Antioxidant activity of environmentally - friendly noble metallic nanoparticles

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Abstract. This research paper presents the environmentally - friendly synthesis of two noble metallic nanoparticles, namely silver (AgNPs) and gold (AuNPs), from ten different plants with proven pharmaceutical benefits, e.g.: Sea buckthorn, Elderflower, Lungwort, Acacia, Cornflower, Ramson, etc. Both AgNPs and AuNPs are prepared via two routes: at room temperature, in the dark, for 24 hours and at 50^0C under a constant stirring of 600 rpm for 30 minutes. UV - Vis spectroscopy was used to investigate the formation of both AgNPs and AuNPs and compared to the UV – Vis spectra of the plain aqueous extracts prepared at 4^0 C. The main purpose of our research was to investigate the antioxidant activity of the aqueous extracts and noble metallic nanoparticles obtained thereof and to determine whether the temperature-conducted environmentally - friendly synthesis of AgNPs and AuNPs influences the antioxidant activity in any way.

1. Introduction

Nanotechnology is a multidisciplinary scientific field that includes elements from physics, chemistry, material science, chemical and mechanical engineering, biology and medicine [1], [2]. Due to their high surface area, nanoparticles can be functionalized with different molecules thus rendering them antibacterial, magnetic, catalytic or magnetic properties [3], [4].

Metallic nanoparticles can be obtained using various conventional methods, involving either "top down" or "bottom up" processes that are usually expensive, time consuming, use toxic chemicals and produce hazardous secondary products [5], [6]. Viable and feasible alternatives are environmentally – friendly methods that use microorganisms, fungi, enzymes or plants [7 – 9] and, among them, aqueous plant extract mediated biosynthesis of silver (AgNPs) and gold (AuNPs) nanoparticles are intensively studied since silver and gold are two noble metals widely known for their strong antibacterial properties [10], [11]. The different phytochemical compounds found in the plants act as catalysts in the reduction mechanism of silver and gold salts to corresponding nanoparticles [12].

Sea buckthorn (*Hippophae rhamnoides*), a fruit that contains a multitude of vitamins (A, C, E), antioxidants and essential fatty acids, has long been known for its therapeutic actions and is used in traditional medicine to prevent aging, reduce inflammation, fights against oxidative stress, free

radicals and bacteria [13], [14]. Acacia (*Robinia pseudacacia*) is also a medicinal herb used in traditional medicine that helps wound healing and recent studies claim that it may help heal ulcers [15]. Acacia gum contains different water-soluble dietary fibers, a good source of fibers, that help keep the cholesterol levels under control [16]. Celandine (*Ranunculus ficaria*), a plant belonging to the buttercup family, is traditionally used as adjuvant in hemorrhoidal affections, may help wound healing and it has been used as an anti-spasmodic and analgesic [17]. Gooseberries (*Ribes grossularia*), although relatively small fruits, are packed with health benefits: contain vitamins (C, B5, B6, E), are a good source of antioxidants, may keep under control blood sugar levels, can act as adjuvants in degenerative brain disorders [18], [19]. Ramson (*Allium ursinum*), its leaves more specific, contain adenosine which can help in regulating high blood pressure and tachycardia, used as tonic to cleanse blood and boost the immune system [20], [21].

Elderflower (*Sambucus nigra*) has been used in traditional medicine due to its antiseptic and anti-inflammatory properties, has diuretic and laxative properties and reduces blood sugar levels [22], [23]. Cornflowers (*Centaurea cyanus*), most commonly known for the ability to revive tired eyes and ease eye strain, are also a tonic and increase immunity [24]. Lungwort (*Pulmonaria officinalis*) is an herbaceous evergreen, used around the world for a variety of respiratory ailments (e.g.: coughs, colds, bronchial detoxification and catarrhal problems) [25]. Japanese raisin tree (*Hovenia dulcis*) has antispasmodic, febrifuge and laxative properties. Its seeds are diuretic and are used to relieve intoxication [26]. Ginseng (*Panax ginseng*) has long been utilized as an herbal medicine that help reduce inflammatory markers and help protect against oxidative stress thus maintaining the health of cells [27].

