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Biosynthesis of Silver Nanoparticles Using *Terminalia Bellirica* Fruit Extract and Their Characterization

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Abstract: Green synthesis is an appropriate method for the synthesis of Ag nanoparticles using *Terminalia Bellirica* fruit extracts. The change in color from light yellow to dark brown was observed by adding $AgNO_3$ solution into fruit extracts. The Ag nanoparticles were characterized by UV-Vis (Ultraviolet-visible), XRD, TEM and FTIR. The reduction of Ag^{+} into Ag^{0} was observed by UV-Vis spectrophotometer. The silver nanoparticles were found to be crystalline in nature when observed in the X-Ray Diffraction analysis. The geometry of these silver nanoparticles is face centered cubic geometry. When observed in the Transmission Electron Microscopy (TEM) analysis, these synthesized nanoparticles were found spherical in shape where their average size was less than 26 nanometers. FTIR peaks confirms the presence of phytochemicals which are responsible for reducing, capping and stabilizing the nanoparticles.

Keywords: *Terminalia Bellirica* • Silver nanoparticles • XRD • Crystalline • FTIR • TEM

Introduction

Recently, synthesis of metallic nanoparticles is most emerging researches in the field of nanoscience and nanotechnology. The field of nanotechnology develops novel therapeutic nanosized materials for biomedical and pharmaceutical applications. Metallic nanoparticles have various properties such as catalytic, optical, magnetic and electrical which is controlled by the size, shape and morphology of nanoparticles (Garima et al, 2011). Nanoparticles has a higher surface area to volume ratio with decrease in size, distribution and morphology. Various metals are being used for the synthesis of nanoparticles such as Ag, Au, Zn, Cu etc. The biosynthesized metallic nanoparticles control the various endemic diseases with less adverse effect (Awwad et al 2012). Recently, many studies have proved that the plant extract act as a potential precursor for the synthesis of metallic nanoparticles in innocuous ways. Green methods for the synthesis of metallic nanoparticles are more favorable instead of chemical and physical methods because it has harmful and highly toxic for living organisms.

To prevent environment from hazardous chemicals and by products the green approaches using natural resources such as plants, algae and microorganisms play a key role for the synthesis of metallic nanoparticles due to simple, cost efficient and environmentally friendly method. Plant resources like leaves, flowers, stems, fruits, roots, barks, peels and seeds have been utilized for the silver nanoparticles synthesis (Jain et. al. 2017; Khan et. al., 2018). Phytochemicals such as alkaloids, flavonoids, saponins, steroids and tannins present in the plant extracts act as capping and reducing agent for green synthesis of silver nanoparticles (Mile et. al. 2013; Kumar et. al. 2014; Tumble et. al. 2017). The synthesis of silver nanoparticles is widely used in various applications such as catalysis, bio emerging. Silver nanoparticles used as effective anti-microbial, anti-fungal, anti-viral, anti-oxidant, anti-cancer agents (Bose 2016; Awwad et. al, 2012). The biological activity of silver nanoparticles strongly depends on their size, shape, concentration, distribution and stability Kandwal et. al. 2019; Praveen et. al. 2021). Some factors depend on the formation of silver nanoparticles such as reaction condition, efficiency of the reducing agent and nature of the capping agent (Mason et. al. 2012; Kumar et. al. 2010; Vivekanandhan et. al. 2009; Kora et. al. 2012; Daisy 2010 and Narayanan et. al. 2008); (Kandwal et. al. 2028). The plant mediated silver nanoparticles are potential remedy for various diseases such as malaria, cancer, hepatitis and other acute diseases (Khajuria et.al. and Purohit et. al. 2020).

Terminalia Bellirica belonging to the combrataceae family and is commonly known as "Bahera" (Sharma et. al. 2020). It is a large deciduous tree to 50 m tall growing on wild throughout indian subcontinent, Srilanka and South East Asia, upto 1200 m in elevation. The bark is ashy-grey and leaves large broadly elliptic to obovate-elliptical and fruits are ovoid grey drupes (Sarawsathi et. al. and Devi et. al. 2014). It has been extensively used in ayurvedic medicine for its anti-diabetic, anti-hypertensive, anti-diarrhoeal, anti-pyretic, anti-microbial, anti-oxidant, lowering cholesterol and blood pressure, anti-cancer and heptaprotactive activities (Kirtikar & Basu 1999; Bardul et. al. 2011; Dev et. al. 2016; Elizabeth et. al. 2005 and Sharma et. al. 2010. These activities are due to the presence of its various types of phytoconstituents such as ellagic acid, gallic acid, glucose, fructose, rhamnose, mannitol, termilignan etc (Saraswathi et. al. 2012; Kirtikar & Basu 1999). Many researchers have reported synthesis of silver nanoparticles using different plant materials (Vivekanandhan et. al. 2009; Kora et. al. 2012; Daisy 2010 and Narayanan et. al. 2008).

