Green synthesis of silver nanoparticles from leaf and flower extracts of *R. indica* and *C. roseus*

ABSTRACT

Silver nanoparticles are playing potential role in various fields such as medical, environmental, food, cosmetics, consumer goods, electronics, textiles etc. Due to which their production is getting hype for their multiple usage. Silver nanoparticles (AgNPs) traditionally can be synsthesized by chemical and physical methods. But in past few decades, biological methods are utilized for the green synthesis of AgNPs. This research article shows the green synthesis of AgNPs which have been synthesized from leafs& flowers extract of *Rosa indica* & *Catharanthus roseus.* It has been reported, *Rosa indica* & *Catharanthus roseus* have possible therapeutic uses in many severe & common diseases such as cancer, diabetes etc. As both plant species have antibacterial, antifungal, antineoplastic & anticancer properties. In this process the leaf extract and flower extract of *Catharanthus roseus* and *Rosa indica* has been used as reducing agent, which was mixed with silver nitrate ($AgNO_3$). Futher, the characterization of synthesized AgNPs was done with the help of UV-Vis spectroscopy & TEM. Based on these results the absorption spectra of *Catharanthus roseus* and *Rosa indica* were both compared to detect which had the most absorption peak.

INTRODUCTION:

Nanoparticles are particles that have a particle size ranging from 1 nm-100 nm, which is one billionth of a meter. Due to their small size and high surface area to volume ratio, nanoparticle provides a tremendous driving force for diffusion, especially at elevated temperatures (1). It is very well known that the metals such as silver and gold is playing important role in biological systems, living organisms and medicine. These metals are primary choice to produce their nanoparticles due to its exclusive properties & potential applications in nanobiotechnology. Silver nanoparticles have potential as it is widely used in chemical reactions as catalysts, electrical batteries and in spectrally selective coatings for absorption of solar energy as optical elements, pharmaceuticals components and in chemical sensing and bio sensing, etc. (2)

The synthesis of silver NPs can be carried out by several methods including chemical (e.g., chemical reduction, micro emulsion techniques, pyrolysis, UV-initiated photo-reduction, photo-induced reduction, electrochemical synthetic method, irradiation methods, microwave-assisted synthesis, polymers and polysaccharides, Tollens method), physical (e.g., evaporation-condensation, laser ablation, arc discharge method, direct metal sputtering into the liquid medium) and biological methods (e.g., use of algae, fungi, bacteria and plants (3) as bioreductant). The chemical and physical processes mostly involve hazardous chemicals, high energy requirements and other strict conditions (4). The sizes and morphologies of silver nanoparticles synthesized from these two methods are quite variable depending on the conditions and methods applied. In contrast to the chemical and physical methods, the biological method, also known as the bottom-up approach, has been used for green synthesis of AgNPs with better sizes and morphologies(5).

Most of the NPs produced by bottom up approach were reported to have a predominantly spherical shape (6).The use of biological reductants, less energy requirements and improved characteristics of the silver nanoparticles are some major advantages of the green approaches. A further advantage is the elimination of the toxic chemicals that are used as surfactants or stabilizers since various proteins present in the plant extracts act as reducing as well as capping agents for silver NPs. This also makes the process fast, efficient, economically effective and ecofriendly (7). A number of plants are reported to synthesize silver NPs. This work give forth about the production of silver NPs through green synthesis, utilizing the ornamental plant *Rosa indica* & *Catharanthus roseus.* For this study, the *Catharanthus roseus* and *Rosa indica* are utilized for that plant extract. *Catharanthus roseus* is an ornamental flower belongs to the Apocynaceae family. It is also known as bright eyes, cape periwinkle, graveyard plant, Madagascar periwinkle, old-maid, old maid flower, pink periwinkle, etc. As its names describes, it is a native species of Madagascar, but found worldwide (8).

C. roseus can be considered as a rich source of alkaloids and phenolics, which possess diverse biological properties including anticancer, antidiabetic, antioxidant, antimicrobial and antihypertensive activities. (9). Due to these medicinal properties, this plant is chosen for green synthesis of nanoparticles like silver nanoparticle.

