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Synthesis of Gold Nanoparticles with Medicinal Plants from Indian Ocean

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Abstract

In the present work, we described the synthesis of gold nanoparticles (AuNP) using aqueous extracts from medicinal plants originated from Indian Ocean. UV-visible spectroscopy, Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) analyses were performed to ascertain the formation of AuNPs and to characterize them. Gold nanoparticles showed different sizes ranging from 100 to 180 nm with the characteristic surface plasmon band around 520 nm. The antioxidant properties of the AuNP formed were assessed by DPPH and KRL tests. The results herein presented clearly demonstrated the redox properties of each studied plants used for the reduction of gold salts. Interestingly this crude plant extracts mediated synthesis led to gold nanoparticles that demonstrated prooxidant properties which could generate Reactive Oxidant Species (ROS) for their use in phototherapy.

Keywords: Green chemistry; Gold nanoparticles; Medicinal plant; Plant extraction

Introduction

Since several years, particular attention was paid on nanotechnology. Indeed, due to their particular properties, metal nanoparticles are increasingly used in multiple applications such as medicine, biotechnology, material sciences, computer sciences, pharmacy and engineering **[1].** Among the metal nanoparticles, gold nanoparticles (AuNPs) have a great importance and were notably studied in the field of chemical analysis, nanoelectronics **[2]** and many studies have shown their potential in the diagnosis and treatment of cancer **[3-5].** They are used as drug carriers, contrast agents and in thermal therapy and have advantages to prevent undesirable off-target and side effects. One of the main approaches to synthetize nanoparticles is the reduction of the metal salts by a chemical reducer. Unfortunately, this method is quite expensive and potentially harmful to the environment [6].

Thus, there is a need to propose an alternative to conventional reduction method to decrease environmental impact. In recent years, biological sources such as bacteria, fungi, yeast and plants were studied for the biosynthesis of AuNPs. Several studies showed notably the potential of plant extracts to bio reduce the metal ions and form nanoparticles. For instance, plants like Hibiscus rosa sinensis **[7]** Murrayakoenigii **[8]** and Trigonella foenum-graecum **[9]** have been reported to form gold nanoparticles. The leaf extract of neem (Azadirachta indica) was used to produce AuNPs **[10].** Several of these nanoparticles formed using plant extracts were also evaluated for their cytotoxic activity. Gold nanoparticles obtained with Couroupita guianensis aqueous extract as reducing agent induced apoptosis **[11].** In a study led by Balashanmugam et al. **[12],** the aqueous leaf extract of Cassia roxburghii produced nanoparticles whose size ranged 25-35 nm. Their IC50 against HepG2 cells was found to be 30 µg/mL whereas no cytotoxicity was observed against normal Vero cell line up to 75 µg/mL **[12].** Moreover gold nanoparticles formed from Acalypha indica leaf aqueous extract induced apoptosis through caspase-3 activation and DNA fragmentation **[13].** In our previous studies1, we focused on an endemic species of Reunion Island, HubertiaambavillaBory (Asteraceae) as the source of reducing agent in AuNPs synthesis. Locally known as "ambaville", this species is traditionally used by local population for circulation, diabetes, eczema, wounds, lichen tropicus, rheumatism, itch, asthma and ulcer **[14].** Phytochemical studies revealed the presence of flavonoids, tannins, leucoanthocyanes, phenols, etc. Methanolic extract of the leaves and stems has shown radical scavenger and antioxidant activities **[14].** In the present study, we report the one step route biosynthesis of nanoparticles using different aqueous extracts of Reunionese plants as reducing agent. Gold nanoparticles formed were analyzed by UV-visible spectroscopy, Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) methods.

Materials and Methods

Chemicals

Tetrachloroauric acid (HAuCl4), water milliQ, ethanol (98%) were purchased from Sigma- Aldrich (Saint-Quentin Fallavier, France). 17 plants of Reunion island were screened **(Table I).** 11 of them are medicinal plants registered in the French Pharmacopeia, 5 are commonly used for food, and one is used for its wood. Medicinal plants were purchased in CAHEB, Le Tampon. Terminalia bentzoe was collected in Saint-Philippe. Tea and turmeric were purchased in Labyrintheen-champ-thé, Saint-Joseph. Psidium cattleianum and Cryptomeria japonica were provided by ONF. Citrus hystrix was collected in Sainte-Marie.

