Environmental Engineering and Management Journal

April 2021, Vol. 20, No. 4, 605-611
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

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PURPLE KOHLRABI PEEL, A NATURAL MATERIAL FOR ECO-FRIENDLY SILVER AND GOLD NANOPARTICLES

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Abstract

Green chemistry is constantly detaching itself as an important alternative to conventional chemical routes since it is environmentally friendly, cost effective, neither does it require the use of toxic chemicals nor does it produce hazardous by-products. For preparing eco-friendly silver and gold nanoparticles, numerous plants have been used and the method involves the following steps: collecting the plant, drying and finely grinding it, mixing the plant with water to obtain the aqueous extract that further reacts with the inorganic salt to form the corresponding metallic nanoparticles. Kohlrabi (*Brassica oleracea* Gongylodes group) is a versatile vegetable rich in vitamin C with a multitude of nutrients beneficial to human health: can help lower the blood pressure and the cholesterol, fights cancer, is an adjuvant in weight loss, etc. This paper presents the eco-friendly synthesis of two metallic nanoparticles, namely silver nanoparticles (AgNPs), gold nanoparticles (AuNPs) from the aqueous extract of purple Kohlrabi peel and the corresponding metallic salts at different temperatures. The qualitative and quantitative screening of phytochemicals from the aqueous extract was carried out using standard analytical methods and the formation of AgNPs and AuNPs was investigated by recording UV-Vis at different time intervals, FTIR and DLS spectra.

Key words: gold nanoparticles, green chemistry, purple Kohlrabi, silver nanoparticles

Received: June, 2020; Revised final: February, 2021; Accepted: March, 2021; Published in final edited form: April, 2021

1. Introduction

Worldwide, there is a growing need for environmentally friendly methods that enable the preparation of a variety of metallic nanoparticles (e.g.: silver, gold, platinum, iron oxide etc.) (Iravani et al., 2015). Therefore, the shift towards green chemistry is constant and has challenged the scientists to research alternative routes that involve the use of microorganisms, biological systems and vegetal materials (Beattie and Haverkamp, 2011). Plant-mediated synthesis, "phytosynthesis", is an important field in nano-biotechnology, the field that where biological processes meet with physical-chemical techniques to obtain well – defined nanoparticles (Husen and Siddiqi, 2014). Green chemistry is used to obtain silver (AgNPs) and gold nanoparticles (AuNPs) from plant extracts having numerous advantages: cost effectiveness, eco-friendlyness, no need for high pressure/temperature and no toxic chemicals are involved (Alsammarraie et al., 2018; Lopes and Courrol, 2018).

Silver is widely recognized as a metal with good conductivity, catalytic activity and enhanced antimicrobial capacity. Silver nanoparticles (AgNPs) have numerous applications that range from water filtration to more complex targeted drug delivery and gene therapy. Gold nanoparticles are intensively used in the biomedical field due to their large surface area and good electron conductivity (Tedesco et al., 2010).

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Silver and gold nanoparticles are well - known for their antimicrobial, antifungal and antimycotic activity making them key components in different applications (Fig. 1) (Geethalakshmi et al., 2010).

Kohlrabi (*Brassica oleracea* Gongylodes group) is a vegetable rich in vitamin C highly recommended for human consumption due to its health benefits, packed with nutrients and easily available since it grows relatively easy in most climates (Fig. 2) (Martinez-Espla et al., 2001; Wiebe et al., 1992). Purple Kohlrabi is an important source of dietary fibers, being considered the perfect vegetable adjuvant in weight loss. It contains significant amounts of potassium, iron and manganese, nutrients that can improve bone strength (Walter et al., 1997). Usually, purple Kohlrabi's peel is not consumed because it is quite tough and is, therefore, thrown away although it contains anthocyanins (from where it takes is purple color). So, this method of preparing both AgNPs and AuNPs also contributes to reducing household wastes.

This paper presents the eco-friendly synthesis of silver (AgNPs) and gold nanoparticles (AuNPs) from the aqueous extract of purple Kohlrabi peel and the corresponding metallic salts at different temperatures and different reaction conditions. The qualitative screening of phytocompounds was carried out using standard analytical methods and, by spectrophotometric determinations, the total content of different bioactive compounds was accurately determined.

