Green Synthesis and characterization of Silver nanoparticles using Cinnamomum tamala leaf extract and its thermo-acoustic investigation

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Abstract
In this study, the aqueous extract of Cinnamomum tamala (CT) was used to prepare silver nanoparticles (AgNPs) by green synthesis.Silver nanoparticles were prepared by using AgNO3 and extract of CT taken in different ratios. The synthesized AgNPs are characterized by using UV-Visible spectroscopy, Fourier Transform infrared spectroscopy (FTIR), Energy dispersive spectroscopy (EDS) and Transmission electron microscopy (TEM).

Antibacterial activities of the synthesized AgNPs were also investigated against Escherichia coli (E. coli). Physico-chemical studies have also been done to find out the extent of interaction between AgNPs and various solvents by deriving the thermodynamic parameters.

Keywords: Antibacterial activity, Cinnamomum tamala, green synthesis, thermo-acoustic parameters, silver nano particle.

Introduction
Nanotechnology is a field which is growing every day by producing different kind of nanoparticles and nanomaterials. Nanomaterials often show unique and significantly changed physico - chemical and biological properties compared to their bulk counterparts, which make them useful for various applications. Nanoparticles (NPs) have wonderful optical, electronic, magnetic and catalytic properties than the bulk material due to high surface area to volume ratio. Nanoparticles are of mainly two types: organic nanoparticles like fullerenes and inorganic nanoparticles like noble metal nanoparticles (e.g. gold and silver nanoparticles).

The demand of inorganic nanoparticles (e.g. gold and silver nanoparticles) is more due to their superior properties. Nanoparticles can be synthesized by various methods like electrochemical, microwave assisted process, facile method, sono chemical method, thermal decomposition method, micro emulsion method and most recent green synthesis. As the nanoparticles synthesized by chemical and physical methods produce pure and well-defined particles but these methods are not cost effective and environment friendly. These problems can be solved by green synthesis in which the plant extracts and plant biomass are used as reducing agent.

As in chemical synthesis, the toxic chemicals are present which are unfavourable in medical field. So, the use of renewable feed stock, safer solvent, energy efficient synthesis and nontoxic chemicals are the main points which must be followed in green synthesis. Due to these important factors, green synthesized biocompatible metal nano particles have gained considerable attention in recent years for potential applications in biomedicine.

Among the noble metals, silver (Ag) is the distinguished one because silver is used in textile fabrics, food additive, food packaging and plastic to eliminate microorganisms. Due to so many important applications, various methods concerning the fabrication of silver nanoparticles (AgNPs) as well as various silver-based compounds containing ionic silver (Ag+) or metallic silver (Ag) have been developed. Silver nanoparticles are used in topical cream to prevent wound infections, anticancer agents and antimicrobial agents in wound dressings.

Various plant extracts jatropha curcas seeds, murraya koenigii (curry) leaf, mulberry leaves, Ocimum sanctum (tulsi) leaf, Luffa Acutangula, soap nuts, capsicum annuum, Arbutus unedo leaf, pomegranate peel have been used for green synthesis of silver nanoparticles and their antimicrobial activities are also studied.

In the present paper, we have characterized the green synthesis of AgNPs using CT (Tejpatta) leaf extract and studied their antibacterial activity. It is mainly used for flavouring food and widely used in pharmaceutical preparation due to its hypoglycaemic, stimulant and carminative properties. It belongs to the family Lauraceae. The Cinnamomum tamala leaves are used as spice having clove like taste and pepper like odour. The essential oils and extract of tejpatta possess antibacterial, antifungal and antioxidant activities.

The studies of thermo-dynamical and transport properties of multi-component (binary and ternary) liquid mixtures and solutions have found wide applications in chemical, textile, leather and nuclear industries. Ultrasonic investigations of liquid mixtures consisting of polar and non-polar components enable to understand the molecular interactions and structural behaviour of molecules and their mixtures. Many research works have been reported in...
this field for liquid mixtures like propylene glycol and hexanol but not in case of nanoparticles. As the structure and shape of molecules is highly affected by the intermolecular interaction when nanoparticles are incorporated in the system. So, there is need to study these parameters in case of nanoparticles.

