Priya and Iyer
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RESEARCH

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Biosynthesis and optimization of highly stable gold nanoparticles, nanoconjugates, nanodrug conjugates and chitosan nanoconjugates using medicinal plants

M. R. Kamala Priya and Priya R. Iyer*

Abstract

Background: In the present study, the medicinal plants were exclusively selected based on their significant anti-HIV and anticancer activities. The green synthesis of gold nanoparticles was carried out using the 15 medicinal plant extracts on reduction with chloroauric acid. The present study also focused on a novel pharmacognostic approach over the usage of plants source. The nanoparticle synthesized through medicinal plants possessed the potential therapeutic properties of the plants. The resultant nanoparticle carried the attributes of a nanomaterial alongside the phytoactivity.

Results: Initially, individual nanoparticle (NP) was synthesized through a single plant extract and studied for its effective anti-HIV and anticancer activity. Finally, a nanoconjugate (NC) comprising of the 3 extracts (trio extract) in one nanoparticle was synthesized. The nanoparticles which exhibited comparatively high anti-HIV and anticancer activity were chosen for the synthesis of nanoconjugate, thereby achieving a synergistic anticancer and anti-HIV activity. Further, a nanodrug conjugate (NDC) was prepared in combination of AuNPs and the chemotherapeutic drug, doxorubicin (Dox) for cancer and AuNPs with antiretroviral drug azidothymidine (AZT) for HIV.

Conclusions: A nanodrug conjugate helps to enhance the efficiency of the drug. The nanodrug conjugate brings about a combinatorial effect of the nanomaterials and the drug. Further, a biocompatible nanocarrier was developed as a conjugate with chitosan and gold nanoparticles using STPP as gelating agent for the drug doxorubicin. The synthesis reaction was optimized under various underlying parameters. The gold nanoparticles proved to be stable at high temperature and different buffers and ensured to be a safe option for bioassays and *in vivo* applications. Upon storing the synthesized AuNPs at different storage conditions, the nanoparticles were observed to be highly stable for a period of more than 48 months. The present study resulted in biosynthesis of highly stable gold nanoparticles using medicinal plant extracts as reducing and stabilizing agents.

Keywords: Gold nanoparticles, Nanoconjugates, Nanodrug, Chitosan, Biosynthesis, Chloroauric acid

Background

Noble metal nanoparticles, such as gold, platinum and silver, are considered as valuable precursors for the production of innovative nanodevices and nanosystems. The green synthesis of nanoparticles has gained much interest due to the high demand for eco-friendly techniques and methodologies. Additionally, the green

*Correspondence: brajuraj@yahoo.com

Post Graduate and Research Department of Biotechnology, Women's Christian College, College Road, Chennai 600006, Tamil Nadu, India

synthesis yields nanomaterials that are biocompatible and biodegradable (Ismail et al. 2018).

The biosynthesis of nanoparticles is achieved through employing plant extracts, vitamins, biodegradable polymers, sugars and microorganisms as reducing and stabilizing agents. Different parts of plants such as leaf, root, latex, seed and stem are used in the synthesis of metallic nanoparticles. Among the various compounds, polyphenols are considered to be the major active components underlying the green synthesis. Out of several metallic nanoparticles, gold nanoparticles are preferred due to their outstanding biocompatibility. Several plants have been successfully reported for synthesis of gold, silver and copper nanoparticles such as broth extracts of neem, aloe vera, Avena sativa, wheat, alfalfa, geranium, Hibiscus and lemongrass. The synthesized nanoparticles exhibit specific physical and chemical properties and activities that are exploited for therapeutic applications in nanobiotechnology (Teimurimofrad et al. 2017).

Methods

Synthesis of gold nanoparticles

Collection of plant samples

Twelve medicinal plants with potential therapeutic properties were chosen for the study and collected from different locations of Tamil Nadu, Pondicherry and Andhra Pradesh as mentioned in Table 1.

The formal identification of the plant material used in the study was done by Dr. G. Jeya Jothi, Taxonomist, Department of Plant Biology and Biotechnology, Loyola College, Chennai. We confirm that no voucher specimen of this material has been deposited in any publicly available herbarium.

Table 1 Plants selected for the study

S. no.	Plants selected for the study
1	*Camellia sinensis*
2	*Calophyllum inophyllum*
3	*Guazuma ulmifolia*
4	*Catharanthus roseus*
5	*Cassia auriculata*
6	*Aegles marmelos*
7	*Andrographis paniculata*
8	*Asparagus racemosus*
9	*Cinnamomum aromaticum*
10	*Annona muricata*
11	*Justicia gendarussa*
12	*Piper nigrum*

Preparation of the plant extracts: 4 methods (hot, cold, solvent and soxhlet)

About 5 g of the plant sample (leaves, bark, fruit and root) was weighed, washed well and boiled in 50 ml of double distilled water. On cooling, the extract was collected and filtered through a Whatman filter paper. This extract was stored at − 18 °C and was used for further synthesis (Vilchis-Nestor et al. 2008). Cold extraction of the sample was obtained by soaking the plant sample in aqueous medium at room temperature overnight. The extract was collected and filtered through a Whatman filter paper. Dried powdered plant material was further macerated in acetone and water and crude extract of the plants were obtained. The extraction was carried out for 8 h at 70 °C. Similar soxhlet extraction was followed for the plant sample using double distilled water (Redfern et al. 2014).

