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BANANA FLOWER NECTAR - MEDIATED SYNTHESIS OF SILVER NANOPARTICLES

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Abstract: *Worldwide, the banana is the most famous fruit on the market. All parts of the plant have been used over the years in medicine and the curative properties of the banana are well known. No reports about the study of the nectar of banana flowers could be find, except those on extracts from different parts of the plant, this being the reason of starting synthesis of silver nanoparticles (AgNPs) from the banana flower's nectar. Because conventional methods for producing NPs are expensive, toxic and non-organic in this study it was used natural sources for NPs synthesis (green synthesis). The AgNPs were characterized by UV-Vis spectrophotometry, optical microscopy (OM), scanning electron microscopy (SEM), Fourier Transform Infrared (FTIR) and Raman spectroscopies.*

Key words: *nanoparticles; UV-Vis; Raman; FTIR; SEM.*

1. Introduction

The plant that was studied in this paper is part of the species *Musa basjoo* [36] which is a herbaceous perennial plant with trunk-like pseudostems which grows to around 2-2.5 m, with a crown of mid-green leaves growing up to 2 m long and 70 cm wide when mature [1]. This species produces male and female flowers on the same inflorescence which may extend to over 1 m. The banana fruits are yellow-green, around 5-10 cm long and 2-3 cm wide; they are inedible, with sparse white pulp and many black seeds. *Musa basjoo* is one of the best known species in the genus because of its ability to grow outdoors in cool climates. Flowers are used to treat dysentery, ulcers, and bronchitis [4, 16].

The natural cycle of the plant's development begins with the appearance of a banana offshoot from the surface of the ground [23]. It grows along with the parent plant (if it arises from the rhizome) or independently if comes from seed. This branch will gradually

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transform in the adult plant, vigorous leaves will grow until it starts forming and developing of the fruit. When the fruit is ripe, the plant dries up and dies [5].

The plant used in this study was grown in pot and kept in laboratory conditions for seven years. During the summer it was taken out of the lab; it flourished (Figure 1) and fructified, but the fruits did not reach maturity [22].

Fig. 1. *Banana flower in the three stages of growth*

The plant is made up of a pseudostem (comprised of concentric layers of leaf sheaths) and an underground rhizome with a fibrous root system [35]. The rhizome is an underground stalk with numerous meristems - growth points - from which appear pseudo-stems, flowering and rooting stems and fibrous roots [31]. The leaf is made up of a longer petiole when reaching maturity (it can reach 1.5 meters) and an oval limbe which is very fragile and breaks at the edges because of to the wind [18].

The inflorescence of the banana tree comes out of the middle of the pseudostem; the flowers appear spirally along the inflorescence axis in groups of 10-20, covered in yellow-green, fleshy petals. The first flowers that appear are female (with petiole), and the last ones on the inflorescence are male (with stamens) [29]. Flowers are spread on two rows, each flower containing a nectar capsule. As the female flowers grow in the fruit, the inflorescence extends and produces bundle of flowers [19]. A third type of flower called hermaphrodite, or neutral, can be found on the stem of the female flowers and the male buds. Generally, they do not grow into a fruit and their stamen does not produce pollen [14].

The biological method offers many resources for the synthesis of silver nanoparticles and this method can be considered as an environmental friendly approach and also a low cost method [27]. The reduction rate of metal ions by using biological agents is higher and also occurs at ambient temperature and pressure. In biological synthesis, cell walls of microorganisms play a major role in the intracellular synthesis of the nanoparticles [8, 9]. The negatively charged cell wall interacts electrostatically with positively charged metal ions and reduces the metallic ions biologically to the nanoparticles. When the microorganisms are incubated with silver ions, extracellular

silver nanoparticles can be generated as an intrinsic defense mechanism against metal toxicity [20, 21]. Organic syntheses of silver nanoparticles were also performed using plant extracts as reducing agents. The nectar from the flower capsules was used in the study for synthesis of silver nanoparticles [33].

Currently, there are several ways of synthesizing silver nanoparticles by chemical, physical, photochemical and biological methods. Each method has its advantages and disadvantages, the main issues being cost, scalability, practice size and size distribution [2, 3]. When silver nanoparticles are produced by chemical synthesis, three main components are required: a silver salt (usually $AgNO_3$), a reducing agent (e.g. ethylene glycol) and a stabilizer or coating agent in order to control the growth of nanoparticles and prevent their aggregation by collision [6]. In the case of biological synthesis of silver nanoparticles, the reducing agent and stabilizer are replaced by molecules produced by living organisms [11, 12]. These reducing and / or stabilizing compounds can be taken from bacteria, fungi, yeasts, algae or plants.