The present research paper describes the environmentally - friendly synthesis of silver (AgNPs) and gold (AuNPs) nanoparticles from ten different plants (e.g.: Sea buckthorn, Elderflower, Lungwort, Acacia, Cornflower, Ramson, etc.) that have multiple pharmaceutical applications and are beneficial to human health. Both AgNPs and AuNPs are prepared via two routes: at room temperature, in the dark, for 24 hours and at 50^0C under a constant stirring of 600 rpm for 30 minutes. UV - Vis spectroscopy was used to investigate the formation of both AgNPs and AuNPs and compared to the UV – Vis spectra of the plain aqueous extracts prepared at 4^0 C. The main purpose of our research was to investigate the antioxidant activity of the aqueous extracts and noble metallic nanoparticles obtained thereof and to determine whether the temperature-conducted environmentally - friendly synthesis of AgNPs and AuNPs influences the antioxidant activity in any way.

2. Materials and methods

2.1. Chemicals

DPPH (2,2 – diphenyl – 1 – picryl – hydrazyl – hydrate), hydrochloric acid (HCl), sulphuric acid (H_2SO_4), copper sulphate ($CuSO_4$), silver nitrate ($AgNO_3$), tetrachloauric acid ($HAuCl_4$), glacial acetic acid (CH_3COOH), aluminum chloride ($AlCl_3$), lead acetate ($Pb(CH_3COO)_2$), catechin standard, gallic acid standard, Folin-Ciocalteu reagent, ferric chloride ($FeCl_3$), Benedict and Millon reagents were purchased from Sigma – Aldricht. Ethanol (C_2H_5OH), methanol (CH_3OH), and sodium hydroxide (NaOH) were purchased from Scharlau. The distilled water used to prepare all the solutions and the aqueous extracts was freshly prepared in our laboratory.

2.2. Preparation of the aqueous extracts

The plants used in the current research were bought either fresh from the local market (Ramson and Celandine) or readily dried from the local natural shops. In the case of Ramson and Celandine, they were thoroughly washed twice with tap water, thrice with freshly prepared distilled water, dried at room temperature for 6 days, finely grinded and used for the preparation of the corresponding aqueous extracts.

The protocol involved in the preparation of all the aqueous extracts involved the following steps: 25 g dried plant were weighted, transferred into a glass “French press” type extractor, infused with 250

mL distilled water for 24 hours in a refrigerator (4^0 C). Then, the aqueous extracts were filtered until all the debris were removed. All ten aqueous extracts are stable at 4^0 C for over 4 months.

2.3. Qualitative screening of carbohydrates, proteins and aminoacids

2.3.1. Qualitative screening of carbohydrates. General test for carbohydrates: in a glass tube, 1 mL Molisch reagent (a solution of α – naphthol in C_2H_5OH) is added to 2 mL aqueous extract and few drops of concentrated H_2SO_4 are dripped. The appearance of a violet ring indicates the presence of carbohydrates.

Several qualitative tests for the qualitative screening of different carbohydrates (e.g.: hexose sugars, glucose, hexose, etc.) are described in the literature [28-31].

a) Benedict test: to 1 mL of aqueous extract 5 ml Benedict's reagent are added and boiled for 5 minutes. Initially the solution is green and after boiling a red, yellow or green precipitate appears.

b) Fehling A test: to 1 mL aqueous extract few drops of Fehling A reagent are added; the solution turns green.

c) Fehling B test: to 1 mL aqueous extract few drops of Fehling B reagent are added; the solution turns brown.

d) Barfoed test: to 1 mL aqueous extract, 3 ml Barfoed's reagent (copper acetate in glacial CH_3COOH) are added, boiled 2 minutes and cooled. A red precipitate should appear.

e) Trommer test: to 3 mL of aqueous extract a ml of 2.5% $CuSO_4$ and 2 ml of 5% NaOH are added, boiled for 3 minutes and a blue precipitate appears that turns red upon heating.