Synthesis of the gold nanoparticles

Various concentrations of chloroauric acid ($HAuCl_4$) stock solutions were prepared and further used for the synthesis of nanoparticles with the required pH adjustments. The pH of chloroauric acid was checked and adjusted to alkaline ranging from pH 6.5 to pH 8.5. The amount of plant extract needed to reduce the chloroauric acid solution was in the ratio of 1:10. The formation of wine red color indicated the synthesis of gold nanoparticles (Vilchis-Nestor et al. (Vilchis-Nestor et al. 2008)). The reduction of gold chloride into gold nanoparticles was indicated by the color produced during the reaction. The formation of pink/wine red/cherry red color indicated the complete synthesis of gold nanoparticles. Depending upon the intensity of the color produced, the synthesized nanoparticles were observed to differ in dimensions (Gavina et al. 2018).

Optimization studies

A range of pH starting from pH 4, pH 6, pH 8 and pH 10 was tested. The optimum pH was observed, where the formation of gold nanoparticles was successful indicated by the color change of the solution. The temperature of the stock solution, chloroauric acid, was set at room temperature (± 25 °C), 45 °C, 65 °C, 85 °C and boiling temperature 100 °C. The optimum temperature was noted to be the temperature at which a complete synthesis of gold nanoparticles was observed. In other temperatures, an incomplete or intermittent reaction was observed through the varied color change (Pandey et al. 2012). Various concentrations of the stock solution were prepared in double distilled water and used for the synthesis of nanoparticles with the required pH adjustments (Boruah et al. 2012). The ratio of plant extract needed

to reduce the chloroauric acid solution was taken in the ratio of 10:1. The synthesis ratio of chloroauric acid/plant extract was varied as 10:1, 10:2, 1:1, 1:2 and observed for the formation of nanoparticles (Boruah et al. 2012).

Sterilization of the synthesized nanoparticles

The synthesized gold nanoparticles were sterilized in autoclave for 15–20 min, at 121 lbs. After sterilization, the nanoparticles were analyzed in UV–visible spectrophotometer. During this process, the nanoparticles were subjected to high pressure and temperature. This test was carried out to understand the thermal stability of the synthesized nanoparticles (Subbarao 2016).

In vitro stability and storage of the synthesized nanoparticles

The synthesized nanoparticles were checked for stability at different pH using phosphate buffer at pH 5.7, pH 6.1, pH 7.0, pH 8.0 and normal saline. To 1 ml of buffer, about 100 µl to 200 µl of AuNPs (Gold nanoparticles) were added and incubated at 37 °C for 24 h. The solution was analyzed in UV–visible spectrophotometer (Wang et al. 2014). The synthesized AuNPs were stored at different storage conditions at room temperature, 4 °C in refrigerator and − 20 °C in deep freezer and analyzed periodically. The AuNPs were then analyzed in UV–visible spectrophotometer (Izak-Nau et al. 2015).

Synthesis of gold nanoconjugate (AuNC) for enhanced bioactivity

As a novel approach in this study, initially individual nanoparticle (NP) was synthesized through a single plant extract and studied for its effective anticancer and anti-HIV activity. Further a nanoconjugate (NC) comprising of the 3 extracts (trio extract) was synthesized based on the best outcome of anticancer and anti-HIV activity. The nanoconjugate (NC) was expected to have a combined synergistic effect and increased anti-HIV and anticancer activity than the nanoparticle (NP).

Nanodrug conjugate for combinatorial effect

Doxorubicin (Dox) is the chemotherapy drug used in the treatment of cancer. Its usage is associated with side effects, and severity is high in some cases. Azidothymidine (AZT) is the antiretroviral drug used in the treatment of HIV/AIDS. Its long-term usage leads to drug resistance and other associated side effects. A nanodrug conjugate can help us to overcome this challenge. The nanoparticle that possesses anticancer/anti-HIV activity was conjugated with the drug such that it brings about a combinatorial effect of the nanoparticles and the drug; thereby, an effective therapeutic index of the drug can be achieved.

Synthesis of nanodrug conjugate

An aqueous solution of sodium tripolyphosphate (STPP) at 1 mg/ml concentration was used as a gelating agent. Equal volumes of AuNPs and doxorubicin (1 mg/ml) were taken. This drug solution was added and homogenized for 30 min at 400 rpm with drop-wise addition of STPP. The prepared nanodrug conjugate was further characterized and studied for anticancer activity. Similarly, azidothymidine nanodrug conjugate was prepared and studied for anti-HIV activity (Tyagi and Pandey 2016).