The simplest way of monitoring the initial formation of both noble metallic nanoparticles is recording their UV – Vis spectra at different time intervals. Furthermore, in order to determine the presence of functional groups, FTIR determinations were performed and the particle size of both AgNPs and AuNPs was evaluated using the DLS technique. DPPH method allowed an accurate determination of the antioxidant activity of the two green synthesized nanoparticles and their aqueous extract precursor.

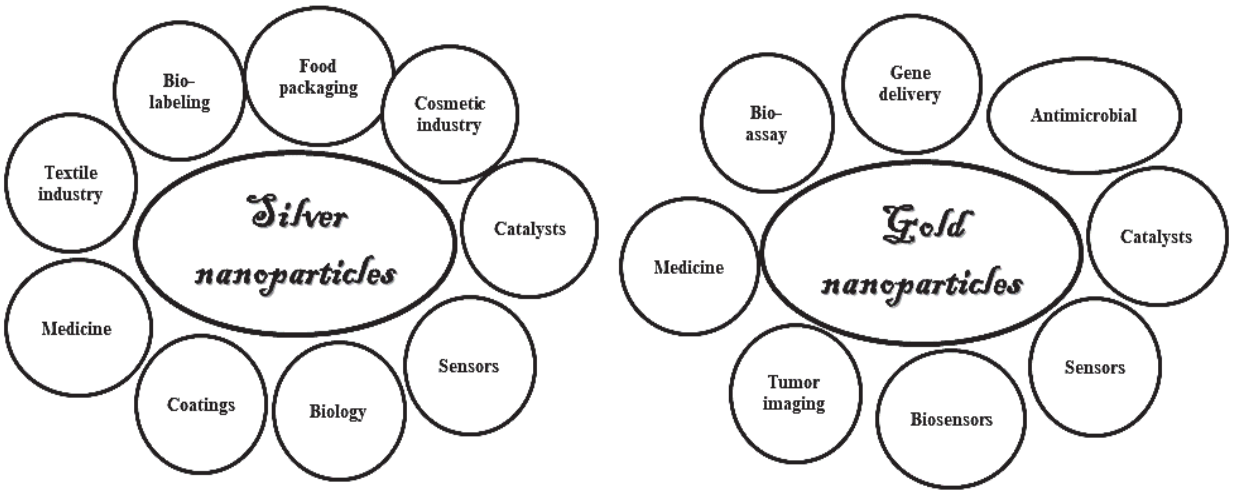

Fig. 1. Applications of silver and gold nanoparticles

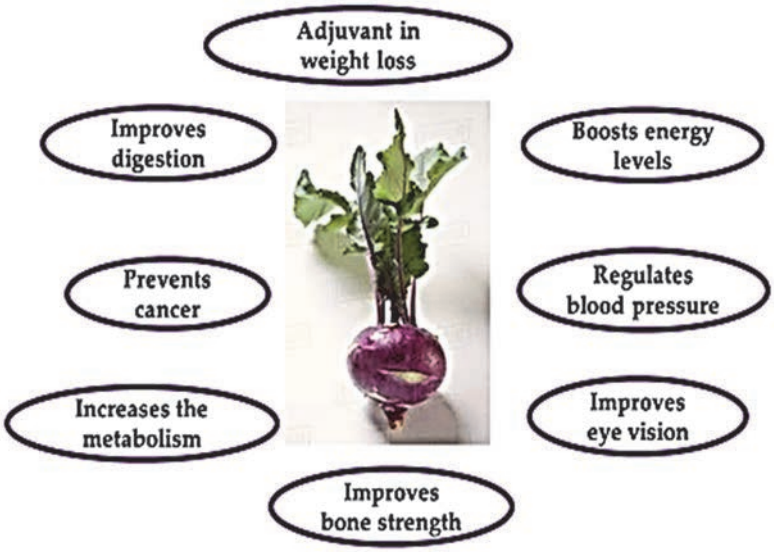

Fig. 2. Health benefits of purple Kohlrabi

2. Materials and method

2.1. Chemicals

2,2 – diphenyl – 1 – picryl – hydrazyl – hydrate (DPPH), hydrochloric (HCl) and sulphuric (H_2SO_4) acid, copper sulphate, silver nitrate ($AgNO_3$), tetrachloroauric acid ($HAuCl_4$), copper sulphate, aluminium chloride, lead acetate, catechin and gallic acid standards, reagents (Folin – Ciocalteu, Benedict and Millon) were al acquired from SigmaAldrich.