On the other hand, *Rosa indica* have many potentials for human health. It is a member of the *Rosaceae* family and is used as a traditional medicine. (10). Some important fatty acids such as Linoleic, α-linolenic, oleic, palmitic, stearic, octadecenoic, eicosenoic, eicosadienoic, erucic, and other minor fatty acids are present in *Rosa indica.* (11) It contain medicinally important bioactive compounds which have their use for treatment of different diseases (12) hence it is a potential herb for green synthesis of nanoparticles which will have applications in many therapeutic way and drug delivery systems.

This biosynthesis of Silver NPs is followed by characterization done with the help of UV-Vis spectroscopy & TEM.

MATERIAL AND METHOD

Biosynthesis of Silver Nanoparticles is performed ***In-vivo.*** Fresh plant of *C. roseus* and *R. indica* were identified and collected. The leaf and flower were used for the experiment. Silver nitrate (1mM) solution is made with Sterile distilled water.

The preparation of plant extract; the leaves and flowers of *Catharanthus roseus* and *Rosa indica* were collected. 10 gm of leaves and flowers from each plant were taken and washed by dripping into tap water and further into distilled water for 30 min. The leaves and flowers were finely cut and kept in the oven to dry at 50 ^{o}C for 2 hrs and later taken out dry overnight. The next day the leaves and flowers were crushed into powder (**fig 1**)

Dried leaf powder	**Dried flower powder**

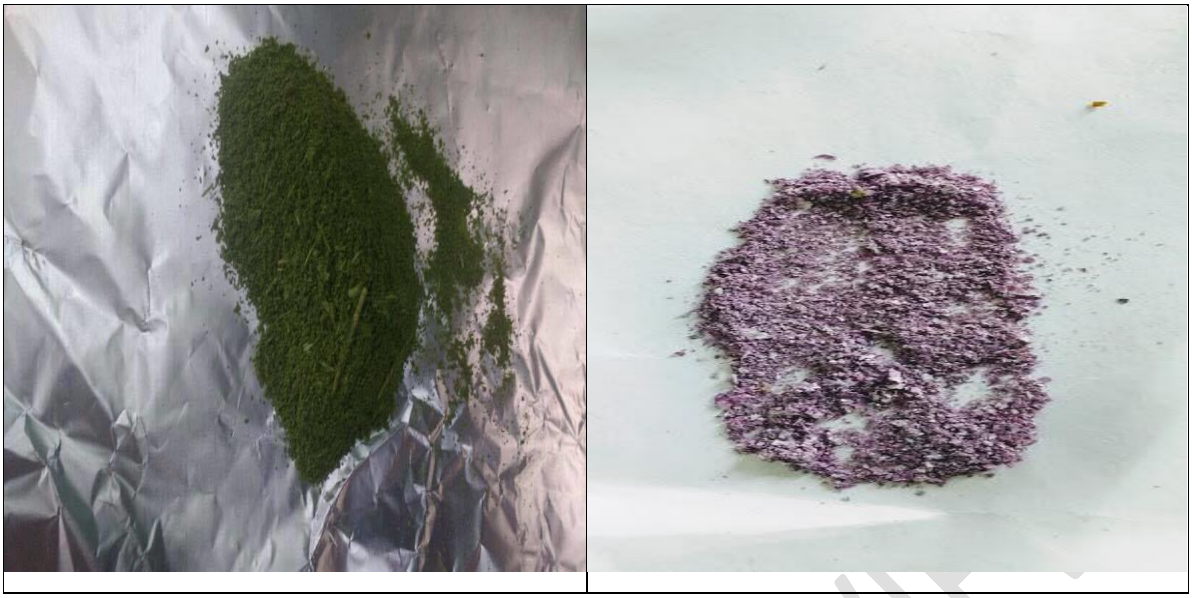


Figure 1Dried leaf powder of Catharanthus leaf and flower

The dried powder was poured into 100 ml distilled water in 500 mL conical flask and boiled for 20 min (20 min was counted only when it began to boil). The extract was separated using a Whatman filter paper and was stored at 4 ^{o}C (**fig2, fig 3a, 3b, 3c**)