Synthesis of chitosan nanodrug conjugate

Chitosan was procured from Loba Chemie and used without further purification. An aqueous solution of STPP (1 mg/ml) was prepared which was added to aid the process of chitosan–nanoparticles–drug nanoconjugate formation (Zambito 2013). A biocompatible nanocarrier was developed as a conjugate using chitosan and gold nanoparticles with the anticancer drug doxorubicin for enhanced biocompatibility. Equal volumes of synthesized gold nanoparticle and chitosan solution were mixed. To this 1 ml of the drug solution was added and homogenized for 30 min at 400 rpm with drop-wise addition of STPP. The prepared chitosan nanoconjugate was further characterized and studied for anticancer activity (Tyagi and Pandey 2016).

Results

All the plant materials collected for the study are listed in Fig. 1.

Preparation of the stock solution—chloroauric acid

The stock solution of chloroauric acid was prepared in different concentrations and used for further synthesis of nanoparticles. A high molar concentration of 25.8 mM preparation has been reported for the synthesis of gold nanoparticles by Boruah et al. (2012). In this study, a successful synthesis of gold nanoparticles has been achieved at very minimal concentrations of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. This minimal concentration of nanoparticles ensures a biologically much safer nanoparticle for in vivo applications.

Preparation of the plant extracts

The plant extracts were prepared through hot extraction, cold extraction, methanolic extraction and soxhlet extraction as shown in Fig. 2. The synthesis of nanoparticles was carried out using the extracts obtained through all the above-mentioned processes. The nanoparticles were successfully synthesized with all the above extracts. When the nanoparticles could be

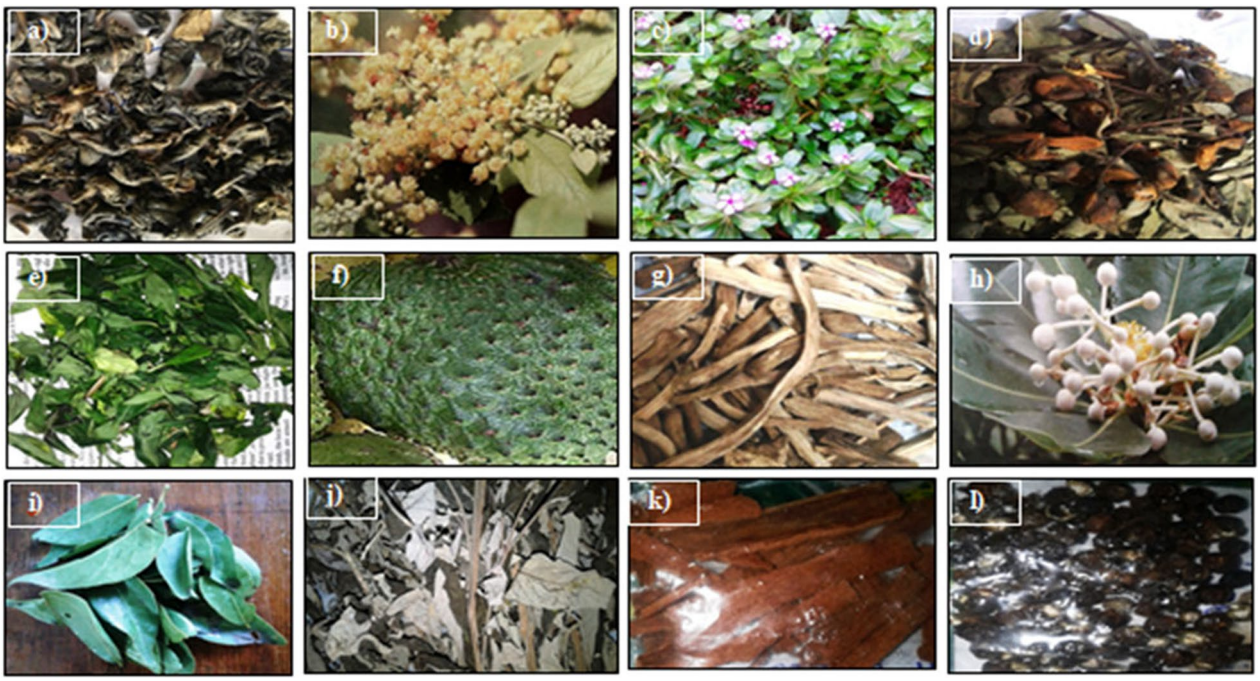

Fig. 1 The plant samples used in the study, **a** *Camellia sinensis*, **b** *Guazuma ulmifolia*, **c** *Catharanthus roseus*, **d** *Cassia auriculata*, **e** *Andrographis paniculata*, **f** *Annona muricata*, **g** *Asparagus racemosus*, **h** *Calophyllum inophyllum*, **i** *Aegles marmelos*, **j** *Justicia gendarussa*, **k** *Cinnamomum aromaticum*, **l** *Piper nigrum*

Fig. 2 Hot extraction, cold extraction, methanolic extraction, stored extracts at − 18 °C

exploited for in vivo applications such as novel therapeutics and drug delivery molecules, the aqueous base nanoparticles would render less toxic or no toxicity effect.