Table I: Plants from Reunion island selected for the green synthesis of AuNP. *plants registered in French Pharmacopeia.

Scientific name	Common name	Family	Status	Use	Main phytochemicals
Aphloiatheiformis*					Saponosids; phenolic compounds: aphloïol, catechic tannins [15]
Ayapanatriplinervis*					Coumarins: ayapanin, ayapin, daphnetin, daphnetin dimethylether, dephnetin 7 methylether, hydrangetin, ombelliferon; stigmasterol; ascorbic acid [15]

Camellia sinensis var	Green tea	Theaceae	Exotic	Food and medicinal	Phenolic compounds: catechins, epigallocatechin gallate, flavonols (rutin, quercetin, kaempferol), gallic acid; alkaloids: theine [19]
assamica	White tea				
Citrus hystrix	Combava	Rutaceae	Exotic	Food	Flavonoids; ascorbic acid [20]
Curcuma longa	Turmeric	Zingiberaceae	Exotic	Food and medicinal	Flavonoids: curcumin [21]
Cryptomeria japonica	Cryptoméria	Taxodiaceae	Exotic	Wood	-
Dodonaea viscosa *	Boisd'arnette	Sapindaceae	Indigenous	Medicinal	Phenolic compounds: catechic tannins, proanthocyanidols, flavans, flavonoids: quercitol and isorhamnetol; saponosids [15,17]
Mussaenda arcuata *	Lingue café	Rubiaceae	Indigenous	Medicinal	Coumarines [15]
Nuxia verticillata*	Bois maigre	Stilbaceae	Endemic	Medicinal	Phenolic compounds: flavonoids, tannins, coumarins, anthocyanins; saponosids; triterpenes; alkaloids; sterols [15]
Olea europeaa fricana*	Boisd'olive noir	Oleaceae	Indigenous	Medicinal	Sterols; saponosids; coumarins; alkaloids; triterpenes [15]
Phyllanthus casticum *	Bois de demoiselle	Phyllanthaceae	Indigenous	Medicinal	Saponosids; catechic tannins [15]
Pittosporum senacia*	Bois de jolicoeur	Pittosporaceae	Endemic	Medicinal	Phenolic compounds: chlorogenic acid, tannins; alkaloids; saponosids [15]
Psidium cattleianum	Goyavier	Myrtacées	Exotic	Food and medicinal	Flavonoids [22]
Psiloxylon mauritia	Bois de pêche	Myrtaceae	Endemi	Medicinal	Phenolic compounds: flavans, gallic tannins, flavonoids: quercetol, vitexin and kaempferol-7-O-glucoside [15]

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Terminalia bentzoe*	Benjoin pays	Combretaceae	Endemic	Medicinal	Phenolic compounds: gallic acid, ellagic acid, pyrogallol, catechins, pyrocatechol, chlorogenic acid, cinnamic acid, caffeic acid, quercetin, rutin [18]
Vepris lanceolata*	Pattepoule	Rutaceae	Indigenous	Medicinal	Alkaloids; triterpenes [15]

Plant extraction and synthesis of nanoparticles

Preparation of the crude extracts: The air-dried leaves of each plant, except turmeric, were reduced in powder and kept dry at room temperature until use. 45 g of powder were extracted with an accelerated solvent extractor in Milli-Q water or macerated in Milli-Q water (ratio 1:10) for 20 hours. The aqueous extracts were then freeze-dried (Lyophilisateur Cosmos 20K Cryotec) and the extraction yield were determined. For turmeric, the powder of the rhizome was directly used and not extracted.