It has been scientifically proven that silver nanoparticles are an effective biocide against a wide range of bacteria, including both gram-negative and positive bacteria, including many strains with a very high virulence degree [24].

It has also been reported that the antibacterial activity of silver nanoparticles against bacteria has been divided into three steps: (1) nanoparticles measuring 1-10 nm attach to the surface of the cell membrane and drastically disrupt its normal functions such as permeability and respiration; (2) the nanoparticles are able to penetrate the bacteria and to cause further damages by interacting with the sulfur and phosphorus compounds, such as the DNA; (3) nanoparticles release silver ions, which will further contribute to the bactericidal effect of silver nanoparticles. Silver nanoparticles have been shown to be a potential antiviral and biocide agent and thus could be helpful in preventing viral and fungal infections [28, 30, 34].

2. Materials and Methods

2.1. Materials

Aqueous solution (1 mM) of silver nitrate ($AgNO_3$) was prepared in 25 mL rated flasks, then 5 mL nectar (undiluted) and 5 mL aqueous solution of silver nitrate was mixed for reduction into Ag^+ ions. The color changed from yellow light to yellow dark in maximum 30 min. The reaction occurred in laboratory conditions (at room temperature and 30 % humidity) [32].

2.2. Methods

UV-Visible spectra of the silver nanoparticles were observed using a Thermo Scientific Evolution 260 BIO spectrophotometer in the wavelength range of 350-700 nm. Scanning was performed at 1 h, 3 h and 24 h, respectively and formation of AgNPs was furthermore confirmed by spectrophotometric analysis.

In order to determine the functional groups of nectar and their possible involvement in the synthesis of silver nanoparticles, FTIR analysis was performed using Vertex 80v spectrometer (Bruker, Germany) equipped with a ATR accessory and high-resolution microscope Hyperion 3000. The IR spectra were collected at 4 cm^{-1} resolution in transmission mode (4000-400 cm^{-1}) with 32 scans per sample. As a reference, the background spectrum of air was collected before the acquisition of the sample spectrum.

Complementary with FTIR spectroscopy was performed Raman analysis using a Xantus-2 portable analyzer (Rigaku, United States of America). Raman spectra were collected in the range of 2000-200 cm^{-1}, with 7-10 cm^{-1} spectral resolution, using 1064 nm LASER wavelength, 490 mW LASER power, 1000 ms integration time, and 3 scans/sample.

Primo Star (Zeiss, Germany) microscope having 100x magnifications was suited for revealing organic microstructures. It has wide optic spectral magnification range and it also allows real time acquisition images through its mounted camera (Axiocam 105). More than that, the acquired images are processed using proprietary software (Zen)

For the actual study, the images for all of the samples were obtained using transmitted light mode along with Plan-ACHROMAT 100x/0.25 dry objective.

The morphological characterization of AgNPs obtained in nectar sample were investigated using field emission scanning electron microscope (FE-SEM) SU-70 (Hitachi, Japan). The mixture of banana nectar with $AgNO_3$ was pipetted on 400 mesh copper grids (Ted Pella, United States of America) and analyzed in STEM mode. The operating conditions were: 10 kV accelerating voltage, ~16 mm working distance, and vacuum $5 \cdot 10^{-8}$ Pa.

3. Results and Discussions

The aqueous solution of silver nitrate acts as a source of silver ions for the synthesis of silver nanoparticles. The silver ions were reduced to silver atoms by nectar of *Musa basjo* flower which nucleate to form nanocrystallites for growth. UV-Vis spectrophotometry, Attenuated Total Reflection - Fourier Transform Infrared (ATR-FTIR) and Raman spectroscopies were used to identifying the chemical composition of the nectar and to characterize the obtained AgNPs.