After thorough literature survey and compilation of data, it was found that no same report has been published on the bio synthesis of silver nanoparticles by using the aqueous leaf extract of *Terminalia Bellirica*. So, in the present investigation, we have attempted the synthesis of silver nanoparticles by green route using the fruit extract of *Terminalia Bellirica* and its characterization by UV-Vis, XRD, FTIR and TEM analysis.

Materials And Methods

Preparation of *Terminalia bellirica* fruit extract: The fresh fruits of *Terminalia bellirica* collected from Advani Forest region of Pauri (Uttarakhand). The collected fruits washed with double distilled water to remove dust. There after washing fruits kept for drying under shade. By the use of mortar-pestle dried fruits grinded to fine powder. Then 10 g of powdered fruits boiled with 200 mL distilled water for 25 min at 65^0 C. Aqueous fruit extract then filtered with Whatman no. 1 filter paper. For further experiment, fruit extract used for the synthesis of Ag nanoparticles.

Synthesis Of Silver Nanoparticles: For the synthesis of Ag NP's, firstly 5mM aqueous $AgNO_3$ solution(1L) were prepared. 100mL aqueous fruit extracts mixed with 5mM aqueous $AgNO_3$ solution(1L) in 1:10 at room tempt. It was observed that the colour of the solution changes from light yellow to dark brown within 10 min, which indicates the Ag nanoparticles are formed. The dark brown solution was centrifuged at 7500 rpm for 12 min. Thereafter obtained Ag nanoparticles were washed 2 times with distilled water followed by acetone. Then Ag NP's were dried at 45^0 C for 24 H in oven. After that, using mortar-pestle dried NP's mashed into fine powder and stored in dry bottles for further analytical study.

Characterization Of Synthesized Nanoparticles: Characterization of synthesized silver nanoparticles was done using some advanced techniques like TEM, XRD, FTIR and UV-VIS spectroscopy. The reduction of silver ions was monitored by analyzing the UV-VIS spectrum of reaction medium after 3 hours on diluting a small aliquot of the sample into double distilled water. This analysis was performed using Perkin Elmer, Lambda 25 UV-VIS spectrophotometer. Diffraction and imaging techniques are used for characterizing crystalline structures and microstructures of materials simultaneously.

At first, the formation of silver nanoparticles was frequently monitored by using Elite-double beam UV-visible spectrophotometer. Thereafter, silver nanoparticles were put through XRD analysis (XRD model: X'PERT-PRO Diffractometer, PANalytical; CuK α radiation, wavelength λ_{max} = 1.54 Å). The spectra of the solution came out to be in the range of 2ɵ from 0^0 to 75^0.

FT-IR spectroscopy (Spectrophotometer Perkin Elmer Model RZX) analysis ranging from 4000-500 cm^{-1} was to identified and to characterize the secondary metabolites in plant extract which are responsible for capping and stabilizing the nanoparticles. With the help of Bebye Scherrer's equation: $D = K\lambda / \beta\cos\theta$, the average size of the silver nanoparticles was calculated

Here,

D = average crystallite size,

K = Scherrer's constant,

λ = X-ray wavelength,

β = full width at half maxima (FWHM)

θ = Bragg's diffraction angle.

The surface morphology of silver nanoparticles was determined by using TEM (JEOL JEM 1400) analysis.

Result and Discussion

In this work, silver nanoparticles were synthesized with the help of aqueous fruit extract of Terminalia bellirica. Secondary metabolites present in the fruit extract, identified and characterized by FT-IR spectroscopy, performed a key role in the synthesis of silver nanoparticles. The colour of solution changed from colorless to dark red due to the formation of these nanoparticles. UV-visible spectrum of the solution showed a broad absorption peak at λ_{max} = 434-442 nanometers (Fig. 1) which confirmed the presence of silver nanoparticles in the solution. The calculated energy band gap came out in between 2.80-2.85 eV.