Alcohols (e.g.: methanol CH_3OH, ethanol C_2H_5OH) and sodium hydroxide (NaOH) were purchased from Scharlau. Distilled water was prepared, freshly, in our own laboratory, for all the experiments.

2.2. Preparation of the aqueous extracts from purple Kohlrabi peel

In this respect, we've used the peel from freshly bought purple Kohlrabi that was subjected to several treatments: the peel was cut off, washed consequently with tap water and double distilled water, dried (away from sunlight) at room temperature for 8 days, cut into very small pieces and grinded using a porcelain grinding mortar.

- 25 g dried purple Kohlrabi peel (pKp) was weighted, transferred into a glass "French press" type extractor and infused with 250 mL distilled water for 24 hours in a refrigerator (4 ^{0}C);
- the aqueous pKp extract was filtered until a clear liquid is obtained;
- the aqueous pKp extract is stable at 4 ^{0}C for more than 4 months.
- The color of the aqueous Kohlrabi peel extract is violet.

2.3. Qualitative screening of carbohydrates

In general, the qualitative screening of phytochemicals (e.g.: saponins, carbohydrates, flavonoids, etc.) is based on standard chemical principles that rely on a color change reaction as a positive response (Caroling et al., 2015).

The literature describes Molisch's test (considered a general test that determines the presence of various carbohydrates) as follows: over 2 mL of aqueous purple Kohlarbi peel (pKp) extract, 1 mL Molisch reagent (α – naphtol in ethanol) is added and some drops of concentrated sulphuric acid are carefully pipeted (Kokate et al., 2002). The positive response translates in the appearance of a violet ring at the surface once all the reactants are added.

Researchers (Oloyede et al., 2014; Sofawora, 1982; Tona, 2005) described different other qualitative test that suggest the presence of various carbohydrates:

- Benedict test: 1 mL aqueous extracts is combined with 5 mL Benedict reagent; the solution is boiled (5 minutes) and the results are: if initially the color is green, after the 5 minutes passed, the solution forms either a red, or yellow or green precipitate (the difference in color resides from the amount of carbohydrates)
- Fehling A test: the principle is that the solution formed by adding few drops of Fehling A reagent to 1 mL pKp aqueous extract should turn green if carbohydrates are present.
- Fehling B test: aqueous extract (1 mL) was mixed with 2 - 3 drops of Fehling B solution and, if the resulted mixture turned brown, carbohydrates are present.
- Barfoed test: 3 mL Barfoed reagent (a solution of copper acetate in glacial acetic acid) were added to 1 mL aqueous extract and boiled for a duration of 2 minutes and then left to cool at room temperature. If carbohydrates are present, the resulted mixture should form a red precipitate.
- Trommer test: aqueous extract (3.5 mL) was mixed with 2.5% $CuSO_4$ and 2 mL of NaOH solution (5%). The resulted mixture was boiled for 3 minutes. A blue precipitate appears which turns red upon heating.
- Tollens test: to 4 mL of aqueous extract a drop of dilute NH_4OH is added and a solution of 0.1 M $AgNO_3$ is poured. After 5-10 minutes of boiling a silver mirror is visible.
- Moore test: 5% solution of sodium hydroxide is added to 2 mL aqueous extract (equal volumes) and boiled for a duration of 5 minutes. The resulted solution should turn red.
- Seliwanoff test (for hexose sugars): 3 mL reagent (resorcinol mixed with HCl) is added to 1 mL pKp aqueous extract and heated (2 minutes). A positive response is when the resulted solution turns red.
- Cobalt chloride test: 2 mL of $CoCl_2$ are added to 3 mL aqueous extract, boiled for 2 minutes and cooled. To the resulted solution, 2-3 drops of NaOH are added and the change in color is observed: a greenish – blue or purple color or the upper part of the solution turns green-blue and lower part purple.
- Ammonium molybdate test: to 2 mL aqueous extract, 3 mL of ammonium molybdate solution are added and boiled. The change in color to bluish – green is a positive response.

2.4. Quantitative determination of phytochemicals

The quantitative analysis of bioactive compounds accurately evaluates the total content of tannins (TCT), total content of flavonoids (TCF), total content of polyphenols (TCP) and total content of terpenoids (TCTp) (Table 1) (Bunghez et al. 2011).