For better understanding of the molecular interactions between the nanoparticles (NPs) and different solvents, thermo-acoustic investigation has been done by measuring the speed of sound together with densities at different temperatures. Knowledge of speed of sound, density at different temperatures of pure nanoparticles and after mixing NPs with different solvents were used to compute important thermodynamic parameter like isentropic compressibility.

Material and Methods
Preparation of plant extract and green synthesis of silver nanoparticles: First of all, 2g of Cinnamomum tamala leaves were washed with distilled water 2-3 times to remove impurities. After washing, leaves were crushed in pestle mortar and mixed with 60 ml of distilled water. The mixture is heated on water bath at 80°C for 2 hours. Then mixture is cooled and filtered out with Whatmann filter paper no. 1. Filtrate is further used for the preparation of NPs.

After that, leaf extract and 1mM aqueous solution of AgNO₃ are mixed in different ratios. The amount of AgNO₃ is kept constant and amount of leaf extract is varied. In this way, we have prepared four samples by mixing leaf extract and AgNO₃ in the ratio 2:10, 4:10, 6:10, 8:10 (in ml) named as T1, T2, T3, T4 respectively. Then we heated it on water bath at 80°C for 30 minutes. At initial stage, samples were colorless but gradually color changed to yellow and finally to orange which indicates the formation of AgNPs. This is due to the reduction of Ag⁺ ions into Ag (0) as shown in figure 1(a) and (b).

Characterization of the synthesized nanoparticles: The formation of AgNPs was monitored under UV double beam spectrophotometer-2202 (Systronics). by measuring the absorption of the reaction medium in the wavelength range between 300-700 nm. Brucker Alpha-T FTIR instrument was used to measure the IR spectrum of the AgNPs in between the 400-4000cm⁻¹. The elemental analysis was done by using Energy dispersive X-ray spectroscopy (EDS) Jeol-JSM6510LV model. For this, a carbon coated copper grid was used on which thin film of the sample was prepared by dropping a very small amount of the sample on the grid and it is dried for 5 min under mercury lamp. The shape and size of AgNPs were examined by using Transmission electron microscope (TEM), model no.-Hitachi H7500 at an accelerating voltage of 90 KV. Few drops of colloidal silver nanoparticles were placed over carbon coated copper grid and it is air dried so that solvent evaporated.

Disc diffusion method was used to study the antibacterial activity. The bacterial strain E. coli (MTCC-443) which was pre cultured in Nutrient Broth (NB) for overnight in incubator at 37°C and Nutrient Agar (NA) media was prepared.

After that, sterilization of Petri plates, discs and media were done in autoclave. Sterilized media in the Petri plates were uniformly spread and allowed to solidify inside the laminar flow. After solidification of media, bacteria were spread on the plates and discs of AgNPs were put in the Petri plates on required places. The plates were incubated at 37°C for 24 hours and resulting zones of inhibition were measured.

Thermo-acoustic parameters: To study the intermolecular interaction between AgNPs and different solvents, we have used density and sound velocity meter (Anton Paar DSA 5000M) to measure speed of sound and densities simultaneously. A density check or an air/water adjustment was performed at 293.15 K with tripilly distilled, degassed water, and with dry air at atmospheric pressure. Before each series of measurements, the densimeter was calibrated with triple distilled and degassed water in the experimental temperature range.

For the different samples of AgNPs T1, T2, T3 and T4 - density and speed of sound were measured at different temperatures in the range of (15-35) °C. Then different samples T1, T2, T3 and T4 were mixed with methanol and propanol in the ratio (v/v) of 1:2 and 1:4 respectively. At the same temperature values, density and speed of sound data were taken again after mixing the solvents. From experimental density and speed of sound data, physico-chemical parameter i.e. isentropic compressibility was calculated by using the formula:

\[ \kappa_s = \frac{1}{\mu^2} \rho \]

where \( \kappa_s \) = Isentropic Compressibility, \( \mu \) =Speed of Sound and \( \rho \) = Density.