Synthesis of AuNP with the plant extracts: Each crude extract was solubilized in distilled water at a concentration of 8 mg/mL. The solution was then filtered on RC 0,20 µm. 20 mL of this solution were added to 50 mL of a 1 mM HAuCl4 solution, heated under reflux at 75°C with an oil bath. After 20 min of reaction at 75°C in darkness, the reaction mixture was cooled in ice and centrifuged three times during 30 minutes at 11 100 rpm (5804R Eppendorf Centrifuge). Between each centrifugation, the supernatant was eliminated and the pellet of AuNP was re-suspended in distilled water. After the third centrifugation, the pellet was re-suspended in 5 mL of Milli-Q water. This AuNP suspension was stored in darkness at 4°C and used for the different characterizations of this study.

Characterization of the AuNP

The synthesis yield was determined by weighing the air-dried AuNP or estimated by optic density measurement.

UV-visible spectroscopy: The UV-visible profiles of the AuNP suspensions were performed with an UVS-99 ACTGene spectrophotometer. After 1 min of sonication, a drop of the suspension was put in the spectrophotometer and the absorbance spectrum was measured between 200 and 848 nm. For each suspension, the width of the Surface Plasmon (SP) was noticed and the wavelength that gave the maximum absorbance (λmax) was noted.

Dynamic light scattering (DLS): The size measurements were performed using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) equipped with a He-Ne laser (633 nm, fixed scattering angle of 173°) at room temperature. After sonication, each AuNP suspension was diluted 1:3 and put in cell. The PdI and the hydrodynamic diameter of AuNP were noted.

Transmission Electron Microscopy: TEM images were acquired with a JEOL JEM 1011 microscope (JEOL, USA) at an accelerating voltage of 100 kV. 2 µL of this re-dispersed particle suspension was placed on a carbon coated copper grid (manufactured by Smethurst High-Light Ltd and marketed exclusively by Agar Scientific) and dried at room temperature.

Stability of AuNP: The stability of AuNP was evaluated 3 months after the synthesis. For each AuNP

suspension, a visual observation, UV-visible and DLS measures were performed after sonication as mentioned above.

In vitro antioxidant activity of AuNPs

DPPH test: This test is a colorimetric test based on the reduction of the free radical DPPH ● (2,2-diphenyl-1-picrylhydrazyl) to a stable molecule DPPHH. The DPPH solution is purple, and this staining will decrease as the DPPH radical is reduced. This decrease in color is measured spectrophotometrically at 515-517 nm. This test is carried out on a 96-well plate. In each well, the test sample (AuNP solution) is brought into contact with the DPPH solution. Control wells are also made: the sample is replaced by water for the negative control and by a standard solution, here ascorbic acid, for the positive control. The oxidation-reduction reaction occurs for 30 minutes, followed by the absorbance measurements. A low absorbance informs about a high reduction rate of DPPH and consequently a high antioxidant activity of the tested sample. Statistical analysis of the results is done by Anova, on the Prisme 6 software.

KRL test: Unlike the DPPH test which is a chemical test, the KRL test is a biological test. The advantage of this test is that it makes it possible to characterize the antioxidant activity of compounds in a biological system. This test is performed *in cellulo* on human erythrocytes. The lysis of these erythrocytes is caused *in vitro* by free radicals generated by thermal decomposition of AAPH. This lysis can be followed by reading absorbance at 450 nm. AuNP solutions have been tested in the same concentration range (100 µg/mL). The following controls were used: water for the negative control and a solution of gallic acid (40 µg/mL) for the positive control. Results are expressed in half-haemolysis time (HT50), in minutes, and are exploited with the Prisme 6 software. Their statistical analysis is done by Anova, also on the Prisme 6 software.

Results and Discussion

Synthesis of AuNP

Gold nanoparticles were obtained with all the tested crude extracts. The macroscopic observation of the coloration of the reaction mixture in red or blue was the first information. The colour of the suspension gives a preliminar indication about the size of the AuNP and/or their polydispersity and their stability. A red colour indicates small nanoparticles (15-20 nm) whereas a blue colour indicates larger nanoparticles. Most of the AuNP suspensions were blue or purple (**Table II**). We assume at this step that the obtained AuNP were rather large. For 4 extracts: *Aphloia theiformis*, *Olea europea africana*, *Psiloxylon mauritianum* and *Curcuma longa*, red and purple-red AuNP were obtained indicating that those AuNP were smaller than the others.