2.5. Green synthesis of silver and gold nanoparticles from purple Kohlrabi peel

For the green synthesis of both AgNPs and AuNPs two reaction conditions were used

- room temperature, no stirring, no heating, 24 hours;
50 ^{0}C, constant stirring of 600 rpm for 30 minutes,
- then the heat is turned off and the reaction mixture is stirred for an additional 30 minutes.

Table 1. Quantitative screening for phytochemicals

Assay	*Reaction parameters*	*Recordings*
TCT: aqueous extract (0.5 mL) was mixed with methanolic vanillin (3 mL of 5% solution) and 1.5 mL HCl were added	incubation time: 15 minutes	500 nm
TCF: distilled water (4 mL) was added over aqueous extract (1 mL) and mixed with 5% $NaNO_2$ solution (5%); after 5 minutes, we added 10% $AlCl_3$ solution (0.3 mL) and 2 mL 1M NaOH together with 2.4 mL distilled water	incubation time:30 minutes	510 nm
TCP: 1 mL aqueous extract was mixed with Folin – Ciocalteu reagent (5 mL); Na_2CO_3 (4 mL aqueous solution) was added after 8 minutes	incubation time: 60 minutes	765 nm
TCTp: 2% H_2SO_4 – vanillin solution (1 mL) is carefully added over 2 mL of aqueous extract	incubation time: 20 minutes	608 nm

In the case of AgNPs, 50 mL of a freshly prepared 10^{-3} M aqueous solution of silver nitrate ($AgNO_3$) was mixed with 5 mL pKp aqueous extract and the next day, the resulted AgNPs were stirred for 30 minutes in an ultrasound bath, at a constant speed of 100 rpm. The same principle is used for the green synthesis of AuNPs with the only difference that a 10^{-3}M aqueous solution of tetrachloauric acid ($HAuCl_4$) was used.

2.6. Physical – chemical characterization

All the UV – Vis absorption spectra were recorded using a Carl Zeiss Jena spectrometer in the range of 250 – 800 nm. Quantitative determinations of TCT, TCF, TCP and TCTp was achieved using a JK VS 721 N Visible spectrophotometer following the details described in Table 1. The green synthesis at 50 ^{0}C of both noble metallic nanoparticles was carried out using a Phoenix Instrument magnetic stirrer. To perfect the green synthesis, a Bioblock Scientific ultrasonic bath was used. Fourier transform infrared spectroscopy (FTIR) spectra were recorded using a Vertex 80 FT-IR spectrometer with high-resolution Hyperion 3000 microscope, in the range of 8000 – 400 cm^{-1}. Dynamic light scattering (DLS) spectra were recorded using a Zetasizer Nano SZ – Malvern with a computer connected equipped with preinstalled Zetasizer software.

Antioxidant activity (AA, %) was evaluated using the DPPH assay: 1 mL ethanolic solution of DPPH (0.02 mg/mL) was mixed with 0.5 mL pKp aqueous extract and we've recorded the absorbance at 517 nm. In parallel, a blank solution was prepared (0.5 mL double distilled water were added to 1 mL 0.02 mL DPPH solution (Mosquera et al., 2009) (Eq. 1):

$$AA\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}}\ x\ 100 \quad (1)$$

In the equation presented above, $A_{control}$ describes the absorbance of the blank DPPH solution while A_{sample} stands for the absorbance of the pKp with 0.02 mg/mL of DPPH solution.

3. Results and discussion

3.1. Qualitative screening of carbohydrates

The general principle of the qualitative screening of carbohydrates is that, when certain reagents are added to the pKp aqueous extract, it changes colour if that specific carbohydrate is present. Carbohydrates, the different sugars that are found in every fruits or vegetables, are in the top three macronutrients that should be part of every day's healthy diet (Giacco et al., 2016). The results for the qualitative screening of carbohydrates of the pKp aqueous extract are presented in Table 2.

In the above – presented table, we used different abbreviations as follows: "+" = weak; "++" = intense; "+++" = very intense; "-" = absent. Molisch's test clearly states that various types of carbohydrates (e. g.: hexose sugars, fructose, glucose, reducing sugars, etc.) are present in the pKp aqueous extract. The cobalt chloride test shows the presence of glucose in pKp aqueous extract. Fehling's test is specific for evaluating the presence of reducing sugars and aldehydes and the results show that these carbohydrates are present in pKp. Benedict's test is a semi-quantitative test and the color of the precipitate indicates that important amounts of reducing sugars are present.