Results and Discussion
Cinnamomum tamala leaf extract was used to prepare AgNPs by green method. When AgNO₃ solution is mixed with Cinnamomum tamala extract then it is colorless. As it is heated on water bath, the reduction of Ag⁺ ions took place and changed into Ag (0). This was confirmed by color change from yellow to orange as shown in figure 1(a) and(b). Final confirmation was done from Surface Plasmon Resonance (SPR).

UV-Visible spectral studies: One of the most common techniques used for the characterization of AgNPs is UV-Visible spectroscopy. UV-Visible spectra of the colloidal solution of AgNPs were taken in the range of 300-700nm. The characteristic absorption peaks for the AgNPs samples T1, T2, T3 and T4 are at 447.2nm, 448.8nm, 450.4nm and 452.7nm respectively as shown in figure 2 which are due to the SPR.
As we know, particle size, shape and absorption of nucleophile or electrophile can affect the position of peaks. If the particle size increases and withdrawal of electron density takes place, then red shift is associated in SPR. It is also well known that adsorption of the nucleophile to the particle surface increases Fermi level of the silver particle owing to its donation of electron density to the particles.

As we are moving from AgNPs’s samples T1 to T4, absorption maximum shifts towards the longer wavelength which causes the red shift. This result shows that some electron donor group like –OH or –NH₂ etc. is present in Cinnamomum tamala.

FTIR Spectral studies: FTIR is an important parameter to find out the functional groups present in the extract. By FTIR of Cinnamomum tamala leaf extract and AgNPs were carried out to find the reducing as well as stabilizing biomolecules present in Cinnamomum tamala leaf extract. Aqueous leaf extract of Cinnamomum tamala acts as reducing and stabilizing agent. The major component of essential oil of Cinnamomum tamala leaves is Eugenol.

The FTIR spectra of Cinnamomum tamala Extract and AgNPs are shown in figure 4 (a) and (b) respectively. Before reduction process, the peak at 3282.20 cm⁻¹ is observed and after reduction it is not visible which shows the presence of –NH₂ or –OH group in extract but the composition of Cinnamomum tamala extract does not contain –NH₂ group which means –OH group is present as shown in figure 3 i.e. structure of eugenol. This –OH group helps in the reduction process of Ag⁺ ions; –OH group is attached to benzene ring. Two electron withdrawing groups i.e. methoxy and allyl groups are at ortho and para position respectively.

Due to –OH group, eugenol releases a proton and changed into anionic form and then resonating structures are formed which help in stabilization. A peak at 1638.18 cm⁻¹ also appeared after reduction process which indicates the presence of C=O group. A peak at 3490.8 cm⁻¹ appears before and after reduction which is due to the –OH (aqueous) because extract is made in aqueous medium.

TEM analysis: The synthesized AgNPs were further characterized by TEM to find out the size and shape. Figure 5 shows the micrographs of AgNPs synthesized from Cinnamomum tamala leaf extract for the sample T1 in different magnifications. The size of the AgNPs is in the range of 7-19 nm and the particles are spherical in shape.

EDS analysis: EDS spectra of synthesized AgNPs are shown in figure 6. As it is clear from the figure that strong signal for Ag is present, so it confirms the formation of AgNPS. Signal for C is also shown in EDS spectra which is due to the carbon coated grid used for the analysis.

Antibacterial Studies: Antibacterial activity of AgNPs prepared by using Cinnamomum tamala leaf extract is shown in figure 7 against E. coli. Ciprofloxin is used as a control as shown in the centre. The zone of inhibition is measured for the samples T1, T2, T3, T4 of AgNPs and recorded as 6mm, 9mm, 10mm and 11mm respectively. It shows that as the amount of extract increases, the inhibition power of AgNPs increases.

Thermo-acoustic parameters: In order to explore the strength and nature of the interaction between AgNPs as well as solvents by deriving thermodynamic parameter such as isentropic compressibility from density and speed of sound, attempt has also been done to explain the thermo-acoustic behaviour after mixing the AgNPs with different solvents like methanol and propanol.

