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Research Article

GREEN SYNTHESIS OF GOLD NANOPARTICLES USING *TOONA CILIATA* METHANOL BARK EXTRACT AND THEIR CHARACTERISATION

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ABSTRACT

In the present study, we identified and justified the use of MeOH extract from *Toona ciliata* bark as a reducing and capping agent for the ecofriendly synthesis of gold nanoparticles on the basis of modern analytical techniques. The reduction of 1.0 mM aqueous solution of aurochloric acid with 1 ml, 1% w/v aqueous solution of MeOH bark extract from *Toona ciliata* has resulted in the formation of stabilised Gold Nanoparticles (AuNPs). The synthesised gold particles showed a surface plasmon band around 550 nm when analysed via UV-Visible Spectroscopy, indicated the gold particles of nano dimensions (10^{-9} m). The Transmission Electron Microscopy (TEM) study of gold nano particles revealed the formation of spherical, poly dispersed nanoparticles of varying sizes ranging from 40-75 nm along with encapsulating cage. The time for the synthesis of gold nano particles was noted to be 30 minute. The preliminary phytochemical analysis of methanol extract form the bark confirmed the presence of alkaloids, glycosides, flavanoids, tannins and reducing sugars. The results of the present study clearly reveal the *Toona ciliata* methanolic bark extract as a new, novel and renewable, cost effective, reducing and capping agent for the application in the field of nanobiotechnology as well as pharmaceutical sciences. Further, the ecofriendly approach developed for AuNPs synthesis with *Toona ciliata* MeOH bark extract is the rapid and cost effective alternative to the traditional chemical methods of AuNPs synthesis.

KEYWORDS: *Toona ciliata*, Methanol Bark Extract, Green Synthesis, Gold Nanoparticles, Transmission Electron Microscopy.

INTRODUCTION

In the last few years the field of nanobiotechnology has received immense attention due to its vast applicability from the kitchen to space. With this development, many new ecofriendly methods for the synthesis of nanoparticles have emerged viz. plant mediated synthesis, microorganism mediated synthesis etc, in order to minimise the risk of pollution to environment and toxicity associated with the use of reductive chemicals in the nanoparticles synthesis. For the development of green chemistry, Raveendran et al. suggested that three main factors in nanoparticle preparation should be considered i.e. solvent choice, the use of an environmentally benign reducing agent, and the use of a non-toxic material for nanoparticle stabilisation[1]. In this respect, plant mediated synthesis of nanoparticles is not only ecofriendly, cheap and safe to handle but the many bio chemicals from the plant posses vast therapeutic potential, which could be employed to alleviate the sufferings of humanity through a suitable drug delievery technique. Metallic gold nanoparticles have tremendous applications in cancer diagnosis & therapy, catalysis and in optoelectronics. For e.g. Gold nanoparticle solutions are bright red/purple colored due to plasmon absorption. Any surface modification of AuNPs results in the shift of plasmon absorption wavelength. This change in optical property of AuNPs when coming in contact with the probe biomolecules is exploited to develop biosensors[2]. Hainfeld et al. demonstrated that the irradiation of AuNPs accumulated in tumor with 250 kVp X-rays caused shrinkage of tumor in mice with subcutaneously grown mammary carcinoma tumor. It was also found that that treatment with X-rays alone had no therapeutic effect on the tumor[3]. Recently a lot of work has been done with regard to plant mediated synthesis of metallic nanoparticles and the respective role of phytochemicals. The main phytochemicals responsible for reduction and stabilisation have been identified as terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids in the light of IR spectroscopic studies. For e.g. Vankar and Bajpai (2010) demonstrated the preparation of gold nanoparticles of 100 nm size of different shapes from *Mirabilis jalapa* flowers extract. The study also suggested that the polyols (Flavanoids, terpenoids and polysaccharides) from *Mirabilis jalapa,* biomass were responsible for reduction of Au^{+++} ions to AuNPs, when analysed through FT-IR spectroscopy[4]. *Toona cilata*, also known as Red Cedar throughout the world was first described from India and is characterised by glabrous filaments is an important medicinal plant. The plant is official in Ayurvedic Pharmacopoeia of India and mentioned as a plant with tonic properties. The stem bark of the tree is used as antiulcer, antileprotic, blood purifier and had been used traditionally to heal wounds[5]. The several pharmacological and ethnobotanical research studies on the various parts of this plant report it to possess antimicrobial[6,7], antiulcer[8], antioxidant[9], antipyretic[10], hypoglycaemic[11] and antitumor & cytotoxic[12] activities. The bright and impelling motive behind this study was to assess the potential of *Toona ciliata* bark as a novel reducing and capping agent for the ecofriendly synthesis of AuNPs. Since the plant material is reported to contain alkaloids, flavanoids, phenols, coumarin glycosides etc[13,] which have been previously identified to be responsible for reduction and stabilisation of metal ions into metal nanoparticles[4,14, 15]. Therefore as a preliminary investigation, we here in this study also performed the phytochemical analysis of the methanolic extract to know the presence of reported phytochemical groups. In fact, this is the first and only research study available on *Toona ciliata* bark till to the date which demonstrates the synthesis of AuNPs using aqueous solution of MeOH bark extract and their characterisation through modern analytical techniques.

