Objective: Current study is aimed at the formulation of silver nanoparticles loaded with the extract of Coleus amboinicus leaf extract by microwave irradiation. A facile and green synthesis is the formulation of silver nanoparticles by using a biological agent such as plant extracts with the aid of microwave irradiation is proposed as an economical and environmentally friendly approach alternative to chemical and physical methods.

Methods: In order to fabricate silver nanoparticles by microwave irradiation, aqueous extract of leaves Coleus amboinicus (CA) were treated with aqueous silver nitrate solution and mixture was placed in the microwave oven for exposure to microwave. Optimizations of the process were carried out by varying the quantity of extract, silver nitrate concentration and duration of microwave irradiation. Formations of nanoparticles were confirmed by UV-visible spectrophotometry for the presence of surface plasmon resonance (SPR) peak. Nanoparticles were characterized by scanning electron microscopy, transmission electron microscopy (TEM) and Fourier transform infrared (FTIR) spectroscopy.

Results: Silver nanoparticle showed the SPR optical absorption band peak at 434 nm by UV-Visible spectrophotometer. Reaction mixture containing 2 mmol silver nitrate and 9 ml of extract subjected to microwave irradiation of 60 sec at a temperature of 60 °C was found to be optimised condition, which produced nanoparticles that were spherical in shape and had an average diameter of 15.685 nm.

Conclusion: This research study opens an innovative design to progress our understanding of how silver nanoparticles behave can be optimized to improve their surface morphology, which is beneficial to improve its therapeutic effect.

Keywords: Coleus amboinicus, Silver nanoparticles, Microwave irradiation

INTRODUCTION

Metallic nanoparticles are being utilized in every phase of science along with engineering, including medical fields and are still charming the scientists to explore new dimensions for their respective worth which is generally attributed to their corresponding small sizes. The interest in silver nanoparticles (SNPs) has gained prominence owing to its excellent plasmonic activity, electromagnetic, optical and catalytic properties, and bacteriostatic and bactericidal effects along with antiproliferative effects compared with other metal nanoparticles [1].

Conventionally silver nanoparticles are synthesized by the chemical method using chemicals as reducing agents, which later on become accountable for various biological risks due to their general toxicity; engendering the serious concern to develop environment friendly processes. Thus, to solve the objective; biological approaches are coming up to fill the void; for instance green synthesis using biological molecules derived from plant sources in the form of extracts exhibiting superiority over chemical and/or biological methods. These plant-based biological molecules undergo highly controlled assembly for making them suitable for the metal nanoparticles synthesis [2].

In recent years, microwave irrigation is considered to be a highly effective technology and is widely used in the synthesis of nanoparticles because of more homogeneous heating process and can speed up the reaction rate by orders of magnitude compared with conventional heating [3].

Coleus amboinicus is a perennial herb belonging to the family Lamiaceae which occurs naturally throughout the tropics and warm regions of Africa, Asia and Australia. The literature survey has emphasized the occurrence of different classes of phytochemicals, including 76 volatiles and 30 non-volatile compounds. Coleus amboinicus oil is rich in oxygenated monoterpenes, monoterpen hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes [4]. Coleus amboinicus is widely used in folk medicine to treat conditions like cold, asthma, constipation, cough, fever, urinary and skin diseases [5].

Hence considering the medicinal importance of silver nanoparticles and the plant Coleus amboinicus, the present study is aimed at quick synthesis of silver nanoparticles through eco-friendly process which is achieved by the use of microwave irradiation and Coleus amboinicus leaf extract as reducing agent respectively.

MATERIALS AND METHODS

Materials

Silver nitrate was procured from Sigma Aldrich (India) and double-distilled water was used for preparing aqueous solutions.

Preparation of Coleus amboinicus leaf extract

Fresh Coleus amboinicus leaves were collected from local areas of Mangalore in the month of December and botanical identity was confirmed by Dr. Jyothi Miranda, Associate Professor, botany department, St. Aloysius College, Mangalore. The A voucher specimen of Coleus amboinicus (15BP063E) was deposited in the institute for future reference. Plant Material was washed thoroughly with distilled water, air-dried.

10 g of finely incised leaves were transferred into 250 ml beakers containing 100 ml distilled water and boiled for about 20 min and
solution was made clear by filtration through Whatmann filter paper.

**Synthesis of silver nanoparticles containing Coleus amboinicus leaf extract**

For the formation of silver nanoparticles with the aid of microwave irradiation, 90 ml of silver nitrate solution was mixed with 10 ml Coleus amboinicus leaf extract and then placed in a microwave oven (CATA-R) working at a 800W irradiation power and 2450MHz frequency.

**Optimisation of microwave assisted synthesis of Coleus–AgNP**

Optimisation of the process was done by varying the concentration of AgNO3, different quantities of Coleus amboinicus leaf extract and exposing to different temperatures and then production of nanoparticles were confirmed by UV-Visible spectroscopic analysis. Further the nanoparticles obtained at optimised conditions were subjected for SEM, TEM for confirming the morphology of nanoparticles.

