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Advances in Bioresearch

ORIGINAL ARTICLE

Synthesis and Characterization of herbal gel on using plants extract

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ABSTRACT
In the present study, dried powdered Tulsi leaves, Neem seeds & Turmeric rhizomes were subjected to extraction with methanol. SNPs of all tree extract were prepared using standard method Confirmation & Characterization of silver nanoparticles were done by U.V. Spectroscopy. The most important application of silver and SNPs is as topical ointments to prevent infection against burns and open wounds. In small concentrations, silver is safe for human cells, but lethal for microorganisms. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, Virus without any side effect. Hence, the present study is to synthesize silver nanoparticles by using neem seed extract, tulsi extract and turmeric extract, characterization of these silver nanoparticles & Converts its gel formulation as well as to observe numbers of activity. The bands of silver nanoparticles were observed around 474 nm in case of Neem Seed SNPs whereas the bands for Turmeric were observed around 284 nm. As well as the bands for Tulsi were observed around 274 nm.
Keyword: Nanoparticles, Spectroscopy and Characterization

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INTRODUCTION

Recently considerable attention has been paid to utilize eco-friendly and bio-friendly plant based products for the prevention and cure of different human diseases. It is documented that most of the world's population has taken in traditional medicine, particularly plant drug for the primary healthcare.
In the present study, dried powdered Tulsi leaves, Neem seeds & Turmeric rhizomes were subjected to extraction with methanol. SNPs of all tree extract were prepared using standard method. Confirmation & Characterization of silver nanoparticles were done by U.V. Spectroscopy.
Herbal drugs are playing a vital role in health care system. This is because they are being cheap and locally available [1]**.** The activity of herbal medicines depends on overall function of a variety of active components, as all the constituents provide synergistic action and thus enhance the therapeutic value [2]**.** Herbal medicines are now in great demand in the developing world for primary health care not because of inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects.
The field of nanotechnology is one of the most active areas of research in modern material science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. New applications of nanoparticles and nanomaterial are emerging rapidly [3]**.**

MATERIAL AND METHOD

Collection and authentication of plant Material

Plant materials of *Azadirachta indica*, *Curcuma longa* & *Ocimum santum* were collected from Shirpur region of Dhule district (Maharashtra) in July 2018. The plants were authenticated at S S V P S, College, Dhule Dr. D A Patil.

Extraction methodology

The extractions of powdered material were done by using Soxhlet apparatus. In solvent extraction, dried material is extracted with methanol. For extraction, 250 gm of powdered material were packed in thimble containing filter paper and extracted with methanol in Soxhlet apparatus for the period till all the substances and others were extracted. The extract thus obtained was concentrated with the help of rotary vacuum evaporator [4].

Synthesis of silver nanoparticles

For synthesis of silver nanoparticles, the conical flask containing 100 ml of AgNO3 (1mM) was reacted with 12 ml of themethanolic extract of A. indica, C. longa&O. santum. This setup was incubated in dark (to minimize the photoactivation of AgNO3), at 37 ◦C under static condition[5].

Preparation of SNP's gel formulation

1 g of Carbopol 934 was dispersed in 50 ml of distilled water with continuous stirring. 5 ml of distilled water was taken and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath. Cool the solution, then to that added Propylene glycol 400. Further required quantity of SNPs was mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water. Finally full mixed ingredients were mixed properly to the Carbopol 934 gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency. The same method was followed for preparation of all SNPs & extract [6].

Evaluation of Gel Formulation

Physical Evaluation

Physical parameters such as Color and Appearance& Homogeneity were checked.

Measurement of pH

pH of the gel was measured by using pH meter.

Viscosity

Viscosity of gel was measured by using Brookfield viscometer with spindle.

Spreadibility

A sample of 0.5 g of each formula was pressed between two slides (divided into squares of 5 mm sides) and left for about 5 minutes where no more spreading was expected. Diameters of spreaded circles were measured in cm and were taken as comparative values for spreadability. The results obtained are average of three determinations.

Linearity study of Phytoconstituents

Calibration Curve of Curcumin

Preparation of Standard

Standard stock solution of Curcumin (2mg/10ml) i.e. 200µg/ml was prepared in methanol passed through 0.45 Millipore filters. Different dilution of stock of Curcumin was prepared.