MATERIAL & METHODS

Collection & Authentication

The plant material i.e. stem bark pieces (Figure 1.0) were peeled off from the matured tree of the *Toona ciliata* from the rocky hill slopes (altitude 1600 msl) of Village:

Patwadangar, District: Nainital (Uttarakhand), India in the month of July-2011. The bark was identified and authenticated vide reference no: NISCAIR/RHMD- 1997/05 by Dr. H.B. Singh, Chief Scientist & Head, Raw Materials Herbarium and Museum (RHMD), National Institute of Science Communication and Information Resources, a constituent establishment of Council of Scientific and Industrial Research, New Delhi, India. The pieces of fresh stem bark soon after collection were washed with running tap water followed by two subsequent washings with distilled water, placed on bloating paper and left for shade drying at room temperature (23±1◦C). After 25 days of complete shade drying, the bark pieces were finally reduced to powder using electrical blendor (Waring Corporation, USA) and passed through sieve #85 to get the fine powder. The powdered material thus prepared was stored in an airtight plastic container till used for further study.

Chemical and Reagents

Aurochloric acid was purchased from HIMEDIA, Mumbai and tri-sodium citrate was purchased from Merck India Pvt Ltd, Mumbai both were of analytical grade. The water was distilled twice to remove impurities. All the other chemicals and reagents used for phytochemical analysis were purchased from HIMEDIA, Mumbai.

Preparation of the MeOH extract

5 gm fine powder of the *Toona ciliata* bark was subjected to hot percolation process into a Soxhlet extraction assembly. The resultant extract was concentrated and evaporated to dryness on to a water bath. The dried extract was weighed, subjected to quantification by calculating the percentage extractive yield and characterised by means of evaluation of colour, odour, consistency, microscopic and fluorescence analysis for its initial standardisation.

Preliminary phytochemical analysis

Preliminary phytochemical tests were performed on the MeOH extract of *Toona ciliata* bark according to standard methods[16, 17] to identify different primary and secondary metabolites viz. carbohydrates, glycosides, alkaloids, tannins, flavonoids, steroids which have previously been reported to be present in the extract[13].

Synthesis of gold nanoparticles

1.0 mM aqueous solution of aurochloric acid was prepared by diluting 2.5 ml of stock solution (10.0 mM) to 25 ml with double distilled water. 20 ml of 1.0 mM aurochloric acid was transferred to an 100 ml capacity Erlenmeyer's flask containing a magnetic stirring bar (Axiva, India) placed on to a magnetic stirrer cum hot plate (Model: WiseStir, MSH 20D, Labtech, Korea). Bring the aurochloric solution to rolling boil (around 60°C) and to the rapidly boiling solution transferred 1.0 ml of 1% w/v aqueous solution of MeOH extract of *Toona ciliata* bark for the reduction of Au^{+++} ions. A change in colour of solution from initial bright yellow to wine red indicated the formation of gold nanoparticles (Figure 10.0), which was later confirmed by UV-Visible spectroscopy and Transmission Electron Microscopy (TEM) analysis.

UV-Visible spectroscopy analysis

The synthesised gold nanoparticles sample diluted with water (1:1) was subjected to UV-visible spectroscopy analysis on to a digital UV-Vis spectrophotometer (Khera Instruments, India) to know the position of the surface plasmon band. The absorbance at different wavelength ranging from 350-750 nm observed from UV-Vis spectroscopic analysis of synthesised nanoparticles were recorded and processed as UV-Vis spectrum.

Transmission Electron microscopic (TEM) analysis

The TEM specimens of the gold nano particles synthesised by using *Toona ciliata* MeOH bark extract were prepared by placing a drop of sample on to a carbon coated copper grid and allowed the specimens to dry in air for few hours. As shown in Figure 11.0 electron microscopic analysis was done on JEOL, JEM-1011 (Japan) transmission electron microscope, which was operated at an accelerating voltage of 80 kV to investigate the morphologic and morphographic pattern of biochemically synthesised gold nanoparticles.

RESULT AND DISCUSSION

Quantification and Characterisation of MeOH extract

The results of quantification and characterisation of MeOH extract from the *Toona ciliata* bark are tabulated in Table 1.0.