2.3.2. Qualitative screening of proteins and aminoacids. The literature describes several testes for the qualitative screening of proteins and aminoacids [32], [33]:

a) Millon test: 1 mL aqueous extract reacts with 5 – 6 drops of Millon reagent and a white precipitate appears that turns red upon heating.

b) Biuret test: to 3 mL aqueous extract, 3 mL 4% NaOH solution and few drops of 1% $CuSO_4$ are added and a purple solution is formed.

c) Ninhydrin test: to 3 mL aqueous extract, 3 drops of 5% $Pb(CH_3COO)_2$ are added and heated for 10 minutes. A purple of blue color is a positive response.

d) Cysteine test: to 5 mL aqueous extract, few drops of 40% NaOH and 5% are added, boiled for 5 minutes and the solution turns purple or blue.

e) Xantoprotein test: to 3 mL aqueous extract, 1 mL conc. H_2SO_4 is added. First a white precipitate is formed that turn yellow upon boiling and orange after adding 1 mL NH_4OH.

2.4. Quantitative determinations

The quantitative determination of bioactive compounds involves the spectrophotometric determination of biocomponents (Table 1) [34].

Table 1. Quantitative determination of **t**otal content of tannins (TCF), total content of flavonoids (TCF) and total content of polyphenols (TCP)

Assay	Recordings
Total tannins content: 0.5 mL extract+3 mL 4% vanillin-MeOH+1.5 mL HCl, 15 min. incubation	Absorbance at 500 nm
Total flavonoids content: 1 mL extract+4 mL distilled water and 0.3 mL 5% $NaNO_2$; after 5 min.: 0.3 mL 10% $AlCl_3$; after 5 min.: 2 mL 1M NaOH and 2.4 L distilled water, 30 min. incubation	Absorbance at 510 nm
Total polyphenols content: 1 mL extract and 5 mL Folin-Ciocalteu reagent; after 8 min.: 4 mL Na_2CO_3, 60 min. incubation	Absorbance at 765 nm

2.5. Environmentally – friendly of silver (AgNPs) and gold (AuNPs) nanoparticles

The eco – friendly synthesis of AgNPs and AuNPs from all the ten plants was achieved using two different routes: at room temperature, in the dark, for 24 hours and at 50^0C under a constant stirring of 600 rpm for 30 minutes. The first stage involves preparing the 10^{-3} M aqueous solutions of silver nitrate ($AgNO_3$) and tetrachloroauric acid ($HAuCl_4$) that will be further used for the phytosynthesis of AgNPs and AuNPs.

The protocol for the phytosynthesis of AgNPs and AuNPs at room temperature is: 5 mL aqueous extracts were mixed with 50 mL 10^{-3} M $AgNO_3$ solution and kept in the dark, at room temperature and no stirring, for 24 hours. The next day, the colloidal solution of AgNPs was stirred for 30 minutes in an ultrasound bath, at a constant speed of 100 rpm.

The protocol for the phytosynthesis of AgNPs and AuNPs at 50^0C involves the following steps: 5 mL aqueous extracts were mixed with 50 mL 10^{-3} M $AgNO_3$ solution and heated, using a magnetic stirrer, at 50^0 C under a constant stirring of 600 rpm for 30 minutes, then the heat was turned off and the stirring was kept on for another 30 minutes. The resulted colloidal suspension of AgNPs was then placed in the dark for 24 hours. The next day, the suspension was stirred for 30 minutes in an ultrasound bath, at a constant speed of 100 rpm.

2.6. Antioxidant activity of aqueous extracts and noble metallic nanoparticles thereof

Antioxidant activity (AA, %) of both aqueous extracts and noble metallic nanoparticles was also spectrophotometrically investigated using the 2,2 – diphenyl – 1 – picryl – hydrazyl – hydrate free radical (DPPH) assay. This method involves the evaluation of the antioxidant capacity of a specific compound, an extract (aqueous, alcoholic, hydroalcoholic) or any other biological material in which the specific compound or extract is mixed together with a DPPH solution and the absorbance is recorded after a well-determined time frame [35], [36].