Synthesis of the gold nanoparticles

The nanoparticles were synthesized using the 15 medicinal plant extracts on reduction with chloroauric acid. The synthesized nanoparticles were pale pink to deep wine red in color as observed in Fig. 3. In this study, the plant materials were exclusively selected based on their significant anti-HIV and anticancer activities. The various phytocompounds such as volatile oils, fatty oils, vitamins, tannins, phenols, flavonoids and others present in the plant extracts acted as the reducing and stabilizing agent for synthesis.

Rate of the synthesis of nanoparticles

The synthesis of nanoparticles took place in varying time durations as mentioned in Table 2. Lal and Nayak (2012) in their study reported that the minimum time of synthesis was recorded as 5 min and maximum time as 5 h. In the present study, the minimum duration was observed to be less than a minute and the maximum duration was

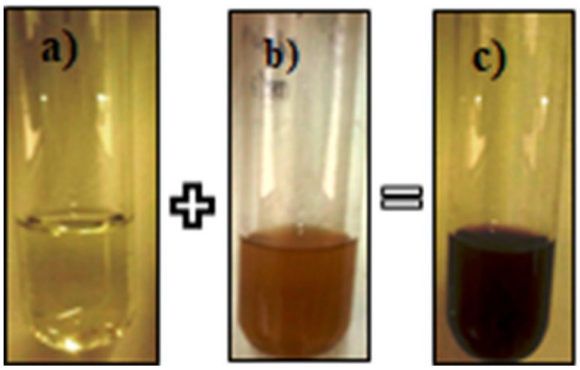

Fig. 3 **a** Chloroauric acid, **b** plant extracts, **c** synthesized AuNPs. a = Precursor solution (chloroauric acid, the standard component in synthesis). b = Plant extracts (variable component, each plant extract resulted in different nanoparticles). c = Synthesized gold nanoparticles (different nanoparticles were synthesized due to different reducing agents, plant extracts)

between 3 and 4 h for synthesis. The synthesis of gold nanoparticles was achieved in a much faster rate than the previous study.

Intensity of the nanoparticles produced

The bioreduction of metallic gold into nanogold resulted in a distinctive shift in color of the colloidal solution. The precursor chloroauric solution was pale yellow in color. The nanoparticles synthesized were predominantly deep red in color, while few exhibited pink color as mentioned in Table 3 and the intensity of the color of the synthesized nanoparticles varied with different plant extracts as observed in Fig. 4. Based on the intensity of the nanoparticles solution, the nanoparticles were further quantified.

A novel attempt of nanoconjugate synthesis

Despite use of biological entities such as plant, bacteria or fungi as an agent for the synthesis of nanoparticles, plants have been exploited only as a reducing agent for nanomaterials. The present study focused on a novel pharmacognostic approach over the usage of plants source. The nanoparticle synthesized through medicinal plants will possess the potential therapeutic properties of the plants. The resultant nanoparticle probably carried the attributes of a nanomaterial alongside the phytoactivity. Initially, individual nanoparticle (NP) was synthesized through a single plant extract and studied for its effective anti-HIV and anticancer activity. Finally, a nanoconjugate (NC) comprising of the 3 extracts (trio extract) in one nanoparticle was synthesized as observed in Fig. 5 based on the results of anti-HIV and anticancer activity. The nanoconjugate (NC) showed a combined synergistic effect and increased anti-HIV/anticancer activity than the nanoparticle.

Synthesis of nanodrug conjugate

It was reported by Czeczuga et al. (2004) that doxorubicin in combination with estradiol, tamoxifen and retinoic acid showed the most effective and statistically significant decrease in the percentage of MCF-7 cells. In the present study, nanodrug conjugate (NDC) was prepared in combination of AuNPs and the chemotherapeutic drug, doxorubicin for cancer and AuNPs with antiretroviral drug azidothymidine (AZT) for HIV as shown in Fig. 6. A nanodrug conjugate helps to enhance

Table 2 Duration of nanoparticles synthesis

S. no.	Plants used for synthesis	Duration of synthesis	Nanoparticles
1	*Camellia sinensis leaves*	Less than a minute	NP1
2	*Guazuma ulmifolia leaves*	3–4 h	NP2
3	*Guazuma ulmifolia Bark*	Less than a minute	NP3
4	*Calophyllum inophyllum leaves*	3–4 h	NP4
5	*Calophyllum inophyllum fruit*	Less than a minute	NP5
6	*Cinnamomum aromaticum Bark*	Less than a minute	NP6
7	*Andrographis paniculata leaves*	30–60 min	NP7
8	*Aegles marmelos leaves*	3–4 h	NP8
9	*Asparagus racemosus roots*	30–60 min	NP9
10	*Catharanthus roseus flowers*	Less than a minute	NP10
11	*Cassia auriculata leaves* and *flower*	Less than a minute	NP11
12	*Annona muricata fruit pulp*	30–60 min	NP12
13	*Annona muricata fruit peel*	30–60 min	NP13
14	*Justicia gendarussa leaves*	3–4 h	NP14
15	*Piper nigrum seeds*	3–4 h	NP15