Figure 8 shows the variation of density with temperature. As temperature increases, density decreases for all the samples T1, T2, T3, and T4. As the amount of nanoparticles is kept fixed and amount of methanol and propanol is increased, density also decreases. But as we increase the amount of CT extract from sample T1 to T4, density increases at the same temperature value against pure nanoparticles. But as solvents are added, the density decreases from sample T1 to T4. This indicates that increase in the amount of extract helps in the increase of density but as solvent is added, it decreases the molecular interaction.

Figure 9 shows the variation of speed of sound with temperature. As temperature increases, speed of sound increases for all the samples T1, T2, T3, and T4. As the amount of nanoparticles is kept fixed and amounts of methanol and propanol are increased, speed of sound decreases with increase of temperature.

This indicates that there is change in molecular interaction after adding solvent to the nanoparticles. The molecular interactions become weak between nanoparticles after adding solvents. But as we move from sample T1 to T4 when solvents are added to the nanoparticles, speed of sound decreases at the same temperature value which implies that the molecular interaction becomes weak.

Figure 10 shows the variation of isentropic compressibility with temperature. As the temperature increases, isentropic compressibility decreases for pure nanoparticles. As amount of nanoparticles is kept fixed and amount of methanol and propanol is increased, isentropic compressibility increases with increase of temperature.

This indicates that there is change in molecular interaction after adding solvent to the nanoparticles. The molecular interactions become weak between nanoparticles after addition of the solvents. But as we move from sample T1 to T4 and solvents are added to the nanoparticles, isentropic compressibility increases at the same temperature which implies that the molecular interactions become weak.
Table 1
Nomenclature used in thermo-acoustic parameters

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nomenclature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>T11</td>
<td>ρ₁, μ₁, κ₁</td>
<td>Density, Speed of sound, Isentropic Compressibility of AgNPs respectively.</td>
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<tr>
<td></td>
<td>[ρ₂a, ρ₃b]</td>
<td>Density after mixing nanoparticles and methanol in the ratio 1:2,1:4 (in ml)</td>
</tr>
<tr>
<td></td>
<td>[μ₂b, μ₃b]</td>
<td>Speed of sound after mixing nanoparticles and methanol in 1:2,1:4 (in ml)</td>
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<tr>
<td></td>
<td>[κ₂b, κ₃b]</td>
<td>Isentropic Compressibility after mixing nanoparticles and methanol in the ratio 1:2,1:4 (in ml)</td>
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<tr>
<td>T12</td>
<td>ρ₁a, μ₁a, κ₁a</td>
<td>Density, Speed of sound, Isentropic Compressibility of AgNPs respectively.</td>
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<tr>
<td></td>
<td>[μ₄c, μ₅c]</td>
<td>Density after mixing nanoparticles and Propanol in the ratio 1:2,1:4 (in ml)</td>
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<tr>
<td></td>
<td>[κ₄c, κ₅c]</td>
<td>Isentropic Compressibility after mixing nanoparticles and Propanol in the ratio 1:2,1:4 (in ml)</td>
</tr>
<tr>
<td>T21</td>
<td>ρ₂a, μ₂a, K₂a</td>
<td>Density, Speed of sound, Isentropic Compressibility of AgNPs respectively.</td>
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<tr>
<td></td>
<td>[μ₂d, μ₃d]</td>
<td>Speed of sound after mixing nanoparticles and methanol in 1:2,1:4 (in ml)</td>
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<tr>
<td></td>
<td>[κ₂d, κ₃d]</td>
<td>Isentropic Compressibility after mixing nanoparticles and methanol in the ratio 1:2,1:4 (in ml)</td>
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<tr>
<td>T22</td>
<td>ρ₂a, μ₂a, K₂a</td>
<td>Density, Speed of sound, Isentropic Compressibility of AgNPs respectively.</td>
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<tr>
<td></td>
<td>[μ₄e, μ₅e]</td>
<td>Speed of sound after mixing nanoparticles and Propanol in 1:2,1:4 (in ml)</td>
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<tr>
<td></td>
<td>[κ₄e, κ₅e]</td>
<td>Isentropic Compressibility after mixing nanoparticles and Propanol in the ratio 1:2,1:4 (in ml)</td>
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<tr>
<td>T31</td>
<td>ρ₃a, μ₃a, K₃a</td>
<td>Density, Speed of sound, Isentropic Compressibility of AgNPs respectively.</td>
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<td></td>
<td>[μ₂f, μ₃f]</td>
<td>Speed of sound after mixing nanoparticles and methanol in 1:2,1:4 (in ml)</td>
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<tr>
<td></td>
<td>[κ₂f, κ₃f]</td>
<td>Isentropic Compressibility after mixing nanoparticles and methanol in the ratio 1:2,1:4 (in ml)</td>
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<tr>
<td>T32</td>
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<td>Density, Speed of sound, Isentropic Compressibility of AgNPs respectively.</td>
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<tr>
<td></td>
<td>[μ₄g, μ₅g]</td>
<td>Speed of sound after mixing nanoparticles and Propanol in 1:2,1:4 (in ml)</td>
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<tr>
<td></td>
<td>[κ₄g, κ₅g]</td>
<td>Isentropic Compressibility after mixing nanoparticles and Propanol in the ratio 1:2,1:4 (in ml)</td>
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<td>T41</td>
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<td>Density, Speed of sound, Isentropic Compressibility of AgNPs respectively.</td>
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<td>[μ₂h, μ₃h]</td>
<td>Speed of sound after mixing nanoparticles and methanol in 1:2,1:4 (in ml)</td>
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<td></td>
<td>[κ₂h, κ₃h]</td>
<td>Isentropic Compressibility after mixing nanoparticles and methanol in the ratio 1:2,1:4 (in ml)</td>
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<tr>
<td>T42</td>
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<td>Density, Speed of sound, Compressibility of AgNPs respectively.</td>
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<td>[μ₄i, μ₅i]</td>
<td>Speed of sound after mixing nanoparticles and Propanol in 1:2,1:4 (in ml)</td>
</tr>
<tr>
<td></td>
<td>[κ₄i, κ₅i]</td>
<td>Isentropic Compressibility after mixing nanoparticles and Propanol in the ratio 1:2,1:4 (in ml)</td>
</tr>
</tbody>
</table>