For that, a DPPH solution was prepared in ethanol and 0.5 mL aqueous extract were mixed with 1 mL 0.02 mg/mL of the DPPH solution and the absorbance of the resulted solution was recorded at 517 nm. A blank was also prepared as follows: 0.5 mL distilled water were mixed with 1 mL 0.02 mg/mL DPPH solution [37], [38].

The antioxidant activity (AA %) was calculated according to the formula:

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

where: $A_{control}$ = absorbance of the blank DPPH solution and A_{sample} = absorbance of the aqueous extracts of the ten studied plants mixed with 0,02 mg/mL DPPH solution.

2.7. Characterization of environmentally – friendly AgNPs and AuNPs

The reduction of pure metal ions (Ag^+ and Au^{3+}) was monitored by recording UV-Vis spectra immediately after the reaction and at well-established time intervals after diluting small aliquots of the samples (aqueous extracts and noble metallic nanoparticles) with freshly prepared distilled water. The absorption spectra were recorded using a M 400 Carl Zeiss Jena UV – Vis spectrometer at a wavelength range of 250 – 650 nm. Quantitative screening of phytochemicals and antioxidant activity measurements were recorded using a JK-VS-721N Visible spectrophotometer at well-established wavelengths.

The environmentally - friendly synthesis of AgNPs and AuNPs at 50^0 C was achieved using an RSM-10HS Phoenix Instrument magnetic stirrer. The ultrasonic bath used in the phytosynthesis stage of the noble metallic nanoparticles was Bioblock Scientific.

3. Results and discussions

3.1. Preparation of the aqueous extracts

All the ten aqueous extracts were prepared in the same temperature conditions, 4^0 C, in the refrigerator, for 24 hours, using a different glass "French-press" type extractor for each specific plant (Figure 1, Figure 2 and Figure 3).

Figure 1. Glass "French-press" type extractor used to prepare Ramson aqueous extract

Figure 2. Glass "French-press" type extractor used to prepare Gooseberry aqueous extract

Figure 3. Glass "French-press" type extractor used to prepare Elderflower aqueous extract

The color of the ten aqueous extracts varies from one extract to another as it can be seen in Table 2.

Table 2. Color of the aqueous extracts prepared from the ten plants

Aqueous extract	Color
Sea buckthorn (*Hippophae rhamnoides*)	Light yellow
Acacia (*Robinia pseudacacia*)	Light orange
Celandine (*Ranunculus ficaria*)	Brown – greenish
Gooseberry (*Ribes grossularia*)	Red - brownish
Ramson (*Allium ursinum*)	Green
Elderflower (*Sambucus nigra*)	Brown
Cornflower (*Centaurea cyanus*)	Indigo
Lungwort (*Pulmonaria officinalis*)	Brown – greenish
Japanese raisin tree (*Hovenia dulcis*)	Light brown
Ginseng (*Panax ginseng*)	Creamy - white

3.2. Qualitative screening of carbohydrates, proteins and aminoacids

Plants owe their pharmaceutic value to the bioactive compounds they contain that have physiological benefits for human health. The screening for bioactive compounds allows detection of biological components that may be used as raw materials for modern drugs [39]. These "metabolic compounds" are also known as "secondary metabolites" and they include alkaloids, flavonoids, coumarins, tannins, terpenes, terpenoids, phenols, polysaccharides and glycosides [40].

3.2.1. Qualitative screening of carbohydrates. The qualitative screening of carbohydrates relies on, as all the other qualitative screening methods, on the visual change of colour of the aqueous extracts when mixed with certain reagents. The general qualitative reaction that indicates the presence of

carbohydrates in with Molisch reagent, when the mixture should turn violet. The results are presented in Table 3.

