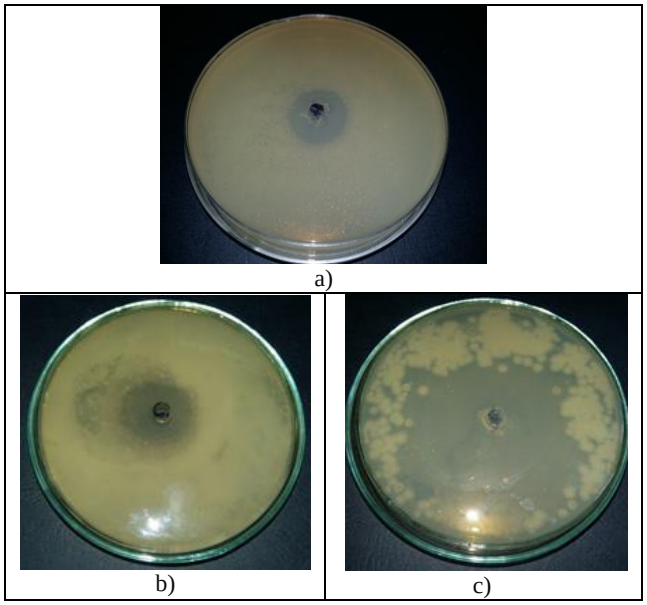

Fig. 4. Antibacterial efficiency of Ch-AgNPs (a) and of silica-based materials without (b) and with (c) Ch-AgNPs, towards Escherichia coli ATCC 8738 bacterium (color online)

Silica material based on mandarin extract & honey (Ch-SiO_2) exhibited an antioxidant activity value (calculated according equation 1) of 80.26 ± 0.63% due to the presence of bioactive molecules arising from mandarin extract and honey in its composition. Even in small content, the presence of Ch-AgNPs enhanced the efficiency of Ch-AgNPs-SiO_2 materials which showed higher AA% (81.73 ± 0.63%).

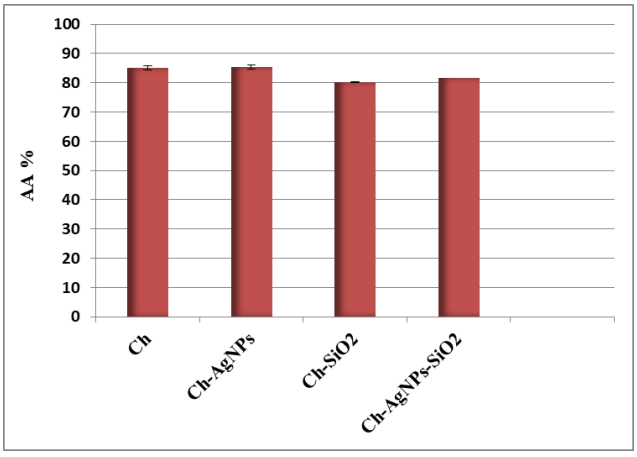

Fig. 5. The antioxidant activity (AA%) of the derived mandarin materials (color online)

4. Conclusions

A simple "green" approach was developed to design silica-based bioactive materials containing mandarin extract, biogenic silver nanoparticles, and honey.

Spectral investigations (UV-Vis absorption and FT-IR ATR spectroscopy) demonstrated that the developed materials were achieved by assembling of their components through hydrogen bonds, electrostatic forces, van der Waals, and hydrophobic-hydrophilic interactions.

The silica–honey–phyto-nanosilver materials developed in this study, presented impressive antimicrobial activity against *Escherichia coli*, exhibiting an inhibition zone diameter of 70 ± 0.54 mm. These biomaterials showed also high ability to scavenge short-life free radicals (81.73 ± 0.63%), evaluated *in vitro* by chemiluminescence technique.

The optical and biological properties of these bio-inorganic materials confer them high potential for biophotonic or bio-applications.

Acknowledgements

The present study was supported by the Projects JINR – Romania (University of Bucharest) collaboration: Project No.48/ 2020 (Theme No.04-4-1121-2015/2020) and Project No. 75/ 2020 (Theme No. 04-4-1141-2020/2022) (IUCN ORDER no.269/20.05.2020).