The primary characterization of silver nanoparticles by absorbance spectral studies has proven to be a UV-Vis technique for the analysis of metal nanoparticles. A defined sharp band, with maximum absorbance at 450 and 439 nm, respectively, for silver nanoparticles synthesized by *Musa basjo*, confirmed the formation of nanoparticles in colloidal state [7, 10]. UV-Vis spectrophotometry, Attenuated Total Reflection - Fourier Transform Infrared (ATR-FTIR) and Raman spectroscopies were used to identifying the chemical composition of the nectar and to characterize the obtained AgNPs.

The UV-Vis spectra (Figure 2) have been recorded after 1 h, 3 h and 24 h since the reaction initiation (silver nanoparticles appear yellowish in color). The not-diluted sample was scanned for analysis; a characteristic peak of silver nanoparticles could be highlighted in the spectrum at: 450 nm / A = 2.12 (1 h), 450.3 nm / A = 2.13 (3 h) and

439.2 nm / A = 1.58 (24 h), respectively. Spectral data obtained suggests that the reduction of silver ions and nucleation processes occurs relatively fast (one hour from initiation); the decrease of peak intensity and slightly blue shifting can be due to stabilization of small nanoparticles due to compounds from natural product (banana flower nectar).

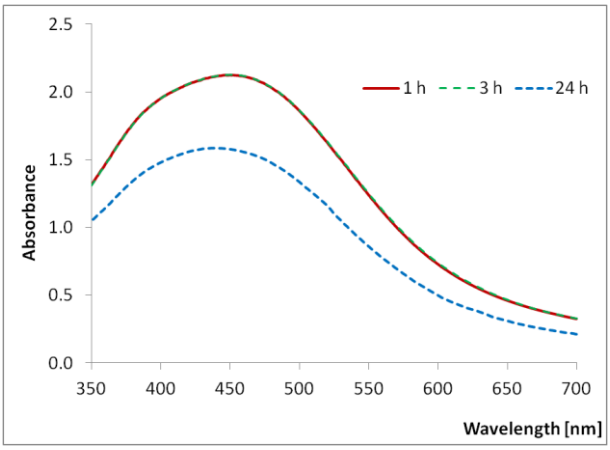

Fig. 2. *The UV-Vis spectrum of AgNP obtained in banana flower nectar*

In the IR recorded spectrum (Figure 3) a strong peak is present at 3256 cm^{-1}, due the stretching vibration of O-H groups from aromatic ring. The medium signal from 1640 cm^{-1} was assigned to ethanol presence or asymmetric and symmetric stretching vibrations of the nitrate group, while the weak signal registered at 996-1106 cm^{-1} can be assigned to C-O stretching and –OH deformation of carbohydrates (polysaccharides). Weak IR bands between 1140-1056 cm^{-1} specific for C-N stretching vibrations of aliphatic amines or C=O stretching vibrations of phenols (which are probable found in the nectar as polyphenols, polysaccharides and proteins) [15, 17, 25, 26].