Table 3. Qualitative screening for carbohydrates of the analyzed aqueous extracts

Aqueous extract	Molisch	Benedict	Fehling A	Fehling B	Barfoed	Trommer
Sea buckthorn	+	-	++	+++	-	+++
Acacia	++	+++	+++	++	-	++
Celandine	++	+++	+++	+++	-	++
Ramson	+	+	+++	+++	-	-
Gooseberry	+	-	++	+++	+++	+++
Elderflower	+	+++	+++	+++	-	++
Cornflower	++	+++	+++	+++	+++	-
Lungwort	+	+	+++	+++	+	+
Japanese raisin tree	+	+	+	+	-	-
Ginseng	++	+	+	+	-	+

In order to explain the results presented in Table 3, the following abbreviations were used: "+" = weak; "++" = intense; "+++" = very intense; "-" = absent, referring to the colour intensity of the resulted solution, after the specific reagents were added. All the ten aqueous extracts contain carbohydrates, which can be easily deducted from the positive response to Molisch test.

Molisch's test reveals that Sea buckthorn and Gooseberry contain different classes of carbohydrates and specific qualitative test reveal the presence of monosaccharides in Gooseberry aqueous extract and of di-, oli- and polisaccharides in both Sea buckthorn and Gooseberry aqueous extracts. Barfoed's test is carried out to distinguish monosaccharides from reducing dissacharides and is positive only for Gooseberry, Cornflower and Lungwort (in a smaller amount).

3.2.2. Qualitative screening of proteins and aminoacids. Proteins are involved in all the natural processes occurring in all the living cells. Proteins are insoluble in neutral salts (e.g.: NaCl, $MgSO_4$) and only solubilize in diluted salts. On the other hand, the majority of the aminoacids are soluble in water. The qualitative analysis of aminoacids involves a colour change or precipitation as a result of a shifting in the structural configuration when reacting with a reagent [41]. The results for the qualitative screening for proteins and aminoacids for the ten aqueous extracts are detailed in Table 4.

Table 4. Qualitative screening for proteins and aminoacids of the analyzed aqueous extracts

Aqueous extract	Millon	Biuret	Ninhydrin	Cysteine	Xantoprotein
Sea buckthorn	+	-	-	+++	-
Acacia	++	+	-	-	-
Celandine	+	-	-	-	-
Ramson	+++	-	-	-	-
Gooseberry	++	-	-	++	-
Elderflower	++	-	-	-	-
Cornflower	++	-	-	-	-
Lungwort	++	++	-	-	-
Japanese raisin tree	+	+	-	-	-
Ginseng	+	-	-	++	-

In order to explain the results presented in Table 4, the following abbreviations were used: "+" = weak; "++" = intense; "+++" = very intense; "-" = absent, referring to the color intensity of the resulted solution, after the specific reagents were added. From Table 4 it can be easily concluded that all ten aqueous extracts are positive to Molisch test, indicating the present of tyrosine, a non-essential

aminoacid with a polar side group. Of the ten aqueous extracts, cysteine test is positive only for Sea buckthorn, Gooseberry and Ginseng, in a greater amount in the fruits of Sea buckthorn.

3.3. Quantitative determinations

All the ten aqueous extracts were analyzed in triplicate. The amount of total tannins (TCT) and the total content of flavonoids (TCF) are presented as mg catechin/L and the total content of polyphenols (TCP) uses gallic acid as standard calibration curve. The results are presented in Table 5.

Table 5. Quantitative spectrophotometric determination of TCT, TCF and TCP

Aqueous extract	Total content of tannins (TCT)	Total content of flavonoids (TCF)	Total content of polyphenols (TCP)
Sea buckthorn	56,266 mg/L	329,26 mg/L	185,31 mg/L
Acacia	120,36 mg/L	850,96 mg/L	963,12 mg/L
Celandine	55,33 mg/L	256,88 mg/L	169,23 mg/L
Ramson	57,43 mg/L	230,56 mg/L	174,75 mg/L
Gooseberry	163,13 mg/L	382,73 mg/L	920,08 mg/L
Elderflower	251,33 mg/L	585,61 mg/L	606,95 mg/L
Cornflower	125,37 mg/L	1466,630 mg/L	1430,25 mg/L
Lungwort	54,23 mg/L	269,55 mg/L	200,36 mg/L
Japanese raisin tree	100,54 mg/l	321,23 mg/L	302,66 mg/L
Ginseng	148,62 mg/l	392,55 mg/L	623,14 mg/L