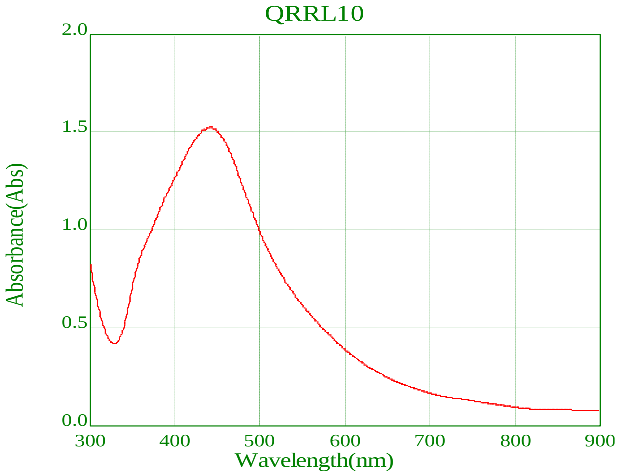

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Fig. 1. UV-visible spectrum of silver nanoparticles

XRD analysis

The XRD spectra of the plant mediated silver nanoparticle showed that the crystalline nature as well as the average crystallite size was calculated using Debye-Scherrer's equation. When calculated, the average size of silver nanoparticles is found to be less than 12 nm. X-Ray Diffraction spectra of plant mediated nanoparticles showed sharp peaks at 2θ = 38.23°, 44.38°, 64.5°, 77.38° and 81.51° Which are indexed to (111), (200), (220), (311) and (222) lattice planes respectively (Table-1) of face centered cubic nano crystals (Fig. 2).

Table-1: Peak List For Average Size Calculation Of Nanoparticles

Pos. [°2θ]	FWHM Total [°2θ]	d-spacing [Å]	Rel. Int. [%]
38.2380	0.3913	2.35184	100.00
44.3884	0.8063	2.03919	22.50
64.5000	0.5634	1.44355	23.92
77.3833	0.7863	1.23222	24.90
81.5174	0.7242	1.17986	7.09

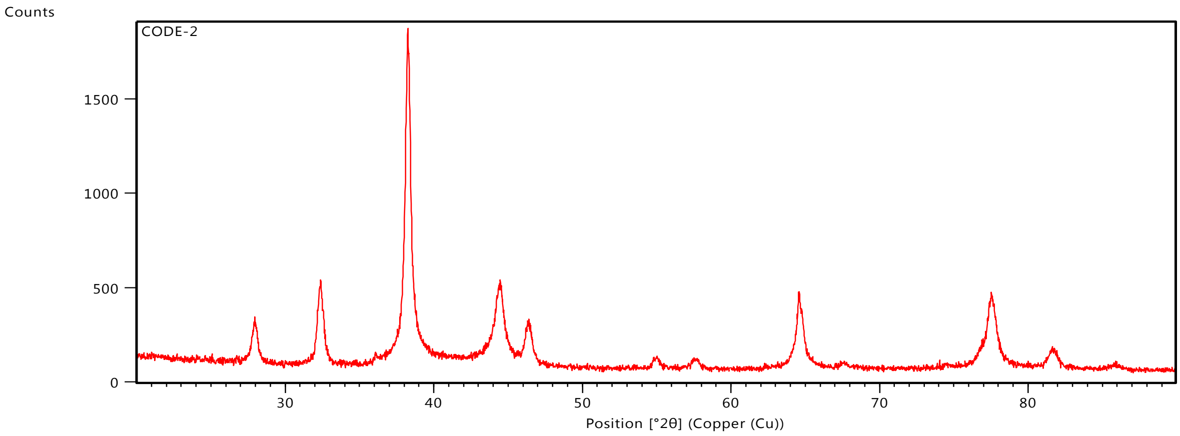

Fig 2. XRD spectra of silver nanoparticles

TEM analysis

TEM analysis of the plant mediated silver nanoparticles, confirmed that silver nanoparticles were spherical in shape with their average size less than 26 nm. Fig.3