Table II: Characteristics of the AuNP obtained with the crude extracts of the selected plants.

AuNP suspension	Colour	Synthesis yield (µg/mL)	Larger of SP	λ_{max} (nm)	Hydrodynamic diameter (nm)	PdI	Shape	Stability over time
AuNP@A. theiformis	red	400	tight	537	61,4	0,229	spherical	stable
AuNP@A.	blue	100	broad	602	225,8	0,	polydisperse in	unstable

triplinervis						17	size and/or shape	
AuNP@Green tea	blue	400	broad	591	128,2	0,085	polydisperse in size and/or shape	stable
AuNP@White tea	blue	400	broad	587	127,6	0,263	polydisperse in size and/or shape	unstable
AuNP@C. hystrix	purple	200	broad	556	311,8	0,212	polydisperse in size and/or shape	stable
AuNP@C. longa	red	200	tight	542	287,3	0,237	spherical	unstable
AuNP@C. japonica	pale violet	400	broad	537	136,6	0,346	polydisperse in size and/or shape	stable
AuNP@D. viscosa	blue	200	broad	546	235,9	0,45	polydisperse in size and/or shape	unstable
AuNP@M. arcuata	blue	200	broad	593	164,9	0,174	polydisperse in size and/or shape	stable
AuNP@N. verticillata	purple	100	broad	558	473,1	0,157	polydisperse in size and/or shape	unstable
AuNP@O. europeaafricana	red-purple	400	tight	541	115,8	0,266	spherical	unstable
AuNP@P. casticum	purple	400	broad	544	113,5	0,219	polydisperse in size and/or shape	unstable
AuNP@P. senacia	blue	200	broad	566	113,3	0,28	polydisperse in size and/or shape	unstable
AuNP@P. cattleianum	blue	100	flat	-	-	-	-	-
AuNP@P. mauritianum	red-purple	400	tight	547	102,3	0,38	spherical	stable

AuNP@T. bentzoe	purple	400	broad	530	158,7	0,183	polydisperse in size and/or shape	stable
AuNP@*V. lanceolata*	purple	200	broad	568	383,5	0,259	polydisperse in size and/or shape	stable

Characterization of AuNP

The characteristics of each AuNP suspension are presented in **Table II.** In our previous work **[24],** extinction band in spectrophotomer UV-Vis of 15 nm AuNP appeared between 500 and 600 nm. This band is attributed to Surface Plasmon band (SP) and provides information about the size, shape and the polydispersity of AuNP. **Table II** gathered the size and shape information obtained by DLS, TEM and UV-Vis spectrophotomer. AuNP show SP band between 530 nm and 602 nm. For the "red suspensions" (AuNP@*A. theiformis*, AuNP@*P. mauritianum*, AuNP@*O. europea africana* and AuNP@*C. longa)*, absorbance is maximum for wavelengths near 540 nm. DLS and TEM results indicate that AuNP@*A. theiformis*, AuNP@*P. mauritianum*, AuNP@*O. europea africana* and AuNP@*C. longa* are small particles, spherical and quite monodispersed in size and shape. Hydrodynamic diameters obtained by DLS were comprised between 61,4 nm (AuNP@*A. theiformis*) and 473,1 nm (AuNP@*N. verticillata*). The smallest particles (<150 nm) were obtained with 9 extracts such as: *A. theiformis*, *P. mauritianum*, *T. bentzoe*, *P.* 8 *senacia*, *P. casticum*, *O. europea africana*, white tea, green tea and *C. japonica*. Those results are correlated with those obtained in MET: AuNP@*A. theiformis*, AuNP@*P. mauritianum* and AuNP@*O. europea africana* had a quite small diameter. Some of the results of TEM analysis are presented in **Figure 1.** AuNP obtained with *A. theiformis*, *P. mauritianum*, *O. europea africana* and *C. longa* were small (20-30 nm, 20-30 nm, 30-40 nm and 10 nm respectively) and spherical shaped. AuNP obtained with *A. triplinervis* were larger and had different sizes and shapes. AuNP obtained with green tea were aggregated. DLS measurement informs about the hydrodynamic diameter as Brownian motion. TEM images show electronic density of the metallic core of the nanoparticles. We assume that the poor electronic density indicates the organic layer on top of the nanoparticles, i.e. the molecules extracted from plants. According to DLS measurement, AuNP@*C. longa* appear as 287 nm sized nanoparticles. These nanoparticles are unstable because of the high viscosity of the plant extract. This could also be explained by Hydrogen bonds between flavonoids which gathered the nanoparticles. The stability of the AuNP was studied for 3 months after the synthesis. The λmax and the size of AuNP are presented in **Figure 2 and Figure 3** respectively. Aggregates were observed in two suspensions: AuNP@*N. verticillata* and AuNP@white tea. A red shift was observed for AuNP@*P. casticum*, AuNP@*D. viscosa* and AuNP@*P. cattleianum*, indeed λmax was increased after 3 months. The hydrodynamic diameter of AuNP obtained with *A. triplinervis*, *P. senacia*, white tea, *O. europea africana* and *C. longa* was strongly increased after 3 months. We qualified as unstable the particles with a variation of diameter size >50 nm. AuNP obtained with *N. verticillata*, white tea, *P. casticum*, *D. viscosa*, *A. triplinervis*, *P. senacia*, *O. europea africana* and *C. longa* were considered as unstable.