The highest value for TCT was calculated for Elderflower aqueous extract (251,33 mg/L), followed by Gooseberry (163,13 mg/L) and Ginseng (148,62 mg/L) while the lowest calculated value was for the aqueous extract of Lungwort (54,23 mg/L). Cornflower has the highest calculated value for TCF (1466,63 mg/L) of all the studied aqueous extracts while the lowest value was calculated for Ramson (230,56 mg/L). regarding the total content of polyphenols (TCP), the spectrophotometric investigations revealed the highest value for the aqueous extract of Cornflowers (1430,25 mg/L).

3.4. Environmentally – friendly of silver (AgNPs) and gold (AuNPs) nanoparticles

UV – Vis spectra were recorded for both aqueous extracts and metallic nanoparticles in the range between 250 – 650 nm. The first proof that AgNPs and AuNPs are phytosynthesized is the visual change of color (Table 6) after adding either $AgNO_3$ or $HAuCl_4$.

Table 6. Color of the eco-friendly AgNPs and AuNPs

Aqueous extract	AgNPs room temperature	AgNPs 50^0	AuNPs room temperature	AuNPs 50^0
Sea buckthorn	Light brown	Light brown	Light violet	Violet-red
Acacia	Orange-brown	Light brown	Light violet	Violet-red
Celandine	Brown	Dark brown	Red	Red
Ramson	Orange brown	Brown	Light violet	Dark violet
Gooseberry	Dark brown	Dark brown	Light violet	Chery red
Elderflower	Brown	Dark brown	Light violet	Cherry red
Cornflower	Grey brown	Orange brown	Light violet	Cherry red
Lungwort	Orange brown	Brown	Light red	Light violet
Japanese raisin tree	Orange	Orange brown	Light red	Light violet
Ginseng	Orange brown	Orange brown	Light violet	Light violet

The formation of green AgNPs if firstly confirmed by the change of color of all the aqueous extracts, independent from the reaction conditions, from orange brown to grey brown, depending on the size and shapes of the nanoparticles. The maximum absorptions for the all the 10-green synthesized AgNPs varies from 427 nm (AgNPs from Ginseng at 50^0 C) to 445 (Cornflower at room temperature) (Table 7).

Table 7. Uv-Vis maximum of the eco-friendly AgNPs

Aqueous extract	AgNPs room temperature (nm)	AgNPs 50^0 (nm)
Sea buckthorn	435	429
Acacia	429	428
Celandine	438	435
Ramson	431	433
Gooseberry	434	436
Elderflower	428	469
Cornflower	445	440
Lungwort	428	431
Japanese raisin tree	430	444
Ginseng	430	427

The color of AuNPs can vary from light violet to dark cherry red, depending on the size and shapes of the nanoparticles. The maximum absorptions for the all the 10-green synthesized AuNPs is from 547 nm (AgNPs from Sea buckthorn at 50^0 C) to 561 nm (Cornflower at 50^0 C) (Table 8).

Table 8. UV-Vis maximum of the eco-friendly AuNPs

Aqueous extract	AuNPs room temperature (nm)	AuNPs 50^0 (nm)
Sea buckthorn	526	547
Acacia	524	560
Celandine	529	555
Ramson	528	550
Gooseberry	526	549
Elderflower	523	555
Cornflower	529	561
Lungwort	529	559
Japanese raisin tree	521	550
Ginseng	530	559

Antioxidant activity of aqueous extracts and noble metallic nanoparticles thereof

The antioxidant activity was determined spectrophotometrically using the DPPH assay, a free radical that possess an odd nitrogen electron that is reduced upon capping a hydrogen atom from the antioxidants, resulting in the corresponding hydrazine [42]. The color of the DPPH solution recorded at 517 nm is due to the odd electron.