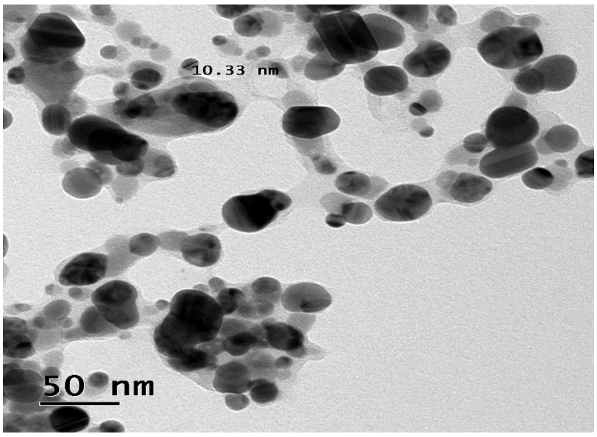

Fig. 3. Transmission electron micro-image of silver nanoparticles

FTIR analysis

FTIR analysis used to identify the functional groups of the biomolecules. These functional groups identify in between 4000 cm^{-1} to 400 cm^{-1} band range which are accountable for the reducing, capping and stabilizing the nanoparticles. FTIR peak in Fig. 4 at 3425.8 cm^{-1} corresponds to O-H stretching vibrations of alcohol and phenol. Other bands at 1619.2 cm^{-1} corresponds to C-C and C-N stretching vibrations and 1384.2 cm^{-1}, 1197.5 cm^{-1} may be corresponding to N=O stretching of the nitro group and C-O stretching of Ar-OH respectively.

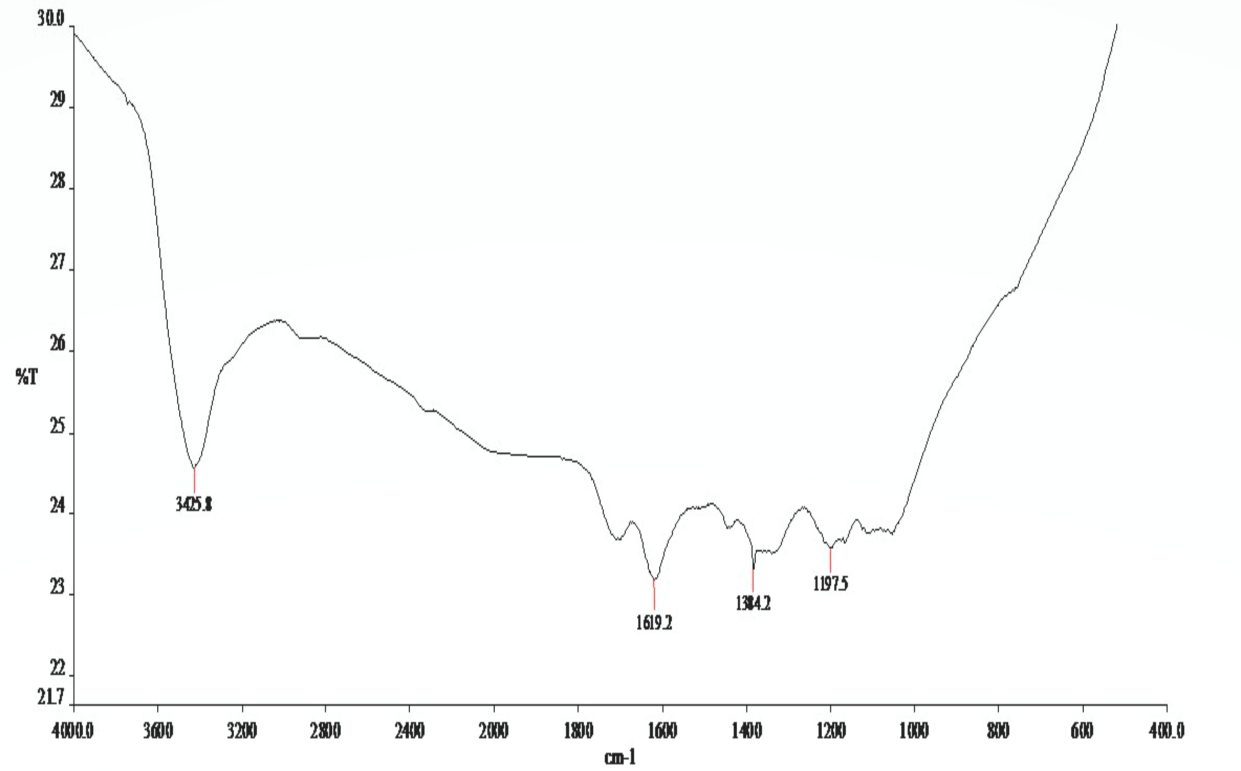

Fig. 4. FTIR spectrum of plant mediated silver nanoparticles

Conclusion

It can be concluded that biosynthesis of silver nanoparticles of *Terminalia bellirica* fruit extract is rapid, non-hazardous, cost effective and eco-friendly. Spherically shaped, crystalline silver nanoparticles with their average size less than 26 nm are synthesized. It is observed that phytochemicals present in fruit extract effectively acts as capping and stabilizing agents of silver nanoparticles. The plant mediated synthesis of silver nanoparticles doesn't require any toxic and hazardous chemicals hence, it is a promising substitute of long-established chemical and physical methods.

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