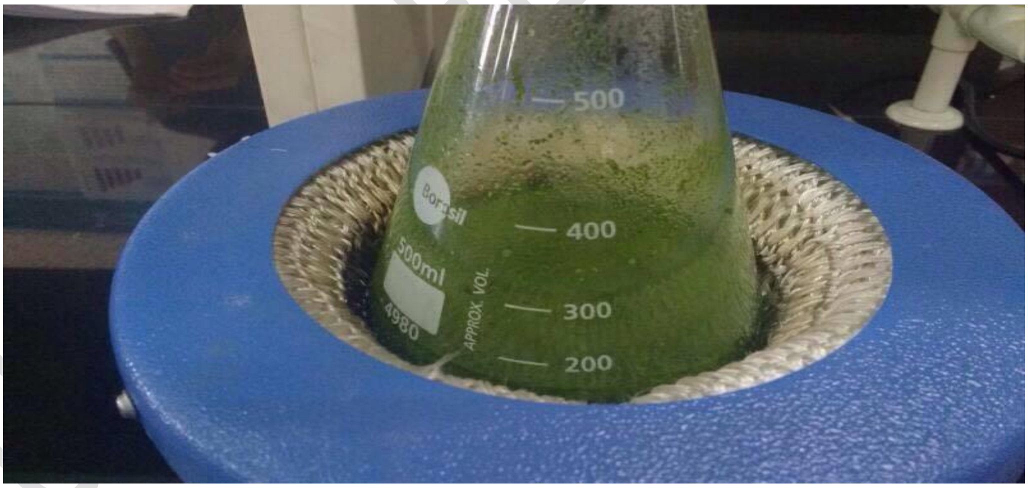
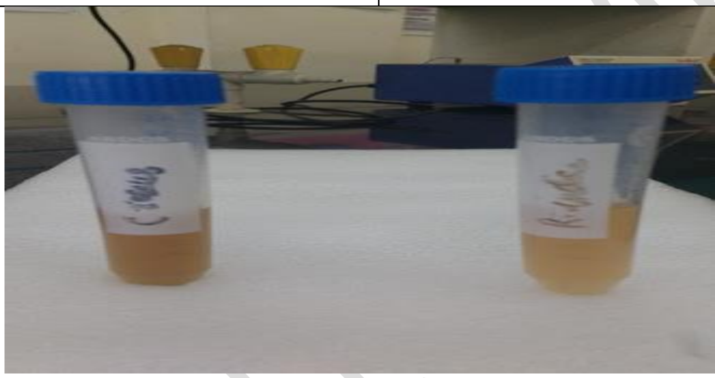


Figure 2 Distil water extraction of leaf extract

a) *C. roseus* leaf extract

b) *R. indica* leaf extract



c) The flower extract of *R. indica* and *C. roseus*

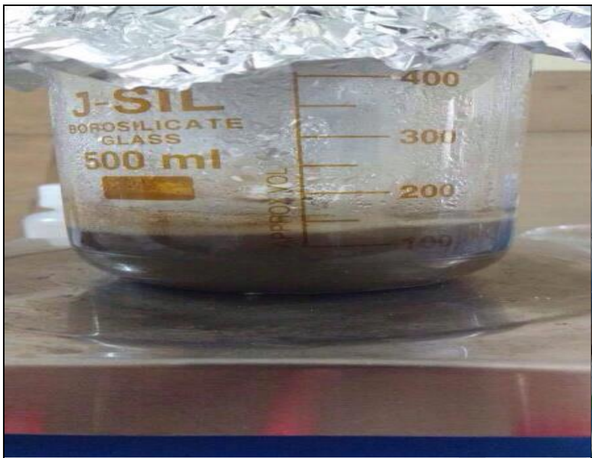
Figure 3: Leaf and flower extract of test plant

<u>Biogenesis of silver nanoparticles</u>

Preparation of aqueous solution of 1mM of silver nitrate ($AgNO_3$) and used for the synthesis of AgNPs. From the 1 mM $AgNO_{3,}$ 90 mL of aqueous solution was taken for synthesis of Silver NPs under vigorous stirring conditions at high temperature of 65 ^{o}C. When the aqueous solution of 1 mM of silver nitrate ($AgNO_3$) temperature had reached to 65 ^{o}C, it is then 10 ml of plant extract was added into 90ml of aqueous solution of 1 mM of silver nitrate ($AgNO_3$) for reduction of Ag^+ ions to Ag^0.