If present, a certain antioxidant donates an electron to the DPPH molecule and quenches the color thus lowering the absorption. There may be a significant decrease in the absorbance for those samples that contain antioxidants (purple color disappearing coupled with yellow color observed by naked eye) the intensity of yellow color will be directly proportional with antioxidant activity compared to the blank.

The results for the spectrophotometric determination of the antioxidant activity are presented in Table 9 (for AgNPs) and Table 10 (for AuNPs).

Table 9. Antioxidant activity of the aqueous extracts and eco-friendly AgNPs

Crt.no	Aqueous extracts	AgNPs room temperature (AA, %)	AgNPs 50^0 (AA, %)
Sea buckthorn	65.39	77.91	69.86
Acacia	35.02	45.54	39.32
Celandine	40.55	41.28	48.98
Ramson	24.35	84.88	25.60
Gooseberry	35.47	60.66	38.74
Elderflower	46.58	61.24	48.98
Cornflower	30,09	42.64	31.57
Lungwort	18.04	18.41	49.66
Japanese raisin tree	15.14	15.70	25.94
Ginseng	10.55	7.75	30.20

From the Table 9 it is clear that, in almost all cases, the antioxidant activity increases for the environmentally – friendly AgNPs whatever the route was used. However, in the case of Ginseng the value of AA at room temperature is lower than that of the corresponding aqueous extracts so further investigations are required. The highest antioxidant activity was calculated for Ramson AgNPs at room temperature (84.88 %) but in the case of AgNPs at 50^0 C additional studies should be carried out. Good values and in the accordance with the literature were obtained for Sea buckthorn, Gooseberry and Elderflower, proving that the formation of the eco-friendly AgNPs increases the value of the antioxidant activity.

Table 10. Antioxidant activity of the aqueous extracts and eco-friendly AuNPs

Crt.no	Aqueous extracts	AuNPs room temperature (AA, %)	AuNPs 50^0 (AA, %)
Sea buckthorn	65.39	29.86	58.87
Acacia	35.02	35.32	62.63
Celandine	40.55	48.98	59.22
Ramson	24.35	25.60	50.85
Gooseberry	35.47	38.74	65.70
Elderflower	46.58	48.98	49.15
Cornflower	30.09	31.57	48.81
Lungwort	18.04	49.66	63.31
Japanese raisin tree	15.14	25.94	60.92
Ginseng	10.55	30.20	47.61

The antioxidant activity of the eco-friendly AuNPs has higher calculated values for the synthesis at 50^0 C, proving that the heat reaction works better in the case of AuNPs. The highest value was calculated for AuNPs at 50^0 C for Gooseberry, followed by Lungwort and Acacia. So, in the case of AuNPs it is clear that heat-conducted green synthesis gives better results for the antioxidant activity.

4. Conclusions

The present paper describes the environmentally - friendly synthesis of silver (AgNPs) and gold (AuNPs) nanoparticles from ten different plants (e.g.: Sea buckthorn, Elderflower, Lungwort, Acacia, Cornflower, Ramson, etc.) with multiple pharmaceutical applications.

Molisch's test reveals that Sea buckthorn and Gooseberry contain different classes of carbohydrates and specific qualitative test reveal the presence of monosaccharides in Gooseberry aqueous extract and of di-, oli- and polisaccharides in both Sea buckthorn and Gooseberry aqueous extracts.

Both AgNPs and AuNPs were prepared via two routes: at room temperature, in the dark, for 24 hours and at 50^0C under a constant stirring of 600 rpm for 30 minutes. The formation of green AgNPs if firstly confirmed by the change of color of all the aqueous extracts, independent from the reaction conditions, from orange brown to grey brown, depending on the size and shapes of the nanoparticles. The maximum absorptions for the all the 10-green synthesized AgNPs varies from 427 nm (AgNPs from Ginseng at 50^0 C) to 445 (Cornflower at room temperature).

UV - Vis spectroscopy was used to investigate the formation of both AgNPs and AuNPs and compared to the UV – Vis spectra of the plain aqueous extracts prepared at 4^0 C. Antioxidant activity of the aqueous extracts and noble metallic nanoparticles obtained thereof was determined using the DPPH assay.

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