The opalescent red solution resulted by performing Barfoed's test show that monosaccharides are present and Seliwanoff test proved the presence of keto hexoses. So, by performing various qualitative tests on the pKp aqueous extract it was observed that carbohydrates are present, whether they are reducing sugars or keto hexoses and that allowed to conclude that pKp is a suitable raw material for the green synthesis of AgNPs and AuNPs.

3.2. Quantitative determination of phytochemicals

TCT and TCF were evaluated as mg catechin/L and thrice measured. Consequently, TCP used galic acid as standard and TCTp used linalool as standard calibration curve.

The results were recorded at different time intervals and are detailed in Table 3. It is clear from the Table above that pKp aqueous extract contains important amounts of polyphenols (between 430 .993 mg/L and 431 .356 mg/L) which comes only to emphasize that purple Kohlrabi peel is a good capping and reductive agent for the green synthesis of noble metallic nanoparticles. Flavonoids are also present in good amounts, varying from 160 .988 mg/L for pKp aqueous extracts analyzed 48 hours after the preparation and 161 .30 mg/L immediately after it was prepared.

Table 2. Qualitative screening for carbohydrates

Phytochemical test	***Pkp aqueous extract***
Molisch	++ (opalescent purple solution)
Benedict	+++ (green precipitate)
Fehling A	++ (turquoise-green solution)
Fehling B	+ (yellow-brownish solution)
Barfoed	+ (opalescent red solution)
Trommer	+++ (blue-greenish precipitate)
Tollens	+ (brown solution with "silver mirror")
Moore	+++ (red solution)
Seliwanoff	+++ (light red solution)
Cobalt chloride	+++ (fructose) (gel-like blue precipitate)
Ammonium molybdate	+++ (blueish-green solution)

Table 3. Quantitative determinations

Aqueous extract	***TCT (mg/L)***	***TCF (mg/L)***	***TCP (mg/L)***	***TCTp (mg/L)***
Purple Kohlrabi (after preparation)	91 .368	161 .30	431 .356	89 .403
Purple Kohlrabi (after 24 hours)	91 .198	161 .05	431 .066	89.105
Purple Kohlrabi (after 48 hours)	91 .006	160 .988	430 .993	88 .989

3.3. UV – Vis spectra and kinetics

UV – Vis spectra were recorded for both pKp aqueous extract and the corresponding AgNPs and AuNPs green synthesized at two temperatures: room temperature (RT) without additional stirring and at a temperature of 50 ^{0}C with an uniform stirring of 600 rpm, for a duration of 30 minutes. The first proof that AgNPs and AuNPs are obtained via green synthesis is the visual change of color (Table 4) after adding either $AgNO_3$ (AgNPs) or $HAuCl_4$ (AuNPs).

Table 4. Color of the pKp aqueous extract, AgNPs and AuNPs green synthesized thereof

No.	***Purple Kohlrabi peel***
Aqueous extract	Purple
AgNPs RT	Light brown
AgNPs 50 ^{0}C	Light brown
AuNPs RT	Light purple
AuNPs 50 ^{0}C	Purple – reddish

The pKp aqueous extract was analyzed using UV-Vis spectroscopy at different time intervals: 0s, 5 min, 30 min, 60 min, 120 min and at 24 hours after the green synthesis. The bio reduction of both AgNps and AuNPs was analyzed using UV-Vis spectroscopy between wavelengths of 250 and 800 nm. The green synthesis' kinetics of AgNPs and AuNPs was spectrophotometrically studied by evaluating how the values of absorbance of the colloidal suspensions shift at different stages of the reaction (Aleksandrova et al., 2017).

Fig. 3 presents the UV-Vis spectra of the AuNPs – pKp green synthesized at room temperature, with the blue line representing the aqueous extract and the orange one the AuNPs thereof. From the spectra it is clear that the AuNPs – pKp are obtained and the maximum absorption is at 548 nm. Similarly, by recording the UV-Vis spectra for the AgNPs-pKp at 50 0C a maximum appeared at 450 nm (Fig. 4).