Table 3 Intensity of color in nanoparticles synthesized

Type of nanoparticles	Intensity of color produced
NP1 CSAuNP	+++++ Deep wine red
NP2 GULAuNP	+++++ Deep wine red
NP4 CILAuNP	+++++ Deep wine red
NP5 CIFAuNP	+++++ Deep wine red
NP6 CAAuNP	+++++ Deep wine red
NP9 ARAuNP	+++++ Deep wine red
NP10 CAAuNP	+++++ Deep wine red
NP11 CRAuNP	+++++ Deep wine red
NP14 JGAuNP	++++ Wine red
NP12 AMPEAuNP	++++ Wine red
NP3 GUBAuNP	+++ Pink
NP7 APAuNP	+++ Pink
NP8 AMAuNP	+++ Pink
NP13 AMPUAuNP	+++ Pink
NP15 PIAuNP	++++ Red

the efficiency of the drug. The nanoparticle (AuNP) and nanoconjugate (AuNC) with anticancer and anti-HIV activity were conjugated with the drugs. Thus, the nanodrug conjugates bring about a combinatorial effect of the nanomaterials and the drug.

Synthesis of chitosan nanodrug conjugate

Chitosan has various characteristics which makes it ideal for in vivo applications. It is biocompatible, biodegradable, non-toxic, non-antigenic and has strong adsorption properties (Hettiarachchi et al. 2011). It was reported by Zambito (2013) that Chitosan nanoparticles conjugated with peptide drug insulin resulted in an enhanced transport of insulin compared to native unconjugated insulin. In the present study, a biocompatible nanocarrier was developed as a conjugate with chitosan and gold nanoparticles using STPP as gelating agent for the drug doxorubicin as represented in Fig. 7.

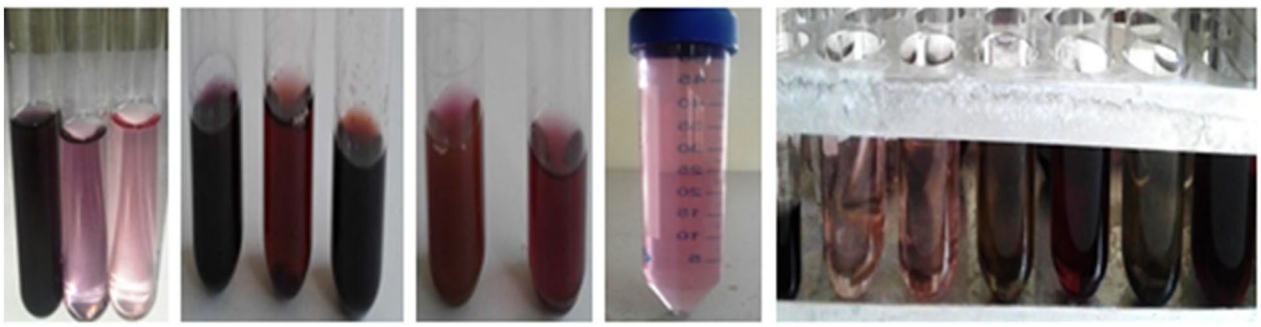

Fig. 4 Synthesized nanoparticles showing varying intensity of color

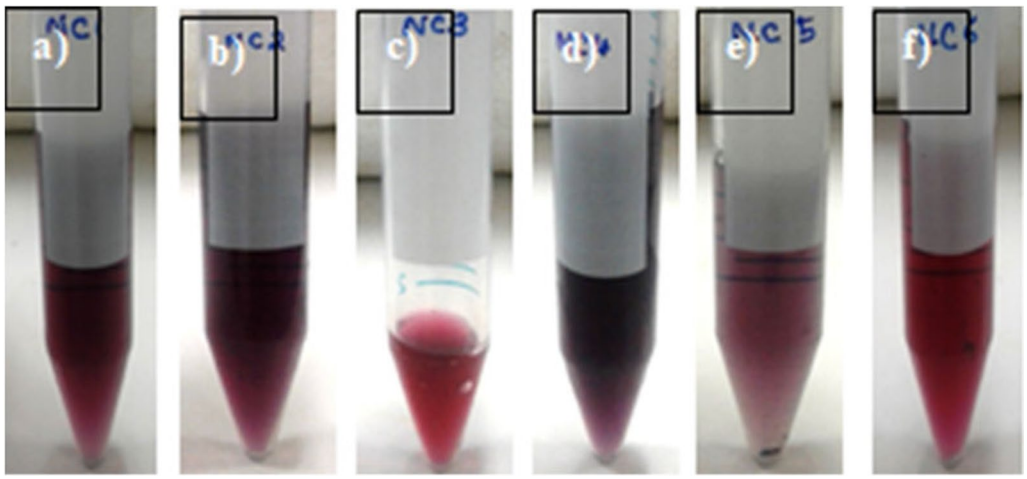

Fig. 5 **a**–**f** Synthesized nanoconjugates using trio extract

Optimization of pH on the synthesis

The pH has a crucial part in the successful reduction of bulk materials and in the synthesis of nanomaterials. The pH range was checked between pH 4, pH 6, pH 8 and pH 10. It was observed that at pH 8, there was a complete reduction of bulk metal into metallic nanoparticles. At the other tested pH gradients, a partial reduction of the bulk metal was observed with varying color changes of the precursor solution. The reaction between chloroauric acid and plant extracts was observed at various pH gradients of pH 4, pH 6, pH 8 and pH 10.