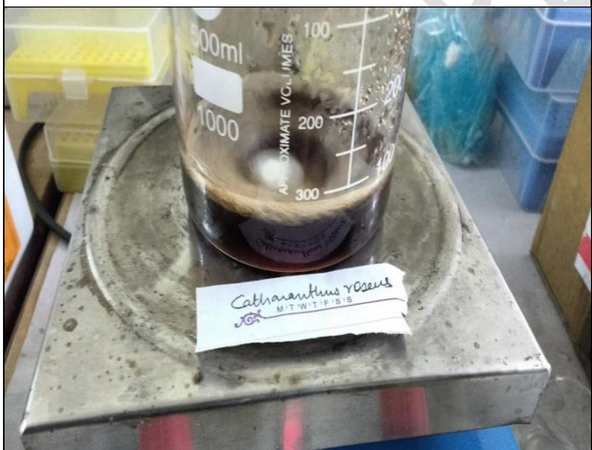
There was a change from a colourless to brownish yellow in 5mins, changes in color occurred in time frame of 30min. A colourless-brownish, yellow-reddish brown, after this no colour change was observed even under vigorous stirring conditions **(fig 4, fig 5a,5b,5c,5d)**

Figure 4 There is bubble formation in the aqueous solution, color changes with time.



5a) from leaf extract of *C. roseus*

5b) from leaf extract of *R. indica*



5c)from flower extract of *C. roseus*

5d) from flower extract of *R. indica*

Figure 5: Reddish brown color is observed

This is the confirmation of silver nanoparticles (AgNPs), as the bubble formation has disappeared. The silver nanoparticles solution mixture was kept for 24 hours under mild stirring and no further changes is observed

The pellets were dispersed in deionized water (1 ml of NPs solution was taken for centrifugation at 10000 rpm for 15 min, followed by dispersion of pellet in deionized water **(fig 6a, 6b)**.

6a **6a** **6b**

Figure 6a, 6b: Nanoparticles in pellets form, from Leaf and flower extract of both plants

<u>Characterization of the synthesized silver nanoparticles</u>

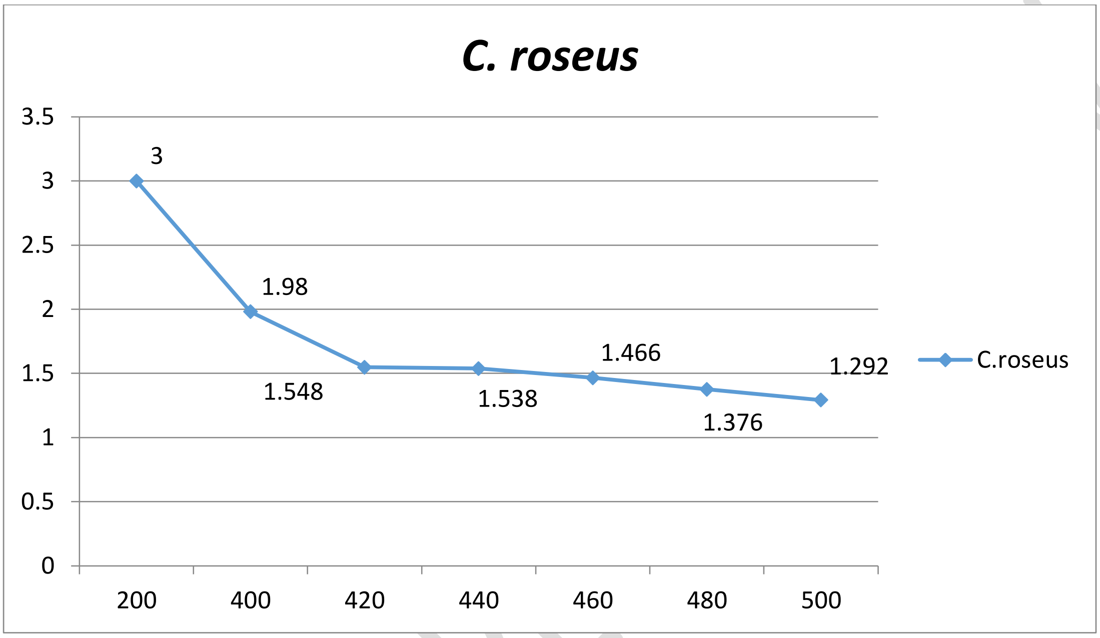
Synthesis of silver nanoparticles solution with leaves extract may be easily observed by UV-Vis spectroscopy **(graph 1A, 1B)**. The bio-reduction of the Ag^+ ions in solutions was monitored by periodic sampling of aliquots (1 ml) of the aqueous component and measuring the UV-Vis spectra of the solution. UV-Vis spectra of these aliquots were monitored as a function of time of reaction on a UV-Vis spectrophotometer in 400–500 nm range1:10 of mixture **(Table 1, 2).**