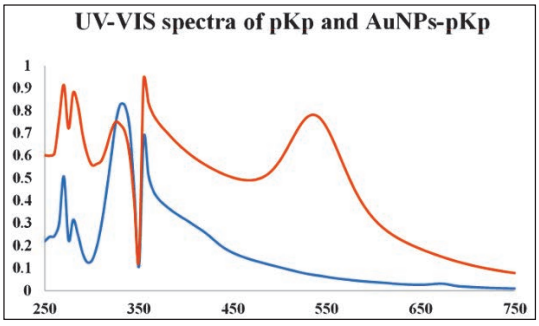

Fig. 3. UV-Vis spectra for the green synthesized AuNPs

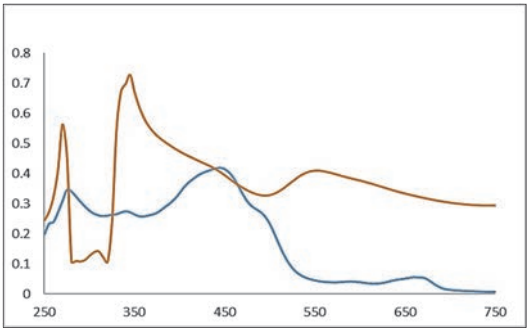

Fig. 4. UV-Vis spectra for the green synthesized AuNPs

Due to the polydispersity of the green synthesized AgNPs, absorption peaks are wide, with a maximum at 435 nm for AgNPs at room temperature and 450 for AgNPs at 50 ^{0}C respectively. From the UV – Vis spectra it could also be concluded that once the reaction time increases, the amount of both green synthesized AgNPs and AuNPs also increases up to a maximum established at 24 hours.

3.4. Antioxidant activity

Antioxidant activity was evaluated spectrophotometric using DPPH assay which mainly means that higher activity reduces absorbances and a

higher value of the concentration is not in particular related to an increased antioxidant activity. The basic principle that stands behind the DPPH assay is that, at 517 nm, an odd electron appears and that enables a color change in the DPPH solution due to electron donation from the antioxidant counterpart. The results are detailed in Table 4. In the case of green synthesized AgNPs, AA (%) varies from 78.6 to 79.33, being considerable higher than the corresponding AA for the pKp aqueous extract. By comparison, the AA (%) values for the green synthesized AuNPs are slightly lower than those obtained for AgNPs but also much higher than the ones of the aqueous extract.

Table 4. Antioxidant activity (%) of pKp aqueous extract, AgNPs and AuNPs

No.	*Purple Kohlrabi peel*
Aqueous extract	52.66
AgNPs RT	79.33
AgNPs 50 ^{0}C	78.60
AuNPs RT	70.35
AuNPs 50 ^{0}C	71.29

3.5. FTIR spectroscopy

All the studied samples (pKp aqueous extract, $AgNO_3$, $HAuCl_4$, AgNPs, AuNPs) were first dried and then analyzed using FTIR to accurately indicate various functional groups found at different wavelengths in the recorded spectra. FTIR spectra for AgNPs exhibit specific peaks at 3325 cm^{-1} assigned to hydroxyl (- OH) groups. The band that appears at 2945 cm^{-1} represents methine (-CH) groups while the bands C = C and C = O were easily identified at 1590 cm^{-1} and 1453 cm^{-1}. The pKp aqueous extract exhibits weak IR bands between 864-765 cm^{-1} (Fig. 5) characteristic to C-N stretching vibrations (aliphatic amines) or C-O stretching vibrations (alcohols or phenols).

The aromatic amide I and amide II group were found in the range of 1386 cm^{-1} and 1321 cm^{-1}. The C – O groups specific for esters, catechins and type III amides were present between 1265 – 1126 cm^{-1}. Specific bands found between 1500 – 1300 cm^{-1} were attributed to amides, proteins and enzymes that contribute to the reduction of Ag ions. All the bio – synthesized AgNPs exhibited specific polyphenols FTIR bands in the range of 1655 cm^{-1} and 1659 cm^{-1}.

3.6. DLS characterization

In order to determine the size of the green synthesized noble metallic nanoparticles, dynamic light scattering (DLS) determinations were carried out. Also, zeta potential was also determined for all the studied samples to analyze the stability of the AgNPs and AuNPs. Fig. 6 presents the DLS spectra for the green synthesized AgNPs and in Table 5 the size and polydispersity index are presented for both AgNPs and AuNPs.

4. Conclusions

In this research study we were able to achieve the green synthesis of two noble metallic nanoparticles, silver (AgNPs) and gold (AuNPs) nanoparticles starting from a renewable source, purple Kohlarbi. More specific, we've used it's peel to prepare an aqueous extract that was further used to green synthesize AgNPs and AuNPs. PKp aqueous extract was characterized using qualitative carbohydrates' content based on standard analytical techniques. The qualitative screening for carbohydrates proved that significant amounts of these macronutrients are present in the pKp aqueous extract and, therefore, pKp is an excellent raw material for the green synthesis of both noble metallic nanoparticles.