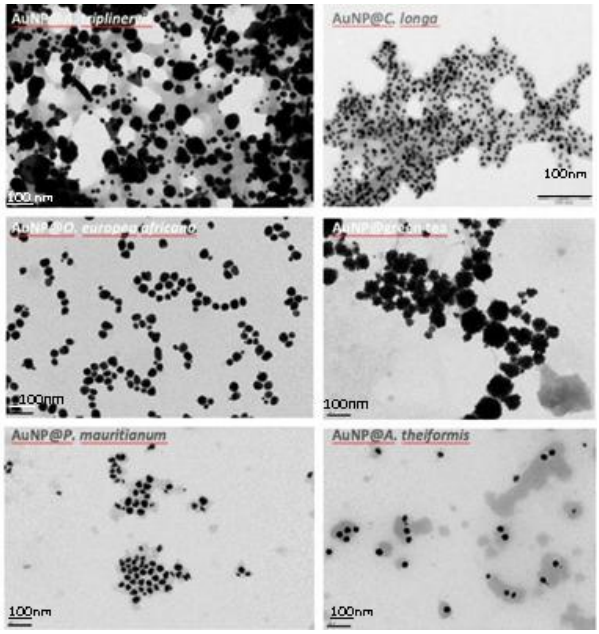

Figure 1: TEM images of gold nanoparticles obtained with (a) A. triplinervis, (b) C. longa, (c) O. europea africana, (d) green tea, (e) P. mauritianum, and (f) A. theiformis.