Calibration Curve of Eugenol

Preparation of Standard

Standard stock solution of Eugenol(100µl/10ml) i.e. 10µl/ml was prepared in methanol passed through 0.45 Millipore filters. Different dilution of stock of Curcumin was prepared.

Confirmation& Characterization of silver nanoparticles

U.V. Spectroscopy

Synthesized silver nanoparticles was confirmed by sampling the reaction mixture at regular intervals and the absorption maximawere scanned by UV–Vis spectra, at the wavelength of 200–700nmin in UV Carry spectrophotometer [7].

RESULT AND DISCUSSION

Linearity study of Phytoconstituents

Calibration Curve of Curcumin

The calibration curve for 6 sequentially and independently prepared stock standard solutions of curcumin that depicts the concentration of curcumin against the absorbance, as presented in figure 11. The absorbance value increased proportionally upon increasing the concentration of Curcumin from 2 to 12 µg/ml.

Nevertheless, for our estimation purposes, the calibration plot was employed to ascertain the total curcumin content of the turmeric SNP.

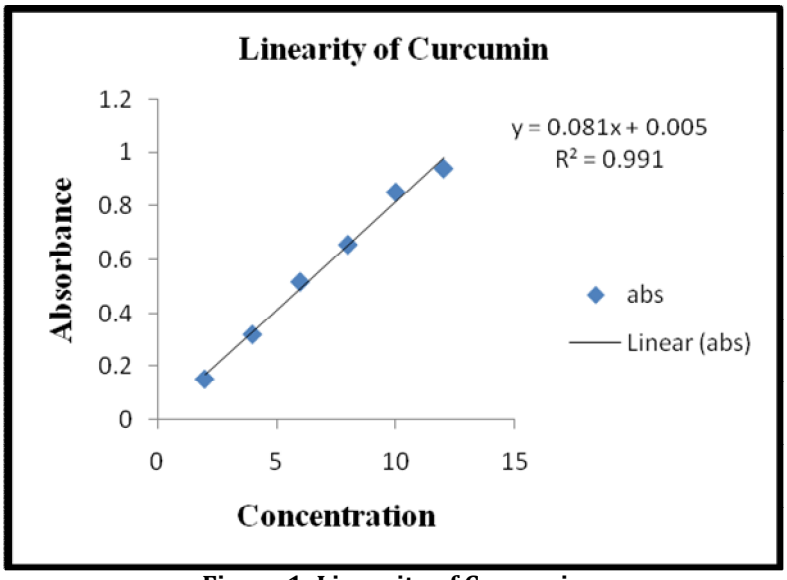

Figure 1: Linearity of Curcumin

Linearity of curcumin (Standard) calibration Curve Y= 0.081x+0.005, Where R^2 = 0.991, SNP of Turmeric contain 5.19 mg/gm of curcumin.

Calibration Curve of Eugenol

The calibration curve for 6 sequentially and independently prepared stock standard solutions of Eugenol that depicts the concentration of Eugenol against the absorbance, as presented in figure. The absorbance value increased proportionally upon increasing the concentration of Eugenol from 2 to12 µg/ml.

Nevertheless, for our estimation purposes, the calibration plot was employed to ascertain the total Eugenol content of the turmeric SNP.