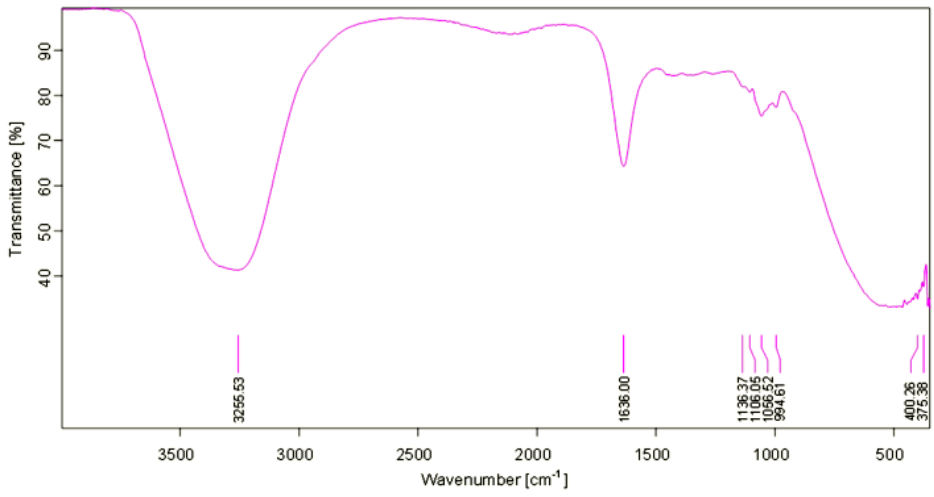

Fig. 3. *The FTIR spectra of banana nectar - $AgNO_3$ mixture*

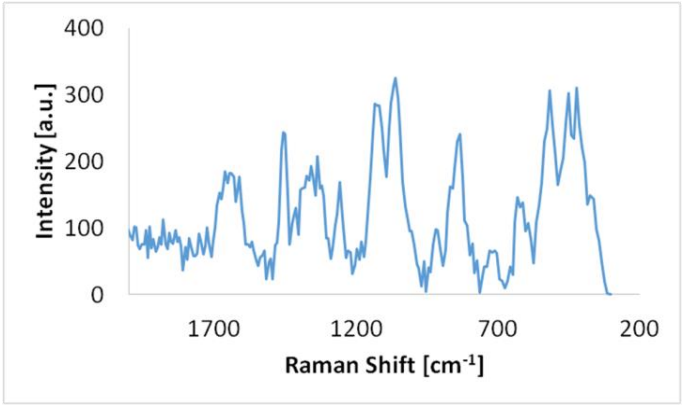

Fig. 4. *The Raman spectra of banana nectar - $AgNO_3$ mixture*

In the Raman spectrum (Figure 4) the peaks recorded at 1610, 1522 and 1448 cm^{-1} can be assigned to stretching vibrations C=C of phenyl ring of flavonoids. The carotenoid esters can be highlighted by the presence of peaks from 1752 cm^{-1} (stretching vibrations of carboxylic group C=O) and 1500 cm^{-1} (C-H symmetrical bending in $–CH_3$ or the carboxyl group COO). The peaks recorded at 961, 911, 860, 834, 518, 452, and 423 cm^{-1} corresponding to D-glucose compound. At 1060-1124 cm^{-1} C-C groups are present while at 1335-1411 cm^{-1} CH_3 groups are found [13].

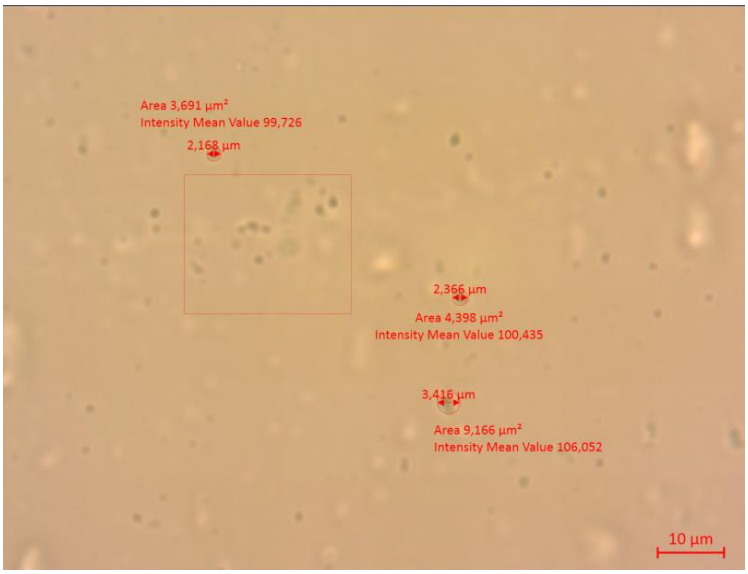

Fig. 5. *The OM image of banana nectar with $AgNO_3$*

The mixture of banana nectar with $AgNO_3$ pipetted on copper grid was investigated by optical microscopy. In Figure 5 can be observed some grey spots characterized by round shape and micrometric sizes (i.e. 2.168 µm, 2.366 µm, 3.416 µm); also, the white spots have unregulated shapes and are bigger than the grey spots.