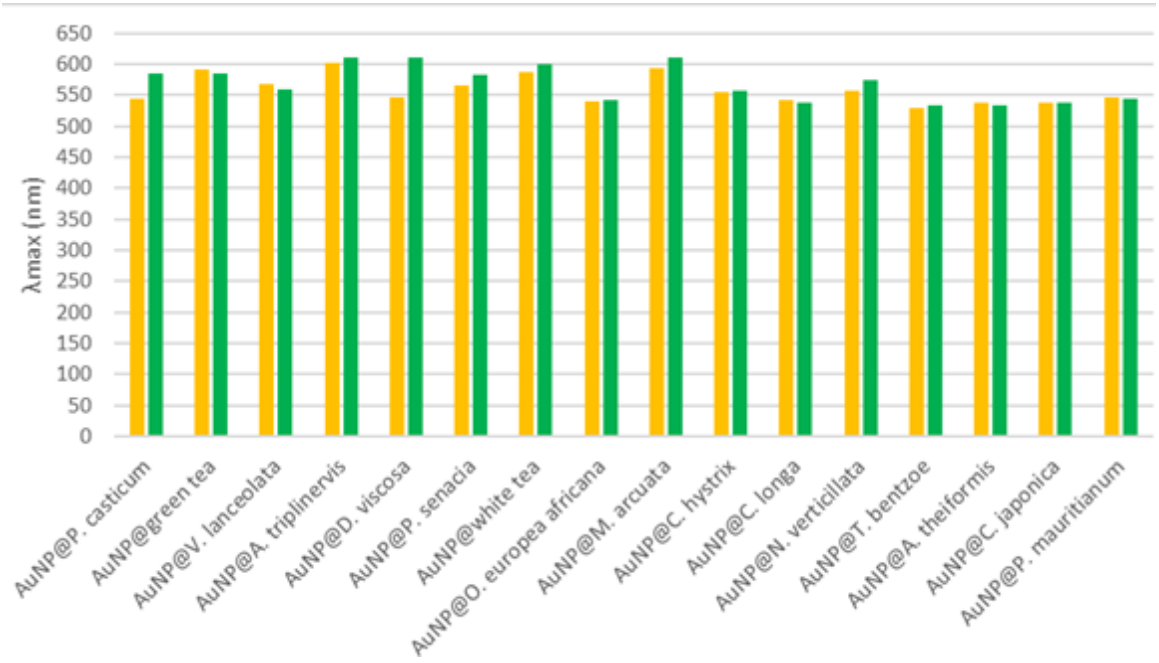

Figure 2: λmax of AuNP suspensions after synthesis (in yellow) and after 3 months (in green).

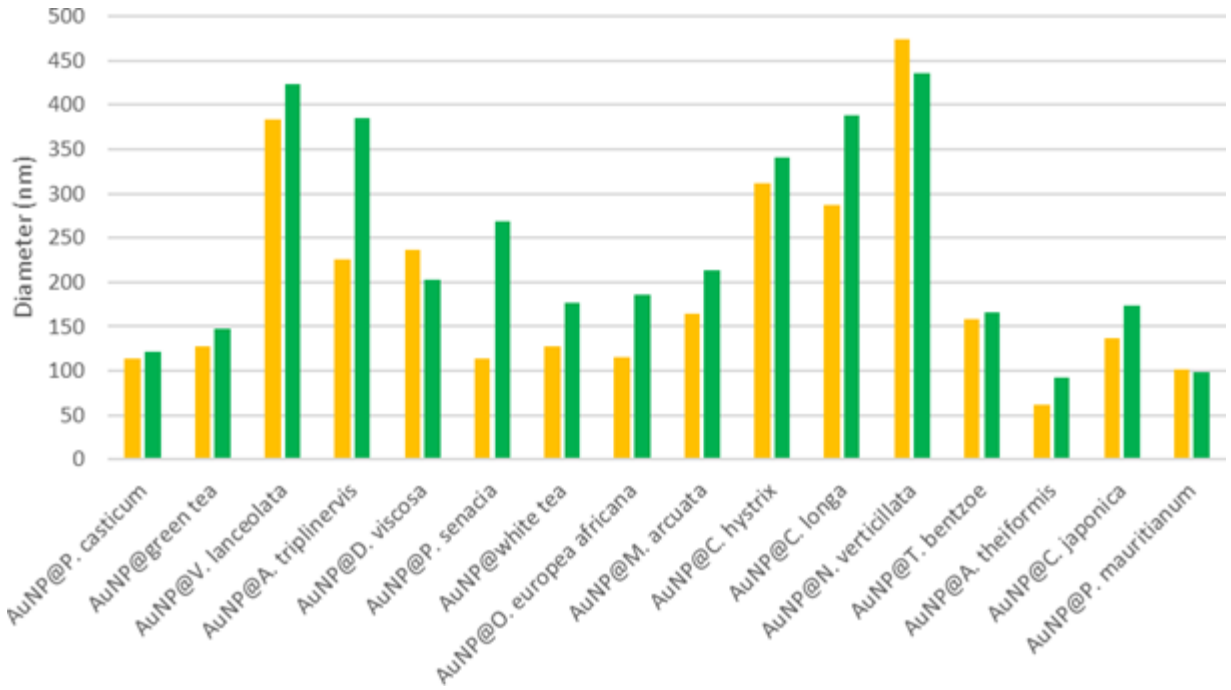

Figure 3: Hydrodynamic diameter (nm) of AuNP after synthesis (in yellow) and after 3 months (in green).