Preliminary phytochemical analysis

The results of preliminary phytochemical analysis of MeOH extract from *Toona ciliata* bark confirmed the presence of alkaloids, glycosides, flavanoids, coumarin glycosides, steroids, tannin, carbohydrate and starch except proteins, amino acids and volatile oils. The results of various tests which were performed are tabulated in Table 3.0 along with respective figures.

UV-Visible spectroscopy analysis

The gold nanoparticles exhibits wine red colour in aqueous solution due to the excitations of their surface plasmon response (550 nm). As the MeOH extract of *Toona ciliata* bark was added to the aurochloric acid, it started to change colour of gold solution from bright yellow to purplish black and finally wine red due to the complete reduction of Au^{+++} ions to AuNPs as shown in Figure 9.0, which demonstrates the property of surface plasmon resonance. UV-Visible spectroscopy could be used to investigate the size and shape controlled nanoparticles in aqueous suspension. Figure 12.0 shows the UV-Visible spectrum of synthesised AuNPs after 30 minutes of the complete bio-reduction by the MeOH extract of *Toona ciliata* bark. The UV-Visible spectrum of AuNPs showed a broad absorption in the UV-Visible range 350-750 nm, having λ_{max} at 550 nm, which indicated the presence of particles of nano dimensions. The broadening of peak also suggested the presence of polydispersity of AuNPs within the dispersion, which was later confirmed by TEM analysis.

Transmission Electron Microscopy (TEM) analysis

As shown in Figure 13.0, TEM analysis of synthesised AuNPs indicated the presence of all most spherical-oval particles of nano dimensions in the range between 40-75 nm. The analysis also revealed the presence of encapsulating cage of extract constituents which were bind to the gold ions (Au^0) and was responsible for the reduction of gold solution, Au^{+++} to Au^0 i.e. AuNPs and their stabilisation. The particles observed were found to be well separated from each other under TEM, which confirmed the presence of polydispersity of AuNPs in dispersion.

CONCLUSION

The reduction of Au^{3+} ions by the MeOH extract from the *Toona ciliata* bark has resulted in the formation of stabilised nanoparticles encapsulated with constituents from methanolic bark extract in 30 minutes after the use of process adopted by us, which is easy, cost effective and more rapid than any of the reported method of AuNPs synthesis to our knowledge. The bio-molecular encapsulation of separated nanoparticles is very important from the nanobiotechnology point of view, since the coating contains the bio-molecules from the naturally obtained extracts and several of them may likely to contribute to a beneficial therapeutic effect, For e.g. The

AgNPs synthesised by tea-leaf extracts showed antibacterial activity against *Vibrio harveyi* suggesting an alternative to antibiotics in controlling *V. harveyi* infection[18].The MeOH extract of the bark in phytochemical analysis showed the presence of different secondary and primary metabolites, from which several like phenolics, reducing sugars, flavanoids and alkaloids are believed to contribute to synthesis and capping of gold nanoparticles (Table 4.0), but to prove this hypothesis to be a truth in our case, it is necessary to characterise both the MeOH extract and extract encapsulated AuNPs by means of FTIR spectroscopy to identify the actual associated functional groups and their interaction with gold ions. Furthermore the encapsulated gold nanoparticles can be subject to appropriate pharmacological screening to cognise their vast therapeutic potential either via *in-vitro* or through *in-vivo* models.

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Table 1.0: Results of quantification and initial characterisation of MeOH bark extract from *Toona ciliata* M. Roem.

Characteristics	Observations
Colour	Shiny dark reddish brown
Odour	Aromatic
Consistency	Solid
Type of solid	Crystalline (Irregular flakes)
Weight of dried extract	0.54 gm
Percentage extractive yield	10.8 %

Table 2.0: Results of fluorescence analysis of MeOH extract from *Toona ciliata* bark.

Material	Visible light	UV-short (254nm)	UV-long (365nm)
MeOH extract	Reddish brown	No Fluorescence	No Fluorescence

Table 3.0, shows the result of preliminary phytochemical analysis of MeOH bark extract from *Toona ciliata* bark

Phytochemical Test	Observation	Inference
For alkaloids		
Tannic acid test	Buff colour precipitate	Positive, Figure 2.0
For glycosides		
Legal test	Pink/Red colour	Positive, Figure 3.0
For flavanoids		
Shinoda test	Light pink colour	Positive, Figure 4.0
For coumarin glycosides		
Alkali test	Blue colour fluorescence	Positive
For steroids		
Salkwoski test	Not confirmed	-
For tannins and phenolic compound		
Ferric chloride test	Greenish black colour precipitate	Positive, Figure 5.0
Lead Acetate test	Pink colour precipitate	Positive, Figure 6.0
Potassium dichromate test	Yellow colour precipitate	Positive
For carbohydrates (Reducing sugars)		
Fehling's test	Brick red precipitate	Positive, Figure 7.0
Benedict's test	Bottle green colour	Positive, Figure 8.0
For proteins		
Biuret test	No violet/pink colour	Negative
Precipitation test (5% $CuSO_4$ solution)	None precipitate	Negative
For amino acids		
Ninhydrin test	No purple/blue colour	Negative