Figure 1: Silver nanoparticles indicated by colour change (a) initial stage (colorless) and (b) final stage (Orange color)
Figure 2: UV-Visible absorption spectra of colloidal solution synthesized AgNPs as a function of the wavelength in the range of 300-700 nm.

Figure 3: Structure of Eugenol (4-allyl-2-methoxy-phenol)

Figure 4: FTIR spectrum of (a) *Cinnamomum tamala* extract and (b) Silver nanoparticles
Figure 5: TEM images of silver nanoparticles prepared by *Cinnamomum tamala* at (a) 20nm scale and (b) 100nm scale

Figure 6: EDS spectra of silver nanoparticles prepared by *Cinnamomum tamala*

Figure 7: Antibacterial activity against E. coli. Of AgNPs for samples T1, T2, T3 and T4
Figure 8: Variation of density with temperature for samples T1, T2, T3, T4 with different proportion of nanoparticles + methanol and different proportion of nanoparticles + propanol.

Figure 9: Variation of speed of sound with temperature for samples T1, T2, T3, T4 with different proportion of nanoparticles + methanol and different proportion of nanoparticles + propanol.
Figure 10: Variation of isentropic compressibility $\kappa_s$ with temperature for samples T1, T2, T3, T4 with different proportion of nanoparticles + methanol and different proportion of nanoparticles + propanol.
Conclusion
In this study, we have done the green synthesis of AgNPs by using Cinnamomum tamala leaf extract instead of chemical methods which are not environment friendly. The characterisation of AgNPs was done by using different techniques like UV-Visible spectroscopy, TEM analysis, FTIR spectra, EDS spectra. AgNPs of size 7-17nm were observed and they were spherical in shape. Antibacterial activity was also studied against E. coli. Isentropic compressibility was calculated from density and speed of sound data.

It was observed that isentropic compressibility increases when solvents were added to AgNPs. This helped us to find out the effect on molecular interaction by the addition of different solvents in aqueous AgNPs. In future, more thermo-acoustic parameters like intermolecular free length can be studied.

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