The observed results are represented in Fig. 8, which were in contrast to the reports stated by Pandey et al.

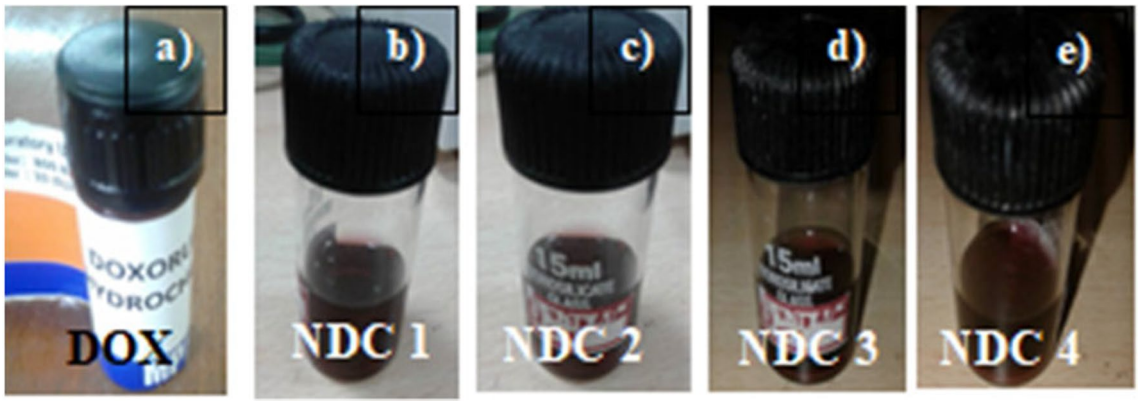

Fig. 6 **a**–**e** Synthesized nanodrug combinations (NDC) using doxorubicin and nanoparticles

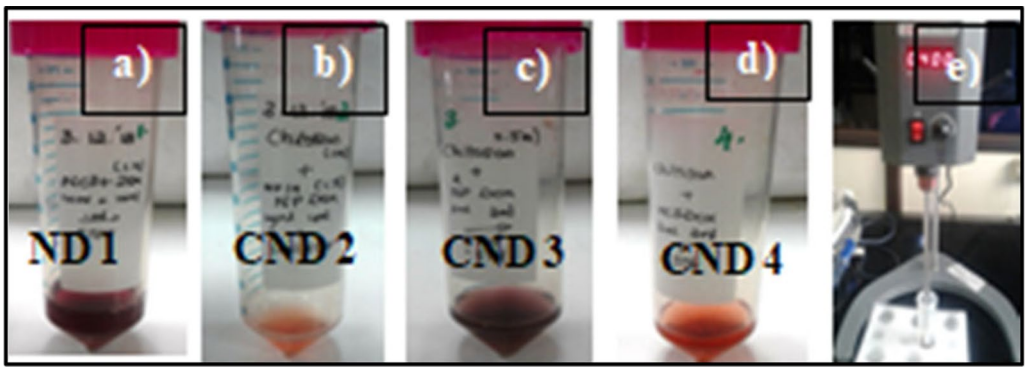

Fig. 7 **a**–**d** Synthesized chitosan nanodrug combinations (CND) and **e** homogenization