Antioxidant activity

DPPH test: The results of the DPPH test are presented in **Figure 4**. Several tests have been carried out. Indeed, since the AuNP solutions are stained, this was a problem for the absorbance readings: the absorbance of the wells containing the AuNP was greater than that of the negative control. To overcome this problem, the absorbance of the AuNP solutions was measured and then subtracted from the results obtained after reaction with the DPPH. This method was carried out three times.

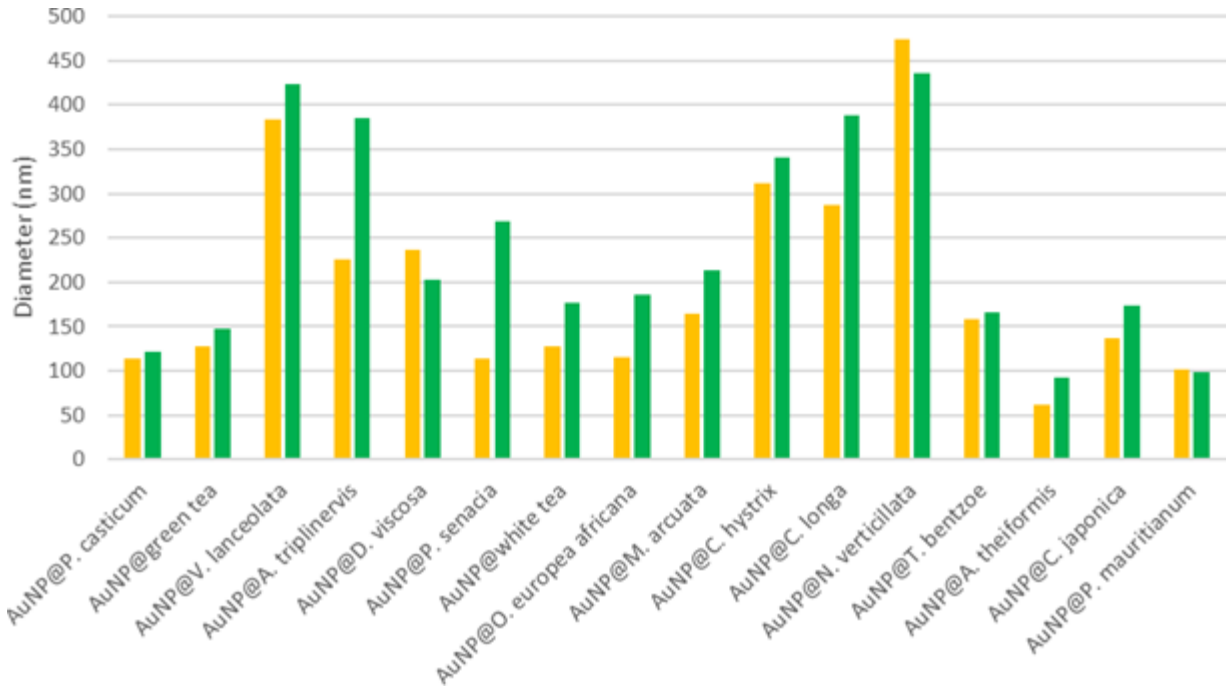

Figure 4: Antiradical activity of AuNP suspensions, assessed by DPPH test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p <0.0001 versus Negative control (ANOVA).

KRL test: The results of the KRL test are presented in **Figure 5**. The AuNP obtained with *M. arcuata*, *T. bentzoe*, *P. mauritianum* and green tea do not have any antioxidant activity according to this test. All other AuNP are prooxidant (HT50<HT50 control). We could explain these results by the generation of Reactive Oxygen Species (ROS). We thus emit the hypothesis that the nanoparticles are photo sensitizers that generate ROS. The use of Low Light Therapy (LLLT) in patients involves red and near-infrared light (600-1100-nm) [22]. Our previous results demonstrated the use of NIR (808 nm) for the thermal destruction of tumor cells [23]. The results of the KRL test could indicate photosensitizer activity of the AuNP coated by plant extracts. This effect could be combined with the plasmonic phototherapy.