Table 1: Showing the UV-Vis absorption spectra of silver nanoparticles of *C. roseus*

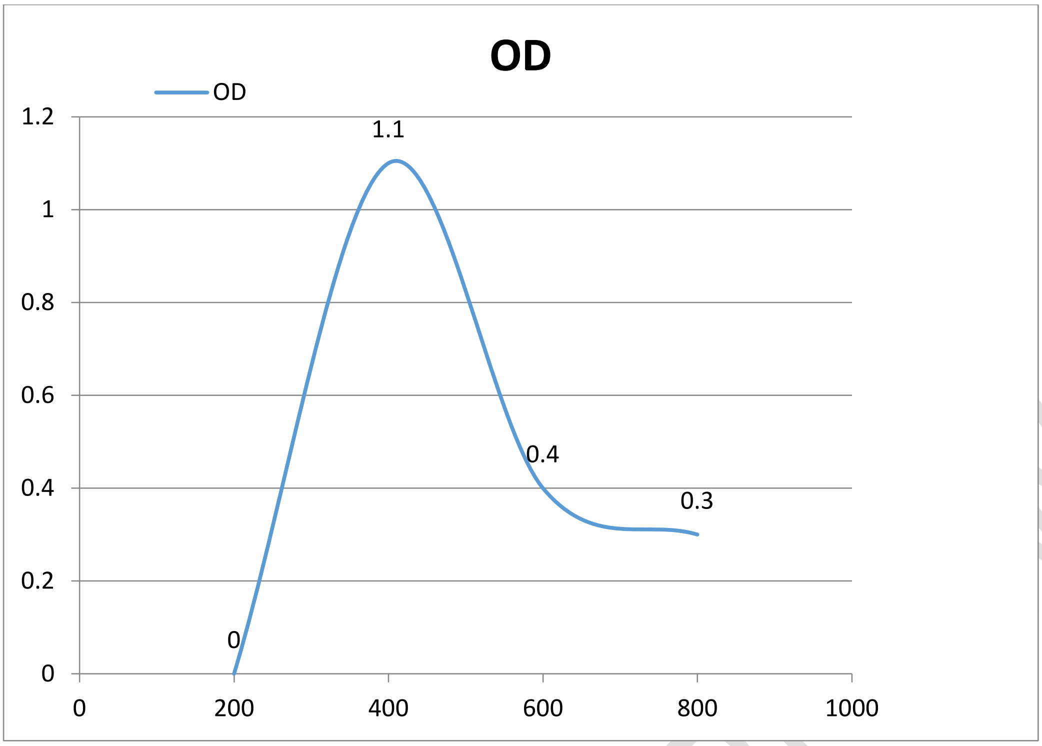
Wavelength	Absorption
200	3.00
400	1.980
420	1.548
440	1.538
460	1.466
480	1.376
500	1.292

Table 2: Showing the UV-Vis absorption spectra of silver nanoparticles of *R. indica*

Wavelength	Absorption
200	**3.00**
400	**1.301**
420	**1.495**
440	**1.490**
460	**1.476**
480	**1.438**
500	**1.408**



Graph 1(a): Graph showing the UV-Vis absorption spectra of silver nanoparticles of C. *roseus*



Graph 1(b): Graph showing the UV-Vis absorption spectra of silver nanoparticles of *R. indica*

For the further characterization the TEM analysis of the silver nanoparticles were done as shown in **Figure 7 (a) and 7 (b).**