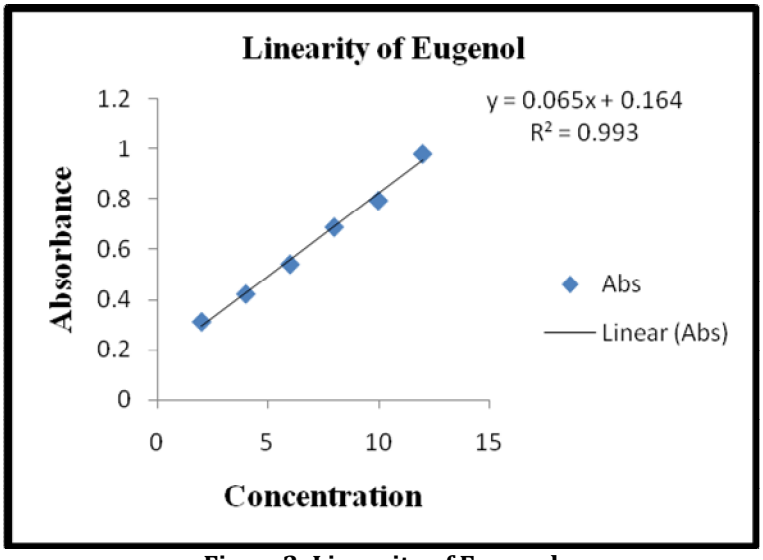

Figure2: Linearity of Eugenol

Linearity of Eugenol (Standard) calibration Curve Y= 0.065x+0.164, Where R^2 = 0.993, SNP of Tulsi contain 4.18 mg/gm of eugenol.

Confirmation & Characterization of silver nanoparticles

The use of plants as the production assembly of silver nanoparticles has drawn attention, because of its rapid, eco-friendly, non-pathogenic, economical protocol and providing a single step technique for the biosynthetic processes. The reduction and stabilization of silver ions by combination of bio-molecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpenoids and vitamins which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures [8]. The protocol for the nanoparticle syntheses involves: the AgNO3 solution, on addition of few mL of plant extract follow the reduction of

pure Ag(I) ions to Ag(0) which can be monitored by measuring the UV–visible spectra of the solution at regular intervals [9].

Nanoparticles are generally characterized by their size, shape, surface area, and dispersity. The common techniques of characterizing nanoparticles are UV–visible spectrophotometer. Preliminary characterization of synthesized silver nanoparticles was carried by following method.

The UV-visible spectroscopy is commonly used techniques with wavelengths in the range 300–800 nm are generally used for characterizing various metal nanoparticles in the size range of 2 to 100 nm. In order to monitor the formation and stability of silver nanoparticles, the absorption spectra of the synthesized silver nanoparticles were recorded. Figure 16, 17 & 18 shows the UV– visible spectra of silver nanoparticle formation using constant $AgNO_3$ concentration with different extract concentrations at room temperature after 24 h. The color of the solutions changed from yellow to yellowish brown in case of turmeric, light brown to dark brown for neem seed & light green to brown for Tulsi; depending on the extract concentration indicating silver nanoparticle formation as the color change observed is due to excitation of surface Plasmon vibration in the silver nanoparticles.The sharp bands of silver nanoparticles were observed around 474 nm, 284 nm & 274 nm in case of Neem Seed SNPs, Turmeric & Tulsi SNP respectively (Figure 3, 4 & 5).

The reduction of the metal ions occurs fairly rapidly; more than 90% of reduction of Ag+ ions is complete within 4 h after addition of the metal ions to the plant extract. The metal particles were observed to be stable in solution even 4 weeks after their synthesis. By stability, there was no observable variation in the optical properties of the nanoparticle solutions with time

So it confirmed that Neem Seed, Turmeric & Tulsi extract has ability to reduce Ag ions into Ag nanoparticles, which lead us for further research on synthesis of silver nanoparticles. Broadening of peak indicated that the particles are polydispersed. The intensity of absorption peak increases with increasing time period.