Silver nanoparticles were also prepared by aging method in which reaction mixture containing silver nitrate and plant extract were kept at room temperature for 24 h and then subjected to analysis by UV-visible spectrophotometer at a time gap of 30 min.

**Characterisation of nanoparticles**

**UV-visible spectroscopy**

The formation of silver nanoparticles were confirmed by the appearance of surface plasmon resonance peak by use of a dual beam UV-Vis spectrophotometer (UV5704S from Electronics, India ltd) in the wave length range of 300–600 nm. Deionized water was used as a blank.

**Measurement of particle size of the nanoparticles**

Particle size distributions of nanoparticles were expressed in terms of Polydispersity index (PDI), Average particle size (r-average) and Polydispersity index (PDI) of the developed nanoparticles were determined using Malvern Zetasizer (Nano ZS, Malvern Instruments, UK).

**Measurement zeta potential**

Zeta potential of silver nanoparticles was measured using Malvern Zetasizer (Nano ZS, Malvern Instruments, UK). Zeta potential gives idea about the surface charge on the nanoparticles and thereby indicates the stability of nanoparticles [6].

**Scanning electron microscopy (SEM)**

Shape and surface morphology of the nanoparticles were studied using SEM. Scanning electron microscopic analysis was carried out by sputtering sample on a small piece of adhesive carbon tape which is fixed on a brass stub and then subjected to gold coating using sputtering unit (model: JFC1600) for 10 sec at 10mA of current. The gold coated sample placed in chamber of SEM (Jeol, JSM 6390LA) and secondary electron/Back Scattered electron images are recorded. Elemental analysis carried out in the scanned area/point/line using EDAX detector (EDAX Make/Model OXFORD XMX).

**Transmission electron microscopy [TEM]**

Transmission electron microscopy was performed on JM 2100 (JEOL, Tokyo, Japan) transmission electron microscope. Samples were prepared by casting the drop of sample solution on carbon-coated grids of 200 mesh, then grid is dried and fixed in the specimen holder. Excess solution was removed by using a piece of soft filter paper. The copper grid was then vacuum dried.

**Fourier transform infrared [FTIR] spectroscopy**

Fourier transform infrared spectroscopy of extract and silver nanoparticles of Coleus amboinicus were performed using a Shimadzu FTIR 8300 spectrophotometer and the spectrum was recorded in the region of 4000 to 400 cm-1. Briefly, samples were diluted with spectroscopic grade KBr (mass ratio of about 1:100) and the spectra were recorded.

**RESULTS AND DISCUSSION**

**Microwave assisted green synthesis of silver nanoparticles**

In the present study, formation of silver nanoparticles by microwave irradiation using the Coleus amboinicus leaf extract was observed at time period of 60 sec which indicates that microwave assisted green synthesis significantly accelerates the process of silver nanoparticle synthesis. Since heating in a microwave is generated by interaction of the permanent dipole moment of the molecule with high frequency (245 GHz) electromagnetic radiations, it is reported to reduce the reaction time by a factor of ~ 20 as compared to conventional heating [7]. The main attraction of microwave synthesis is that it yields small, uniform sized nanoparticles in much lesser reaction time. The speedy consumption of starting materials reduces the formation of agglomerates in microwave assisted methods and provides nanoparticles with narrow size distribution [8, 9].

**Optimisation of microwave assisted synthesis of Coleus–AgNP**

Optimisation of the process was done by varying the concentration of AgNO3 (1, 2, 4, 6 and 8 mmol), different quantities of Coleus amboinicus leaf extract (1,3,5,7 and 9 ml), different temperatures (40, 50, 60, 70, 80 °C). Optimised condition for nanoparticle synthesis was determined by the nature of SPR peak obtained by UV analysis.

**Effect of concentration of silver nitrate**

The concentration of silver nitrate was varied from 1,2,4,6 and 8 mmol and their effect on the synthesis of silver nanoparticle were studied based on the pattern of SPR peak which is shown in fig. 1(a). Results showed that sharp and intense SPR peak was obtained when the nanoparticles were prepared using 2 mmol silver nitrate solution which indicates that the nanoparticles had comparatively lesser particle size. Studies have proved that the SPR peak of silver nanoparticles in aqueous solution modifies to shorter wavelengths and sharpness of peak increases as the particle size decreases [10]. When the concentration of aqueous silver nitrate solution was increased from 1 to 8 mmol, the optimal concentration was found to be 2 mm and when the concentration was increased to 4 mm and beyond that, it resulted in the formation of large sized nanoparticles. This is due to the fact that increasing the metal concentration initially resulted in synthesis of smaller particles due to availability of different functional groups for the reaction; however larger particles were formed at higher concentration of metal which may be due to non-availability of functional groups responsible for the reduction reaction [11].