The TEM analysis gave the size of nanoparticles**.**

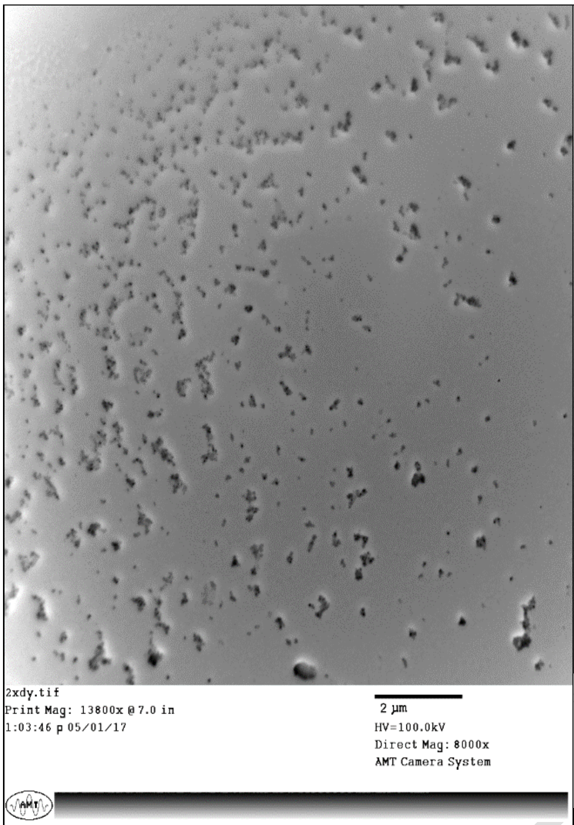


Fig 7(a): TEM micrograph of synthesized silver nanoparticles

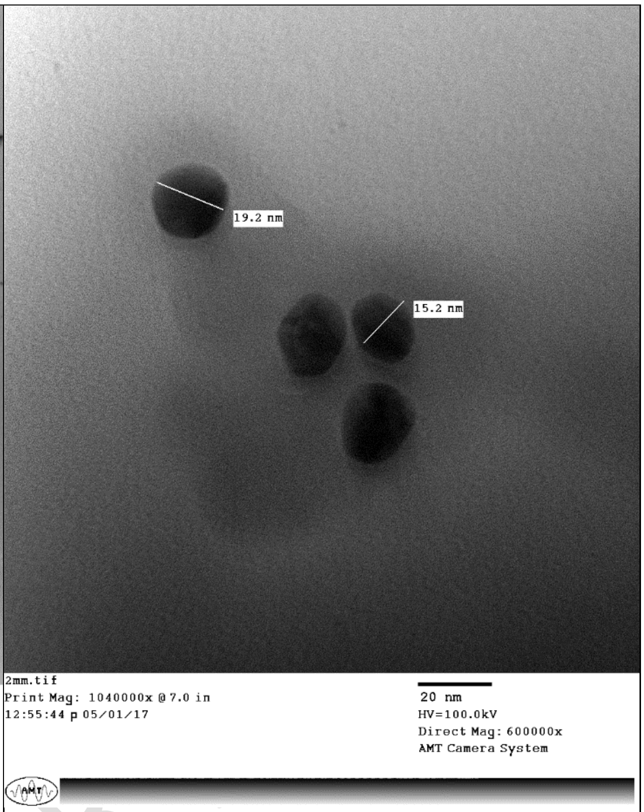


Fig 7(b): TEM micrograph showing size of synthesized nanoparticles

Figure 7: Electrographic image of nanoparticles

RESULT

Silver nanoparticles reveal dark reddish-brown color in aqueous solution which was due to the biochemical reaction of $Ag(NO)_3$ reacts with plant broth that leads to the formation of AgNPs by following reaction **(fig. 8)** explains the proposed mechanism of biological synthesis of nanoparticles.