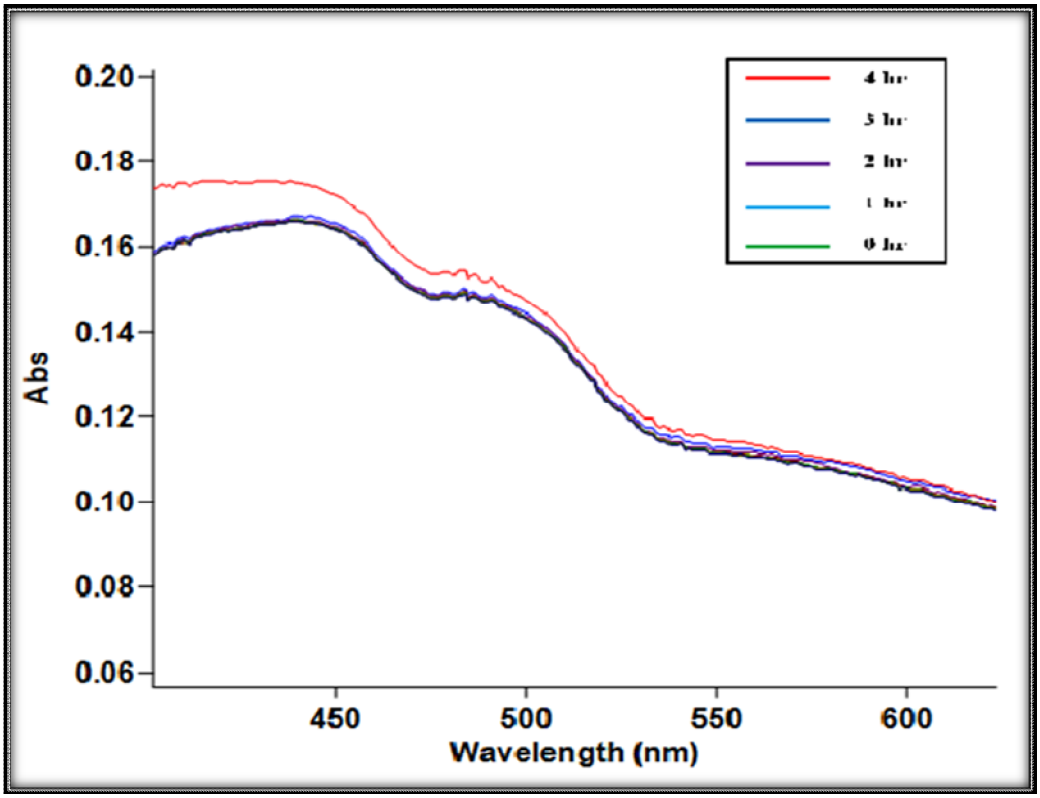

Figure 3: UV-Vis spectra of Neem Seed SNPs at different time interval

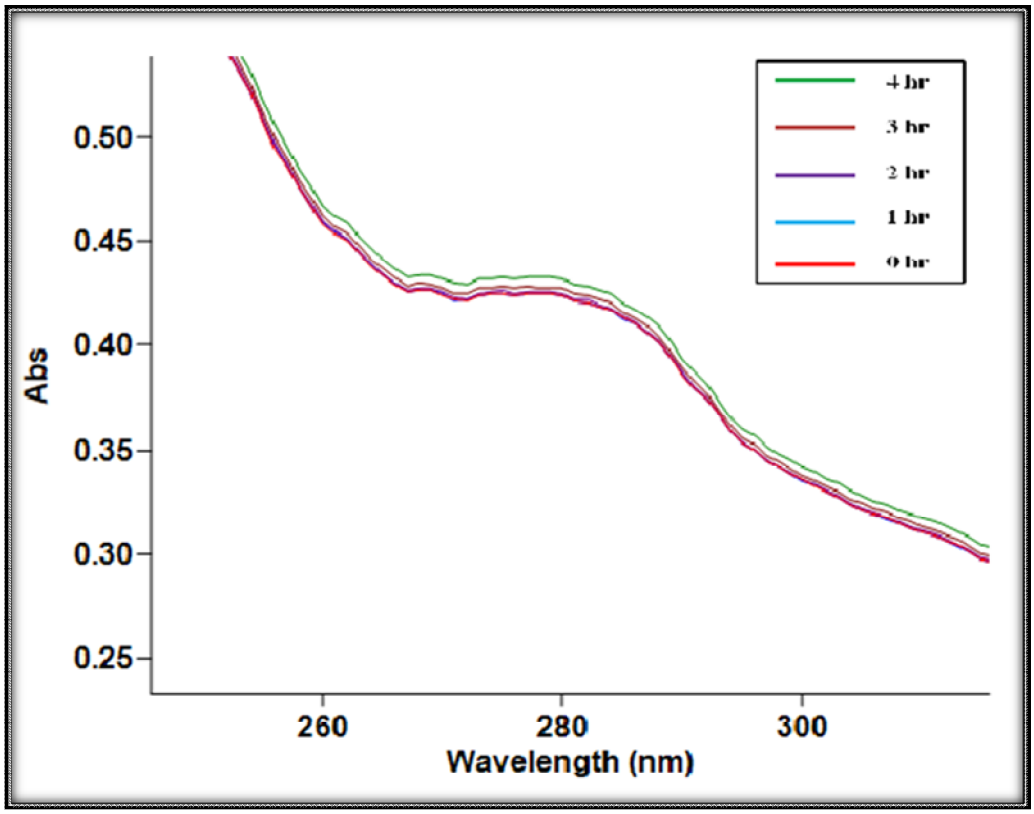

Figure 4: UV-Vis spectra of Turmeric SNPs at different time interval

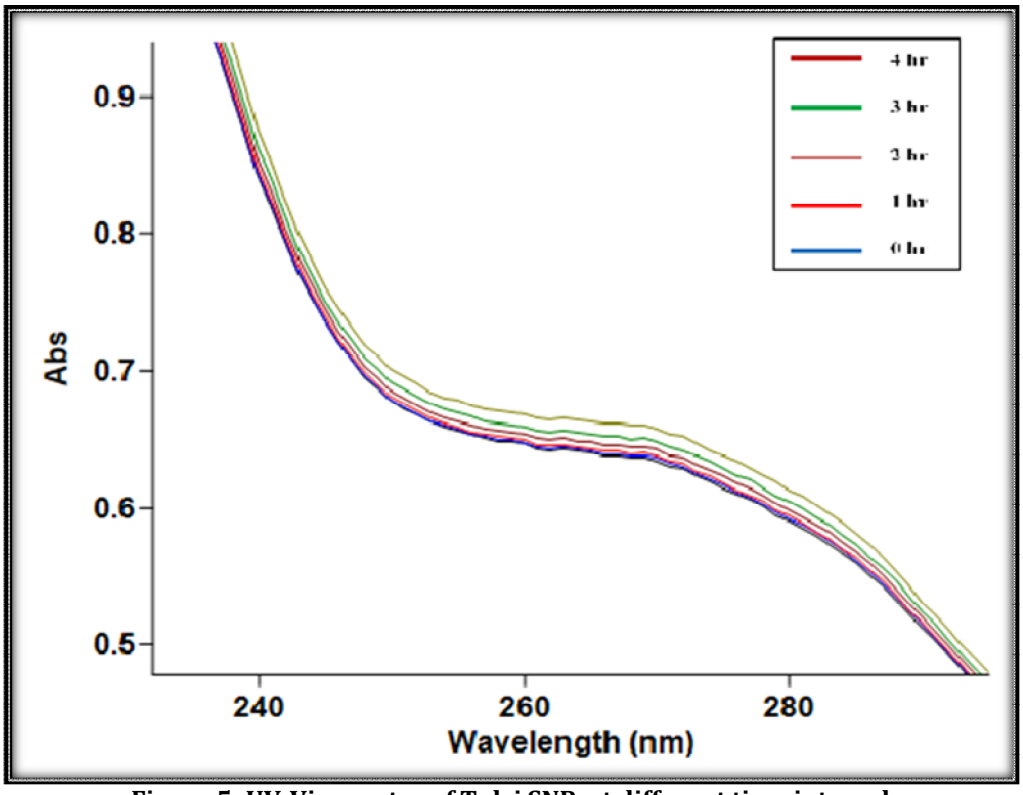

Figure 5: UV-Vis spectra of Tulsi SNPsat different time interval

CONCLUSION

In conclusion, it has been demonstrated that the extract of Tulsi leaves, Neem seeds & Turmeric rhizomes are capable of producing Ag nanoparticles extracellularly and the Ag nanoparticles are quite stable in solution. The UV-vis. spectra showed strong absorption bands due to surface Plasmon resonance (SPR) of

metal nanoparticles form in the reaction media has absorbance peak in the range of 250-480 nm thereby confirming their formation. The bands of silver nanoparticles were observed around 474 nm in case of Neem Seed SNPs whereas the bands for Turmeric were observed around 284 nm. As well as the bands for Tulsi were observed around 274 nm.

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