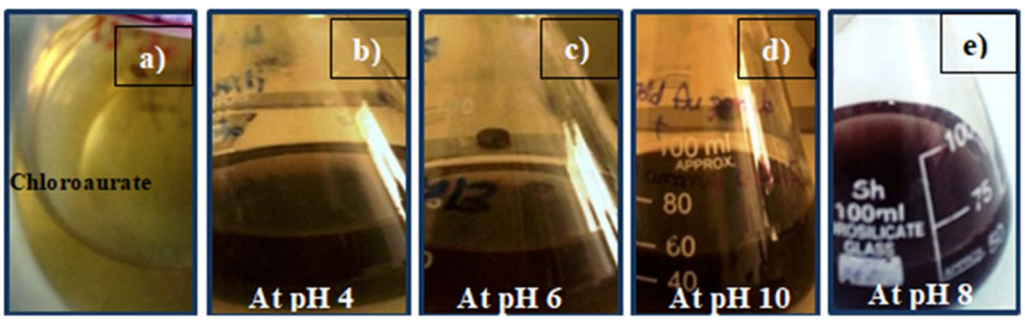

Fig. 8 **a**–**e** Chloroaurate and nanoparticles synthesis at different pH

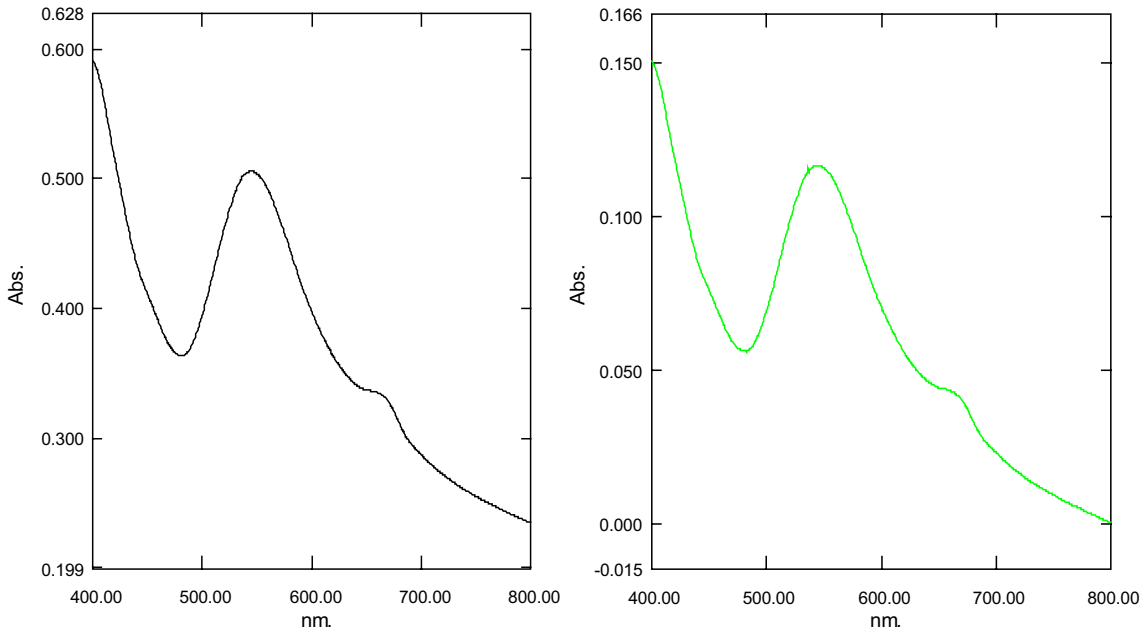

Fig. 9 **a** Before sterilization, **b** after sterilization

(2012) and Sharon et al. (2012) where it has been stated that pH 10 was the optimum condition for the synthesis. In the present study, pH 8 was observed to be the optimum pH that resulted in successful synthesis of nanoparticles.

Optimization of temperature on the synthesis

The reaction was carried out in varying temperatures ranging from room temperature (28°C) to 100°C. It was found that a complete reduction that yielded the nanoparticles was observed at room temperature. In other temperatures, the reduction was incomplete or intermediate which was indicated by varied color changes.

Sterilization of the synthesized nanoparticles

The synthesized gold nanoparticles were subjected to sterilization in autoclave for 15 to 20 min, at 121 lbs. The nanoparticles were analyzed before and after the process under UV–visible spectrophotometer as represented in Fig. 9.

In vitro stability of the synthesized nanoparticles

The synthesized nanoparticles were checked for stability at different pH mimicking the physiological environment using phosphate-buffered saline (PBS). The various conditions included pH 5.7, pH 6.1, pH 7.0, pH 8.0 and normal saline. The synthesized gold nanoparticles were incubated in different buffers and analyzed in UV–visible spectrophotometer as represented in Fig. 10.

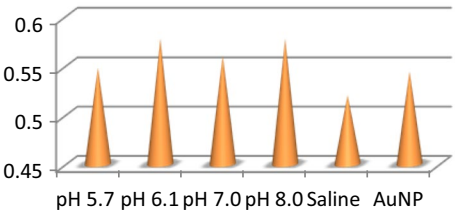

Fig.10 Representation graph of in vitro stability of nanoparticles

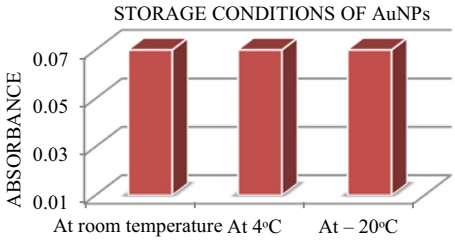

Fig. 11 Representation graph of storage conditions of nanoparticles

Storage conditions of synthesized nanoparticles

Upon storing the synthesized AuNPs at different storage conditions such as room temperature (±28), 4 °C

(refrigerator) and -20 °C (deep freezer), the nanoparticles were analyzed in UV–visible spectrophotometer as shown in Fig. 11 and compared with the standard spectrum. It was observed that the AuNPs were stable in all these 3 stored conditions without any significant difference in the state of the nanoparticles. Hence, the nanoparticles could be stored at any appropriate storage conditions.