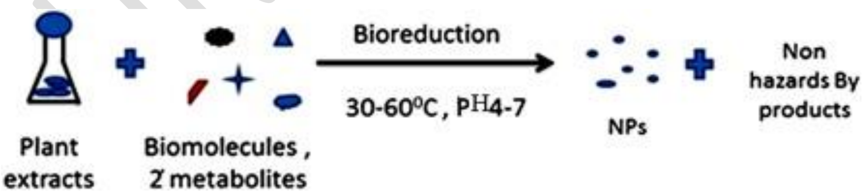


Figure 8: Biosynthesis of Nanoparticles

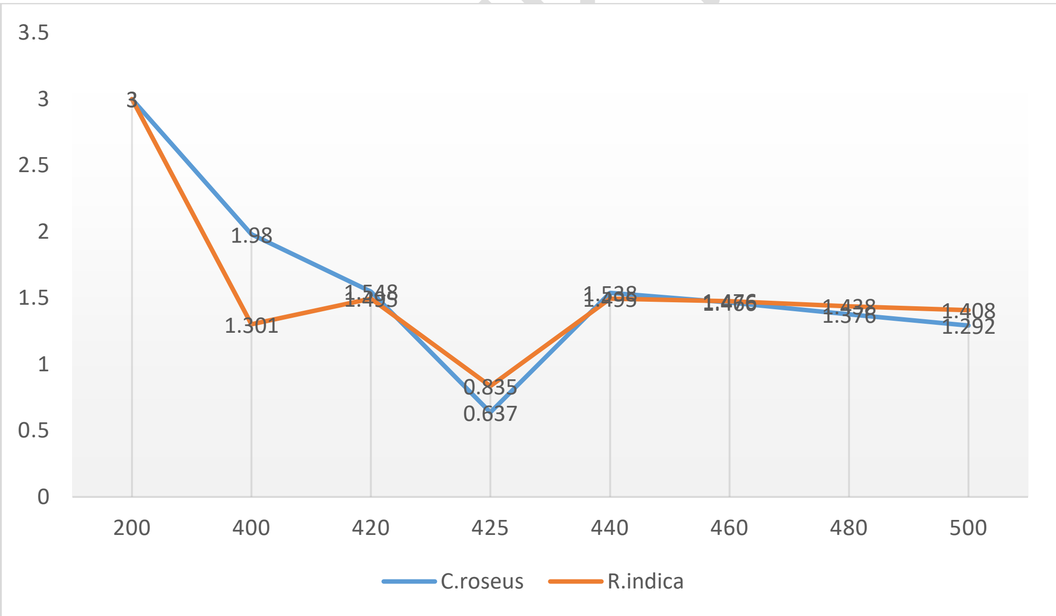
When the mixture of the extract was added with aqueous solution of silver nitrate complex under vigorous stirring by magnetic stirrer at 65 ^{o}C, the color changes from colourless to brownish yellow within 5 min and after 15 min it turns to dark reddish brown. It was left for

24 hr under mild stirring but no change occurred. This was due to reduction of Ag^+ ions which confirms the analysis for the formation of Ag-nanoparticles.

The biosynthesis of Silver nanoparticles is characterized by UV-Vis spectroscopy. The results from the UV- Vis spectrophotometer of silver nanoparticles synthesized from *C. roseus* is compared with that of results of Absorption spectra of silver nanoparticles synthesized from *R. indica* **(Table 3)**

Table 3: Comparative study of absorption spectra of silver NPs from two plants

Wavelength	**Absorption spectra C. *roseus***	**Absorption spectra *R. indica***
200	**3.00**	**3.00**
400	**1.980**	**1.301**
420	**1.548**	**1.495**
440	**1.538**	**1.490**
460	**1.466**	**1.476**
480	**1.376**	**1.438**
500	**1.292**	**1.408**
425 (from Flower)	**0.637**	**0.835**



Graph 2: This graph shows the differences of UV-Vis Absorption spectra of silver nanoparticles synthesized using leaf and flower extracts of *Catharanthus roseus* and *Rosa indica.*

Based on the analysis it is clear that both *C. roseus* and *R. indica* are medium for the reduction of Ag^+ that generates silver nanoparticles Ag^0.

Also based on the analysis of Silver NPs from flower extracts of two plants, *R. indica* has higher absorption spectra as compared to *C. roseus* shown in the graph above **(Graph2)**. Absorption spectra of silver nanoparticles from leaf extract of two plants shows similar pattern with *R. indica* having maxima absorbance at 420 nm (1.490) and *C. roseus* also having maxima absorbance at 420 nm (1.538) **(Table 3)**. Similarly the TEM micrograph confirmed the segregated round and big nanoparticles of two sized 19.2 nm and 15.2 nm stating that the plant extract has better potentials to produce big nanoparticles in large amounts in less time and least cost (**fig 7(a) & 7(b)**).

DISCUSSION

This method was very facile for the synthesis of silver nanoparticles. The use of eco- friendly approach for the production of sliver nanoparticles by using extract of *C. roseus* and *R. indica.* The developed process was fast and better from previously known processes. The primary confirmation was done by qualitative analysis that is change of brownish color within 5 min and after 15 min it turns in to dark reddish brown. The reduction of silver nanoparticles was also confirmed by UV-Vis spectrophotometer- as the maximum absorbance of UV-Vis spectra were found at around ~ 420 nm. Silver Nanoparticles unveil dark reddish-brown color in aqueous solution which is due to the excitation of Surface Plasmon Resonance, which is a signature feature of AgNPs. When the extract was added with aqueous solution of silver nitrate under vigorous stirring by magnetic stirrer at room temperature, the color changes from colourless to brownish yellow within 5 min and after 15 min it turns in to dark reddish brown. After 24 hours, no color change was observed, signifying the stability of the synthesized nanoparticles. It was reported that the synthesis of silver nanoparticles can be within 30 min of incubation period by using leaves extract of *Acalypha indica* and its antibacterial properties is well established (13,14). Similarly, Ag-NPs synthesized were reported brown in color (15, 16). Recently synthesis of silver nanoparticle were from *A. indica* leaf extract and the synthesis of Ag-NPs were in less than 15 min with highest absorbance at 445 nm. The reduction of Ag^+ to Ag^0 confirms the qualitative analysis for the formation of Ag nanoparticles as shown in **figure 9 (a, b).**