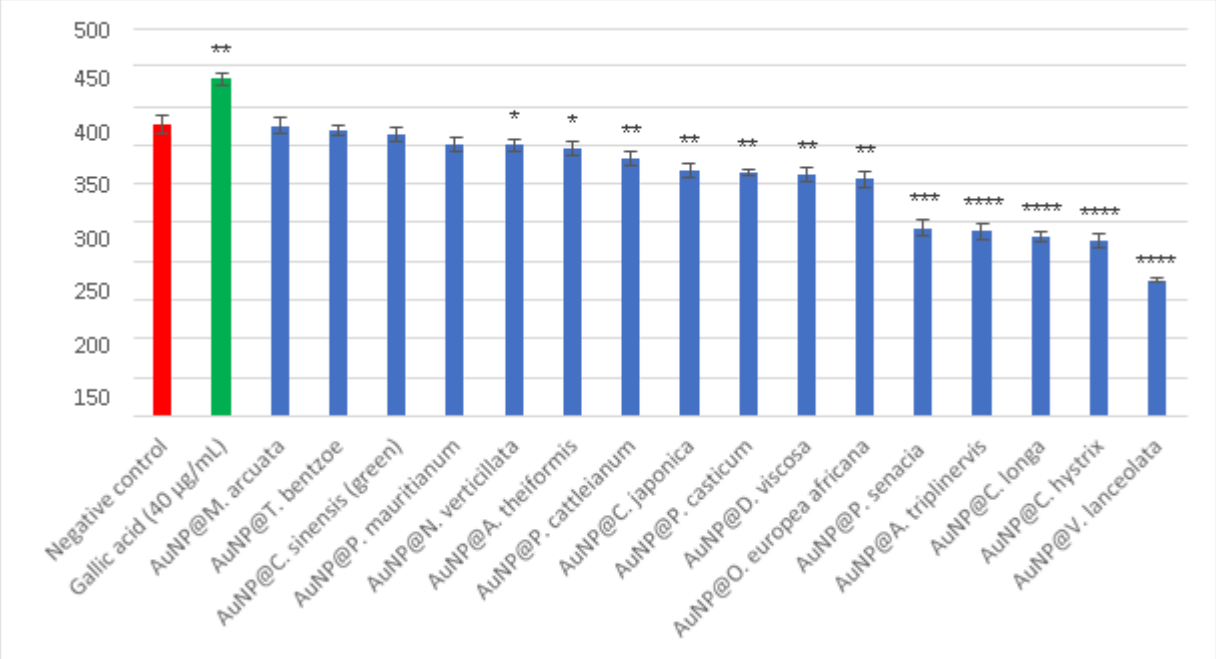

Figure 5: Antiradical activity of AuNP suspensions, assessed by KRL test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p <0.0001 versus Negative control (ANOVA).

Conclusion

All crude extracts tested in this study contained reducing compounds required for AuNP synthesis. Low Light Therapy (LLT) by plasmonic resonance or photodynamy requires narrow band of Surface plasmon (SP) located in the visible area between 400-800 nm. For photodynamic property, photosensitizer molecules are necessary on the top of the nanoparticles. Two mediated plant syntheses provide AuNP with interesting characteristics for light therapy: *A. theiformis* and *P. mauritianum*. Quick synthesis was observed with a quite good yield (400 µg/mL), leading to spherical and small (20-100 nm) AuNP, monodisperse in size and shape. As shown by the KRL test, AuNP@*A. theiformis* are prooxidant and could represent good sensitizer property for photodynamic phototherapy. Moreover, those AuNP were stable over time. Therefore, *A. theiformis* and *P. mauritianum* are both good candidates for the green synthesis of AuNP. The next challenge will consist in identifying the molecules involved in the gold nanoparticles synthesis for the application of the photodynamic therapy combined with the existing plasmonic phototherapy capability.

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