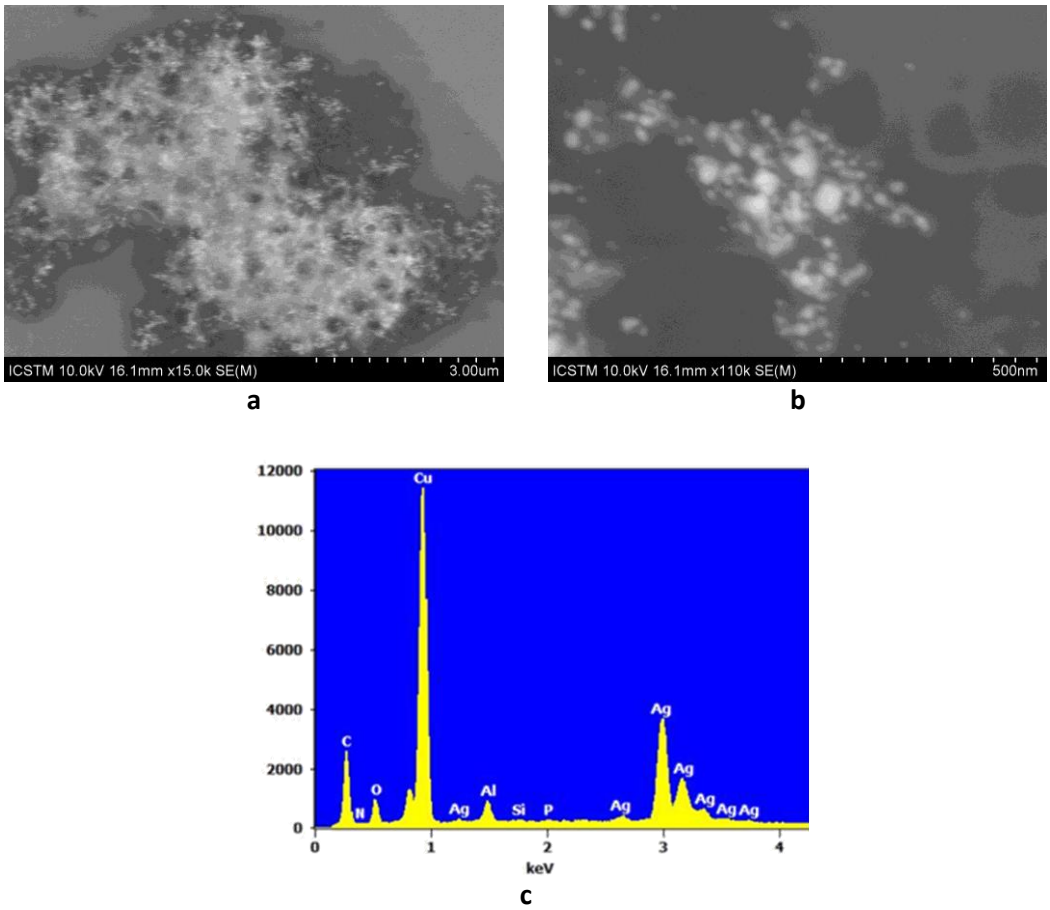

Fig. 6. *The SEM-EDS analysis of AgNPs obtained in banana flower nectar: a*) *SEM image at 15kx; b*) *SEM image at 110kx; c*) *EDS spectrum*

The morphology details, as size and shape of AgNP-banana flower nectar, were certified by using SEM-EDS analysis. The SEM image (Figure 6 a-b) showed a very high density of silver nanoparticles. It was observed the development of silver nanostructures is uneven in size (17-80 nm) but uniform in shape (spherical).

If it is desired that obtained nanoparticles to be stable (not agglomerated), some substances (i.e. stabilizers) should be used. The EDS analysis (Figure 6c) confirms that in banana nectar, silver nanoparticles were obtained. Also, the presence of Al and Si (from stub), C and Cu (from STEM grid) is also highligted. Other chemical elements, such as N and P are present in the sample but in very small quantities.

4. Conclusion

AgNPs synthesis in banana nectar was investigated using UV-Vis spectrophotometry at 430 nm and the phenolic compounds were revealed by ATR-FTIR and Raman spectroscopies. SEM analysis of sample highlights formation of spherical silver nanoparticles with sizes in the range of 17-80 nm. SEM and UV-Vis data confirm the

presence of silver nanoparticles uneven in size (the relatively large peak from UV-Vis spectra) and relatively spherical in shape. Images obtained both from optical microscopy and SEM shows a clear layer around the nanoparticles due to the natural stabilizers (the compounds presents in natural product); the small clusters of nanoparticles can be formed mostly due to sample preparation, the UV-Vis spectra of colloidal solution at 24 h suggesting disperse nanoparticles without agglomeration. In the same time both FTIR and Raman data suggest the presence of natural stabilizers around silver nanoparticles.