	
9(a) Ag (NO) $_3$ solution	**9(b) Synthesized Ag NPs**

Figure 9: Synthesis of Silver Nanoparticles

Characterization of Metallic Nanoparticles by UV-Vis Spectrophotometeric Analysis and TEM analysis

Spectrophotometric absorption measurements in the wavelength ranges of 400–450 nm (17) are used in characterizing the silver nanoparticles, respectively. The UV–Vis spectrum of metallic nanoparticles showed the distinct peak was at 425 nm for AgNPs (18, 19) which corresponds to Surface Plasmon Resonance of the metallic nanoparticles. The presence of the dark brown color and the peak at 425 nm confirms the production of AgNPs (20). The efficiency of synthesized nanoparticles from flower extract act as an efficient catalyst, the activity that is studied by reduction of dye methylene blue that is confirmed by the decrease in absorbance with time and is the property attributed to electron relay effect (21)

Thus, this technique is invaluable in the terms of environmental protection since green synthesis doesn't require toxic chemicals for reduction. Furthermore, the synthesis was accomplished under high temperature with vigorous stirring, without addition of any further capping materials.

The TEM analysis helps in characterizing the nanoparticles further where electron transmission generates a micrograph that helps in knowing the presence of the nanoparticles. Apart from that the characteristics like shape, cluster and size of nanoparticles is also determined. The TEM micrograph in this case helped in knowing the size and shape of nanoparticles that were spherical, big, segregated and of two sizes 19.2 nm and 15.2 nm that shows that the dried flower extract had potential to form huge, spherical and uncluttered nanoparticles better than that from leaf extract of the plant.

CONCLUSION

Nanotechnology is facilitating technology that deals with nanometer sized items. The exercise of nanomaterial in biotechnology unites the fields of biology and material science. In recent research large number of synthesis of nanoparticles is being done by greener technologies and ecofriendly approaches.

Recently biological reduction of bulk metal compounds was achieved by the application of leaf extracts where *R. damascene* leaf extract was used for reduction of $AgNO_3$and formation of AgNPs, and *M. piperita* was used for production of AuNPs from Choloroauric acid (22, 23). Although the green synthesis is far more economical and environmental friendly as compared to the other technologies, the commercial viability of the process for the synthesis of nanoparticles using these swiftly obtainable plant extracts must be assessed before employing them on a large scale.

The therapeutic potential of these synthesized silver nanoparticles has also been studied extensively as synthesis of silver nanoparticles from the flower extract *Achillea biebersteinii* flowers extract, which do not involve any harmful chemicals were well-dispersed and stabilized through this green method and showed potential therapeutic benefits against angiogenesis in rat aortic ring model (24)

The antioxidant and scavenging properties of silver nanoparticles from flower extract of *R. dauricum* further establishes its role in therapeutics and also in cosmetics (25). However, the few constraints are still there in this green technology and ecofriendly approach for production of silver nanoparticles.

In regards of biological synthesis for metallic nanoparticles, the nearest challenge is to overcome technical limitations of synthesis and implementing the process on large scale. Moreover, the synthesis improvement will get a thrust only when applications of NPs become established on large scale to propagate a demand for NPs. In this sense, the applications in medical and pharmaceutical research will play a major role. The selective effect of NPs on cancer cells may very well be the application which forces nanotechnology out of the confines of the laboratory and thrust it into spotlight. The other important application could be transcending the trend of antimicrobial resistance by using NPs in addition to drugs, which is proving to be one of the greatest challenges of the 21^{st} century. It is hoped that nanotechnology will continue to gain momentum in the coming years and fuel another technological revolution for the benefit of the humankind.

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