Discussion

The molar concentration of the precursor solution plays a very crucial role in the development of any product. At this minimal concentration, the nanoparticles could be probably eliminated easily from the body. On the other hand, if there is any possibility of retention of nanoparticles within the body, the condition could be probably negligible, since the nanoparticle would be administrated at nanomolars, the quantity being very low and very much negligible. It was reported by Song et al. (2009) that the plant extracts were stored at − 4 °C and utilized within short periods. Various other studies stated on a similar usage of plant extracts. In the present study, when the plant extracts were stored at − 18 °C, the extracts were functionally stable and used throughout the study for synthesis. Thereby, an efficient and maximum utilization of the biological resource has been achieved. It was reported by Gavina et al. (2018) that the color of the nanoparticles indicated about the aggregation of the particles in the colloidal solution. The nanoparticles were observed to be red in color in dispersed state, and in case of aggregation of the nanoparticles, a blue color was observed. In the present study, the nanoparticles were observed to be red color, thereby demonstrating well-dispersed nanoparticles without aggregations. The synthesis of nanoparticles was observed at different temperatures. The observed results were in contrast to the reports stated by Pandey et al. (2012) and Sharon et al. (2012), where it has been reported that boiling temperature or 100 °C was the optimum condition for the synthesis of nanoparticles. In the present study, room temperature was observed to be the optimum temperature that resulted in successful synthesis of nanoparticles. The sterilization of nanoparticles by autoclaving or moist heat has been reported to be an effective procedure by Subbarao (2016). Masse et al. (2019) have studied on the ultrastability of gold nanoparticles through various sterilization techniques such as freeze drying, heating, ultracentrifugation and autoclaving. It was reported that gold nanoparticles exhibited high stability under all the subjected sterilization conditions by comparing UV–visible spectrum of gold nanoparticles before and after sterilization. It was observed that there was no significant difference in the SPR peak absorption of the synthesized gold nanoparticles before and after sterilization which implied the high stability and thermal resistivity of the biosynthesized gold nanoparticles.

It was reported by Wang et al. (2014) that the gold nanoparticles were stable in water and biobuffer, but were unstable at varying pH. In the present study, the AuNPs were incubated in different buffer solutions for 24 h and analyzed under UV–visible spectrophotometer. It was observed through the absorbance spectrum of the nanoparticles that only minimal changes (< 10%) were recorded in the SPR band at 525 nm. The gold nanoparticles proved to be stable at different buffers, thereby ensuring to be a safe option for bioassays and *in vivo* applications. A report on various storage conditions has been stated by Izak-Nau et al. (2015). The nanoparticles were stabilized with 6 different capping agents and stabilizers before storage. In the present study, the nanoparticles were stored without any capping agents and stabilizers. The nanoparticles were observed to be highly stable for a period of more than 48 months.

Clinical applications

When the effect of a drug is enhanced, the enhancement of side effects is generally a problem. When it comes to doxorubicin, it is a drug that poses a high risk of cardiotoxicity. There arises a question on its increased side effect or fatality. But in the present study, the drug is enhanced with a biosynthesized nanoparticle, so the enhancement is achieved with the same amount of drug without any further increase in its concentration and since the drug is proportionately combined with the nanoparticles, the amount required can be reduced to half of the efficient dose. A less dose of the drug would probably lower its side effects. Azidothymidine is a drug that requires multiple doses during the day. Doxorubicin is withdrawn for 7 to 10 days after administration. This study will definitely contribute to the improvement of the present administration methods of these drugs. A further extended research will definitely help us to develop better administration methods and minimize the number of doses required. Further, there are wider opportunities for this study to be taken forward and applied to other drugs in place of doxorubicin and azidothymidine. Indeed, the nanoparticles are excellent drug carrier and drug delivery molecules, which can be exploited for various drugs and compounds of interest.

Conclusions

The plant materials were exclusively selected based on their significant anti-HIV and anticancer activities. The green synthesis of gold nanoparticles was carried out using the 15 medicinal plant extracts on reduction with chloroauric acid. The present study also focused on a

novel pharmacognostic approach over the usage of plants source. The nanoparticles which exhibited comparatively high anti-HIV and anticancer activity were chosen for the synthesis of nanoconjugate, thereby achieving a synergistic anticancer and anti-HIV activity. Further, a nanodrug conjugate (NDC) was prepared in combination of AuNPs and the chemotherapeutic drug, doxorubicin (Dox) for cancer and AuNPs with antiretroviral drug azidothymidine (AZT) for HIV. A nanodrug conjugate helps to enhance the efficiency of the drug. The nanodrug conjugate brings about a combinatorial effect of the nanomaterials and the drug. Further, a biocompatible nanocarrier was developed as a conjugate with chitosan and gold nanoparticles using STPP as gelating agent for the drug doxorubicin. The synthesis reaction was optimized under various underlying parameters. The gold nanoparticles proved to be stable at high temperature and different buffers and ensured to be a safe option for bioassays and *in vivo* applications. Upon storing the synthesized AuNPs at different storage conditions, the nanoparticles were observed to be highly stable for a period of more than 48 months. The present study resulted in biosynthesis of highly stable gold nanoparticles using medicinal plant extracts as reducing and stabilizing agents.

Abbreviations
NP: Nanoparticles; NC: Nanoconjugate; NDC: Nanodrug conjugate; Dox: Doxorubicin; AZT: Azidothymidine.

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None.

Author contributions
PI designed and directed the study, and KP executed the entire research work from synthesis of NPs, its characterization and in vitro studies and was the major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data and material are available upon request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
There are no competing interests to declare.

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