The AgNPs and AuNPs showed a significant increase of the antioxidant activity compared to the aqueous extract. Recordings following the DPPH method showed a highest value for the AgNPs at room temperature (79.33 %) and AuNPs at 50 ^{0}C (71.29 %).

From the FTIR – ATR spectra it was clear that the phytocomponents (especially proteins and flavonoids) act as reducing agents. The narrow adsorption band at 1660–1692 cm^{-1} was assigned to carbonyl group while stretching vibrations of C-O groups were detected at 1030 cm^{-1}.

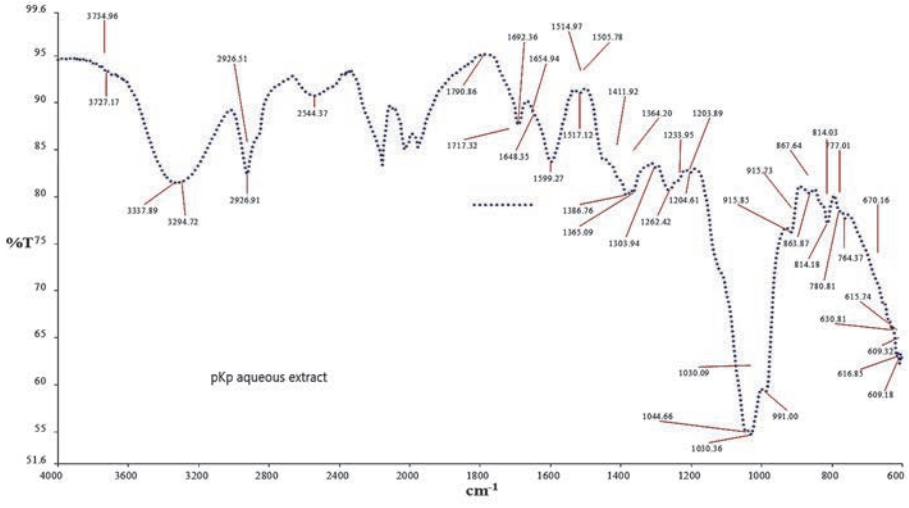

Fig. 5. FTIR spectra for the pKp aqueous extract

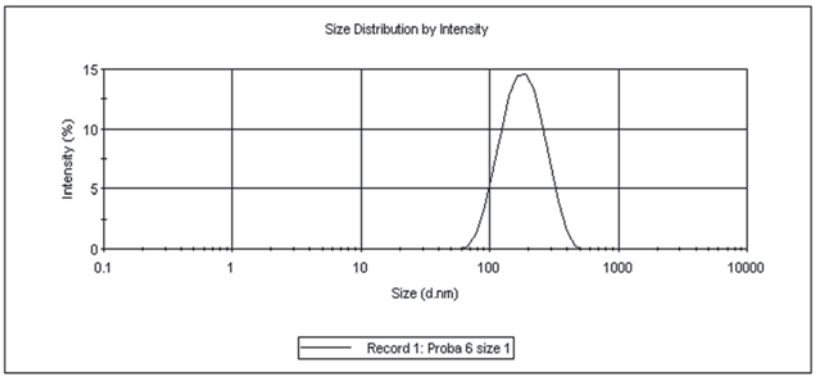

Fig. 6. DLS spectra for the green synthesized AgNPs at room temperature

Table 5. Particle size and zeta potential for AgNPs – pKp

Sample	***Dm (d.nm)***	***P1...i (d.nm)***	***PdI***	***PZ (mV)***
AgNPs RT	153	P1 = 191	0.199	- 18
AgNPs 500 C	158	P1 = 180	0.186	-16

The visual change of color was observed for both AgNPs and AuNPs and the green synthesis was confirmed by recording the UV-Vis spectra at different time intervals. The absorption peaks for AgNPs – pKp are wide, with a maximum at 435 nm for AgNPs at room temperature and 450 for AgNPs at 50 ^{0}C. From the UV – Vis spectra it could also be concluded that once the reaction time increases, the amount of both green synthesized AgNPs and AuNPs also increases up to a maximum established at 24 hours. Also, the DLS spectra proved that the size of the green synthesized AgNPs andAuNPs is in the nanometer range.

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