References

[1] S. B. Levy, B. Marshall, Nat. Med. **10**, 122 (2004).
[2] S. Mandal, M. Deb Mandal, N. K. Pal, K. Saha, Asian Pac. J. Trop. Med. **3**(12), 961 (2010).
[3] P. E. Lusby, A. L. Coombes, J. M. Wilkinson, Arch. Med. Res. **36**, 464 (2005).
[4] M. Blassa, M. Candracci, A. Accorsi, M. P. Piacentini, M. C. Albertini, E. Piatti, Food Chem. **97**, 217 (2006).
[5] I. Canan, M. Gündoğdu, U. Seday, C. A. Oluk, Z. Karaşahin, E. Ç. Eroğlu, M. Ünlü, Turk. J. Agric. For. **40**(6), 894 (2016).
[6] K. Ghasemi, Y. Ghasemi, M. A. Ebrahımzadeh Pakistan J. Pharma. Sci. **22**, 277 (2009).
[7] M. E. Barbinta-Patrascu, C. Ungureanu, I.-R. Suica-Bunghez, A.-M. Iordache, S. Milenković Petrović, A. Ispas, I. Zgura, J. Optoelectron. Adv. M. **20**(9-10), 551 (2018).
[8] M. E. Barbinta-Patrascu, N. Badea, M. Constantin, C. Ungureanu, C. Nichita, S. M. Iordache, A. Vlad, S. Antohe, Rom. J. Phys. **63**(5-6), 702 (2018).
[9] N. Basavegowda, Y. Rok Lee, Mater. Lett. **109**, 31 (2013).
[10] E. D. B. Santos, N. V. Madalossi, F. A. Sigoli, I. O. Mazali, New J. Chem. **39**(4), 2839 (2015).
[11] S. Deb, D. Sarkar, J. Optoelectron. Adv. M. **21**(3-4), 275 (2019).
[12] N. Nasr, M. H. Sayyad, J. Optoelectron. Adv. M. **20**(11-12), 618 (2018).
[13] A. M. Mebert, C. J. Baglole, M. F. Desimone, D. Maysinger, Food Chem. Toxicol. **109**, 753 (2017).
[14] N. Fernández-Bertólez, C. Costa, F. Brandão, G. Kiliç, J. A., Duarte, J. P. Teixeira, B. Laffon, Food Chem. Toxicol. **118**, 13 (2018).

[15] I. Lacatusu, N. Badea**,** R. Nita, M. Giurginca, D. Bojin, I. Iosub, A. Meghea, J. Phys. Org. Chem. **22**, 1015 (2009).
[16] I. Lacatusu I., N. Badea, D. Bojin, S. Iosub, A. Meghea, J. Sol-Gel Sci. Tech. **51**(1), 84 (2009).
[17] I. Lacatusu, N. Badea, R. Nita, A. Murariu, F. Miculescu, I. Iosub, A. Meghea, Opt. Mater. **32**, 711 (2010).
[18] M. E. Barbinta-Patrascu, M. Constantin, N. Badea, C. Ungureanu, S. M. Iordache, V. Purcar, S. Antohe, Rom. J. Phys. **64**(3-4), 701 (2019).
[19] W. T. Tsai, K. J. Hsien, J. M. Yang, J. Colloid Interface Sci. **275**(2), 428 (2004).
[20] M. E. Barbinta-Patrascu, N. Badea, C. Ungureanu, A. Ispas, Optoelectron. Adv. Mat. **13**(1-2), 131 (2019).
[21] G. More, T. E. Tshikalange, N. Lall, F. Botha, J. J. M. Meyer, J. Ethnopharmacol. **119**, 473 (2008).
[22] A. R. J. Azar, S. Mohebbi, Micro Nano Lett. **8**(11), 813 (2013).
[23] C. Petcu, V. Purcar, R. Ianchis, C. I. Spataru, M. Ghiurea, C. A. Nicolae, H. Stroescu, L. I. Atanase, A. N. Frone, B. Trica, D. Donescu, Appl. Surf. Sci. **389**, 666 (2016).
[24] V. Purcar, R Somoghi, S. G. Nițu, C-A. Nicolae, E. Alexandrescu, I. C. Gîfu, A. R. Gabor, H. Stroescu, R. Ianchiş, S. Căprărescu, L. O Cinteza, Nanomaterials **7**, 2 (2017).
[25] V. Purcar, O. Cinteza, M. Ghiurea, A. Balan, S. Căprărescu, D. Donescu, Bull. Mat. Sci. **37**, 107 (2014).
[26] G. D. Manrique, F. M. Lajolo, Postharvest Biol. Technol. **25**, 99 (2002).
[27] Y.-Y. Yu, W.-C. Chen, Mater. Chem. Phys. **82**, 388 (2003).
[28] X. F. Wen, K. Wang, P. H. Pi, J. X. Yang, Z. Q. Cai, L.-J. Zhang, Y. Qian, Z. R. Yang, D.-F. Zheng, J. Cheng, Appl. Surf. Sci. **258**, 991 (2011).
[29] Y. Gao, C. He, Y. Huang, F. L. Qing, Polymer **51**, 5997 (2010).
[30] S. Kaviya, J. Santhanalakshmi, B. Viswanathan, J. Muthumary, K. Srinivasan, Spectrochim. Acta A Mol. Biomol. Spectrosc. **79**(3), 594 (2011).
[31] A. S. Alqarni, A. A. Owayss, A. A. Mahmoud, Arab. J. Chem. **9**(1), 114 (2016).
[32] A. Mignani, S. Fazzini, B. Ballarin, E. Boanini, M. C. Cassani, C. Maccato, D. Barreca, D. Nanni, RSC Adv. **5**(13), 9600 (2015).
[33] A. A Golub, O. Biliaieva, V. V Neshta, F. Sams- Dodd, Compositions for treating wounds and skin conditions, Kiev, 36-260, WO2010079209A2 (2010). https://patents.google.com/patent/WO2010079209A2/en
[34] K. A. Abd-Elsalam, M. A. Alghuthaymi, J. Nanotech. Mater. Sci. **2**(2), 38 (2015).

*Corresponding authors: ungureanucamelia@gmail.com; elipatras@gmail.com; nicoleta.badea@gmail.com; purcarvioleta@gmail.com; ana24ispas@yahoo.com