References

1. Agrawal, A., Verma, S., Sharma, N., et al.: *Cryoconservation of some wild species of Musa L.*. In: Indian Journal of Genetics and Plant Breeding (2014) 74(4S), p. 665-669.
2. Banerjee, P., Satapathy, M., Mukhopahayay, A., et al.: *Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis*. In: Bioresources and Bioprocessing (2014) Vol. 1, Article no. 3.
3. Bankar, A., Joshi, B., Kumar, A.R., et al.: *Banana peel extract mediated novel route for the synthesis of silver nanoparticles*. In: Colloids and Surfaces A: Physicochemical and Engineering Aspects (2010) Vol. 368, p. 58-63.
4. Chang, S., Wu, Z., Sun, W., et al.: *Factors Related to Physical Strength of Banana Pseudoste.* In: Advances in Bioscience and Biotechnology (2018) Vol. 9, p. 136-146.
5. Čížková, J., Hřibová, E., Christelová, P., et al.: *Molecular and Cytogenetic Characterization of Wild Musa Species*. In: PLOS One (2015) Vol. 10, Article no. e0134096.
6. Durán, N., Marcato, P.D., De Conti, R., et al.: *Potential Use of Silver Nanoparticles on Pathogenic Bacteria, their Toxicity and Possible Mechanisms of Action*. In: Journal of the Brazilian Chemical Society (2010) Vol. 21(6), p. 949-959.
7. Florescu, M., Stihi, C., Radulescu, C., et al.: *Mineral composition of Lavandula angustifolia flowers and Hippophae rhamnoides fruits extracts*. In: Journal of Science and Arts (2017) No. 4(41), p. 789-794.
8. Gogoi, K., Saikia, J.P., Konwar, B.K.: *Immobilizing silver nanoparticles (SNP) on Musa balbisiana cellulose.* Colloids and Surfaces B: Biointerfaces (2013) Vol. 102, p. 136-138.
9. Ibrahim, H.M.M.: *Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms.* In: Journal of Radiation Research and Applied Sciences (2015) Vol. 8, p. 265-275.
10. Krishnamurthi, P.P., Ramalingam, H.B., Raju, K.: *FTIR studies of hydrogen bonding interaction between the hydroxyl and carbonyl liquids*. In: Advances in Applied Science Research (2015) Vol. 6(12), p. 44-52.
11. Kubyshkin, A., Chegodar, D., Katsev, A., et al.: *Antimicrobial Effects of Silver Nanoparticles Stabilized in Solution by Sodium Alginate.* In: International Journal of Biochemistry and Molecular Biology (2016) Vol. 2(2), doi:10.21767/2471-8084.100022.