**Effect of concentration Coleus amboinicus leaf extract**

Productivity of nanoparticle synthesis also based on the amount of plant extract. Henceforth, so as to decide ideal concentrate required to frame nanoparticles with desired morpholgy and size, quantity of plant extract was varied from 1, 3, 5, 7 and 9 ml in 50 ml of 2 mmol silver nitrate solution. Intensity and sharpness of SPR peak was found to increase with the increase in the amount of Coleus amboinicus leaf extract which is observed in fig. 1(b). Sharp narrow shape of SPR band indicates the formation of spherical and homogeneous distribution of silver nanoparticles. Maximum production with small particle size were obtained using 9 ml of the extract the reason being that at higher extract concentrations the biomolecules act as reducing agent and cap the nanoparticle surfaces protecting them from aggregation [12]. Similar studies were reported in which comparatively higher extract ratio is responsible for the synthesis of symmetrical nanoparticles [13].

**Effect of irradiation temperature**

Reaction Temperature plays a key role in particle growth and shape/size control, especially for silver nanoparticles. In order to study the effect of temperature, the solution containing 9 ml of plant extract and 50 ml of 2 mmol silver nitrate was subjected to microwave irradiation at different temperatures of 40, 50, 60, 70, 80 °C and subjected to UV analysis. UV spectra indicated that wavelength shifted to a lower value at a temperature of 60 °C resulting in the formation of smaller silver nanoparticles. The rate of synthesis of nanoparticles increased with increase in temperature till 60 °C.
because the increasing of the reaction temperature will lead to a rapid reduction rate of the silver ions and the subsequent homogeneous nucleation of silver nuclei allowing for the formation of silver nanoparticles with small size [14]. Beyond 60 °C there was slight increase in particle size which may be due to aggregation of small nanoparticles which was reported with the studies done with the E. coli [15]. From the fig. 1(c) it is clearly evident that temperature of 60 °C is the optimum requirement for formation of silver nanoparticles.

**Synthesis of nanoparticles at room temperature by aging method**

Fig. 1(d) shows the UV spectrum of silver nanoparticles prepared by aging method and results indicate that formation of silver nanoparticles occurs at slow rate hence more time consuming when compared with synthesis by microwave irradiation which takes only 60 Seconds. Microwave assisted method of production of nanoparticles causes increase in the kinetics of reaction by two orders of magnitude, rapid initial heating and generation of localized heat at reaction sites, thereby increasing the rate of reaction. This experiment suggests that the slow rate of formation of silver nanoparticles at room temperature can be replaced by the use of microwave irradiation which is less time consuming [16].

Hence based on SPR peak obtained by UV spectroscopy, Optimised parameter required for the production of silver nanoparticles are mixture of 2 mmol silver nitrate and 9 ml of Coleus amboinicus leaf extract which should be exposed to microwave irradiation at 800W power, temperature of 60 °C for 60 seconds. Silver nanoparticles synthesised at optimised condition was further characterized for shape and surface morphology by SEM and TEM analysis.

**Characterization of silver nanoparticles**

**Visual inspection**

When Coleus amboinicus leaf extract when exposed to Ag+ions (AgNO3), the colour of the reaction mixture turned from transparent colour to brown which was due to the formation of silver nanoparticles as shown in fig. 2. The primary evidence for the formation of silver nanoparticle is the change in colour of the sample solution which occurs due to collective oscillation of the conduction electrons of the silver nanoparticle produced in the reaction mixture [17].
UV-visible spectroscopy analysis

In the present study, reduction of silver ions present in the aqueous solution of silver nitrate during the reaction with the ingredients of *Coleus amboinicus* leaf extract has been seen by the UV-Vis spectroscopy ranging from 300 to 800 nm.

The plant extract solution exposed to AgNO₃ ions showed a distinct absorption at around 434 nm indicated in fig. 3 which corresponds to surface plasmon resonance (SPR) of silver nanoparticles which arises due to the collective oscillations of the conduction electrons of nanoparticles in presence of visible light which is and it is highly influenced by shape and size of the nanoparticles [18].

Scanning electron microscopy (SEM)

SEM images of silver nanoparticles showed that nanoparticles were spherical in shape as observed in fig. 4.

Particle size, PDI & Zeta Potential

The optimized silver nanoparticles shows the particle size, PDI and Zeta potential 17.23 nm, 0.153 and - 23.56 mV respectively. Particle size and shape was further determine by TEM.

Transmission Electron Microscopy [TEM]

TEM technique has greater magnification and resolution than SEM and the images provide more accurate information regarding size, shape and crystallography of the nanoparticles. TEM images of silver nanoparticles shows that nanoparticles have average diameter of 15.685 nm which are predominantly spherical in shape as shown in fig. 5.
Fourier Transform Infrared [FTIR] spectroscopy

FTIR spectroscopy of synthesized nanoparticle formulation were carried out in order to find out the nature of the functional groups present in plant extract and also to determine their involvement in the bio reduction of silver during the formation of nanoparticles.

CONCLUSION

The bio-reduction of aqueous silver ions by the plant extract of Coleus amboinicus leaf extract is a good source for green environment approach towards the synthesis of silver nanoparticles. Microwave synthesis has the potential to selectively heat either the solvent or the precursor molecules for nanomaterial preparation. Microwave irradiation and its mode of heating make the synthesis of the metallic nanoparticles fast, uniform, reproducible and yields smaller nanoparticles in much lesser reaction time.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

No conflict of interest

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