12. Küünal, S., Kutti, S., Rauwel, P., et al.: *Biocidal properties study of silver nanoparticles used for application in green housing.* In: International Nano Letters (2016) Vol. 6(3), p. 191-197.
13. Lingegowda, D.C., Kumar, J.K., Prasad, A.G.D.: *FTIR spectroscopic studies on cleome gynandra - comparative analysis of functional group before and after extraction*. In: Romanian Journal of Biophysics (2012) Vol. 22(3-4), p. 137-143.
14. Lý, N.S., Lê, C.K., Trieu, T.D., et al.: *A distinctive new species of wild banana (Musa, Musaceae) from northern Vietnam.* In: Phytotaxa (2012) Vol. 75, p. 33-42.
15. Liyanage, R., Gunasegaram, S., Visvanathan, R., et al.: *Banana Blossom (Musa acuminate Colla) Incorporated Experimental Diets Modulate Serum Cholesterol and Serum Glucose Level in Wistar Rats Fed with Cholesterol.* In: Cholesterol (2016) Vol. 2016, Article ID 9747412.
16. Liu, A.Z., Li, D.Z., Li, X.W.: *Taxonomic notes on wild bananas (Musa) from China.* In: Botanical Bulletin of Academia Sinica (2002) Vol. 43, p. 77-81.
17. Mahmood, A., Ngah, N., Omar, M.N.: *Phytochemicals Constituent and Antioxidant Activities in Musa x Paradisiaca Flower.* In: European Journal of Scientific Research (2011) Vol. 66(2), p. 311-318.
18. Manilal, V.B., Sony, J.: *Banana Pseudostem Characterization and ItsFiber Property Evaluation on Physical and Bioextraction*. In: Journal of Natural Fibers (2011) Vol. 8, p. 1-12.
19. Murphy, M., Clare, E.L., Rydell, J., et al.: *Opportunistic use of banana flower bracts by Glossophaga soricina.* In: Acta Chiropterologica (2016) Vol. 18(1), p. 209-213.
20. Murugan, T., Ravikumar, V., Chitra, B., et al.: *Synthesis and Characterization of Environment Friendly Silver Nanoparticles.* In: Journal of Pharmaceutical Sciences and Research (2017) Vol. 9(9), p. 1545-1548.
21. Narayanamma, A.: *Natural Synthesis of Silver Nanoparticles by Banana Peel Extract and As an Antibacterial Agent.* In: Journal of Polymer and Textile Engineering (2016) Vol. 3(1), p. 17-25.
22. Ogbonna, O.A., Izundu, A.I., Ikeyi, A.P., et al.: *Proximate Compositions of fruits of Three Musa Species at Three Stages of Development.* In: Journal of Dental and Medical Sciences (2016) Vol. 15(6), p. 107-117.
23. Padam, B.S., Tin, H.S., Chye, F.Y., et al.: *Banana by-products: an under-utilized renewable food biomass with great potential*. In: Journal of Food Science and Technology (2014) Vol. 51(12), p. 3527-3545.
24. Pulit-Prociak, J., Banac, M.: *Silver nanoparticles - a material of the future...?*. In: Open Chemistry (2016) Vol. 14 (1), p. 76-91.
25. Radulescu, C., Stihi, C., Ilie, M., et al: *Characterization of Phenolics in Lavandula Angustifolia.* In: Analytical Letters (2017) Vol. 50(17), p. 2839-2850.
26. Radulescu, C., Olteanu, R.L., Stihi, C., et al.: *Chemometric Assessment of Spectroscopic Techniques and Antioxidant Activity for Hippophae rhamnoides L. Extracts Obtained by Different Isolation Methods.* In: Analytical Letters (2019) Vol. 52, doi: 10.1080/00032719.2019.1590379.

27. Rafique, M., Sadaf, I., Rafique, M.S., et al.: *A review on green synthesis of silver nanoparticles and their applications*. In: Artificial Cells, Nanomedicine, and Biotechnology (2017) Vol. 45(7), p. 1272-1291.
28. Sahni, G., Panwar, A., Kaur, B.: *Controlled green synthesis of silver nanoparticles by Allium cepa and Musa acuminata with strong antimicrobial activity*. In: International Nano Letters (2015) Vol. 5(2), p. 93-100.
29. Sharma, M., Patel, S.N., Sangwan, R.S.: *Biotransformation of banana pseudostem extract into a functional juice containing value added biomolecules of potential health benefits.* In: Indian Journal of Experimental Biology (2017) Vol. 55, p. 453-462.
30. Siddiqi, K.S., Husen, A., Rao, R.A.K.: *A review on biosynthesis of silver nanoparticles and their biocidal properties*. In: Journal of Nanobiotechnology (2018) Vol. 16, Article no. 14.
31. Singh, S.: *Banana blossom-an understated food with high functional benefits.* In: International Journal of Current Research (2017) Vol. 9(01), p. 44516-44519.
32. Suica-Bunghez, I.R., Ion, R.M., Teodorescu, S., et al.: *Fitochemical and antioxidant characterization of Autumn crocus (Colchicum autumnale) flowers and roots plant extracts*. In: Journal of Science and Arts (2017) No. 3(40), p. 539-546.
33. Sun, Q., Cai, X., Li, J., et al.: *Green synthesis of silver nanoparticles using tea leaf extract and evaluation of their stability and antibacterial activity*. In: Colloids and Surfaces A: Physicochemical and Engineering Aspects (2014), Vol. 444, p. 226-231.
34. Tran, Q.H., Nguyen, V.Q., Le, A.T.: *Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives.* In: Advances in Natural Sciences: Nanoscience and Nanotechnology (2013) Vol. 4, Article no. 033001.
35. Xiao, Y., Chen, J., Kuang, J., et al.: *Banana ethylene response factors are involved in fruit ripening through their interactions with ethylene biosynthesis genes*. In: Journal of Experimental Botany (2013) Vol. 64(8), p. 2499-2510.
36. Zheng, K., Fan, X., Mao, Y., et al.: *The well-designed hierarchical structure of Musa basjoo for supercapacitors.* In: Scientific Reports (2016) Vol. 6, Article no. 20306.