'Positive = Present, Negative = Absent'

Table 4.0: The phytochemicals from different plants responsible for the formation of gold and silver nanoparticles

Plant Texa with part used	Nanoparticles	Phytochemicals	References
Azadirachta indica leaf	Ag/ Au	Terpenoids	Shankar et al., 2004; Tripathy et al., 2009
Black tea leaf	Ag/Au	Polyphenols/ flavanoids	Begum et al., 2009
Mirabilis jalapa flowers	Au	Flavones/ terpenoids/ polysaccharides	Vankar et al., 2010
Zingiber officinalis rhizome	Ag/Au	Alkaloids/ flavanoids	Singh et al., 2011

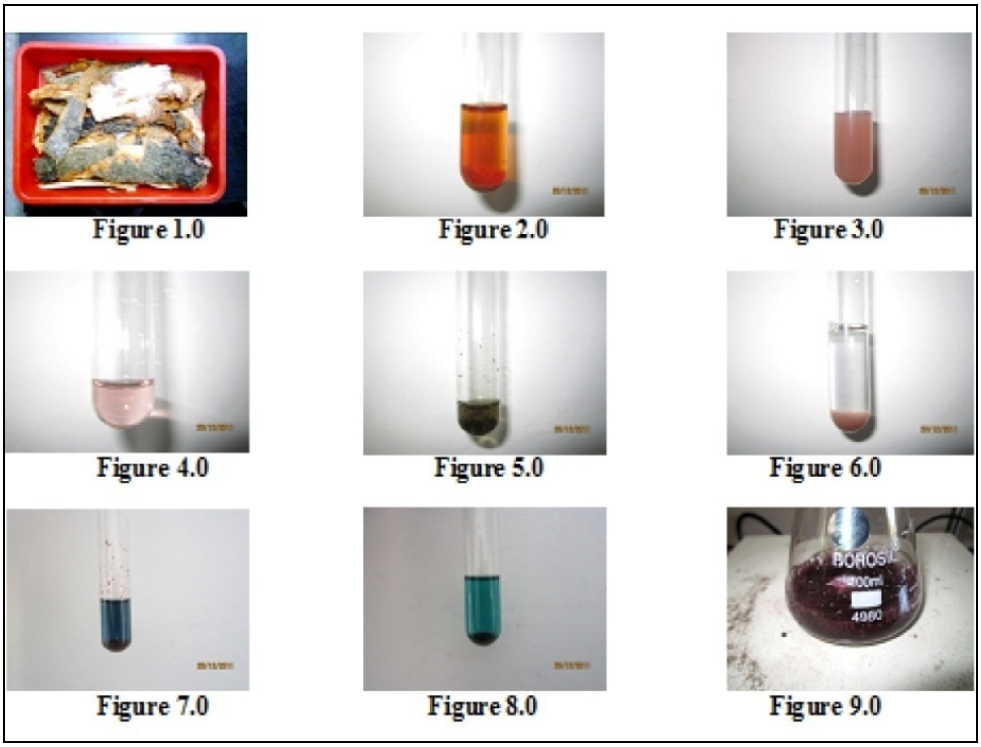

Figure 10.0: Freshly collected bark pieces of *Toona ciliata*; Figure 1.0, Results of phytochemical tests; Figure 2.0-8.0 and Synthesised Gold nanoparticles; Figure 9.0

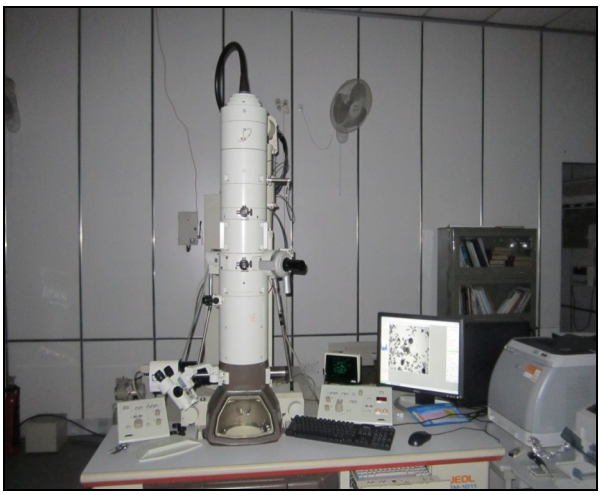

Figure 11.0: Transmission Electron Microscope (Model: JEM 1011, JEOL Corporation, Japan)

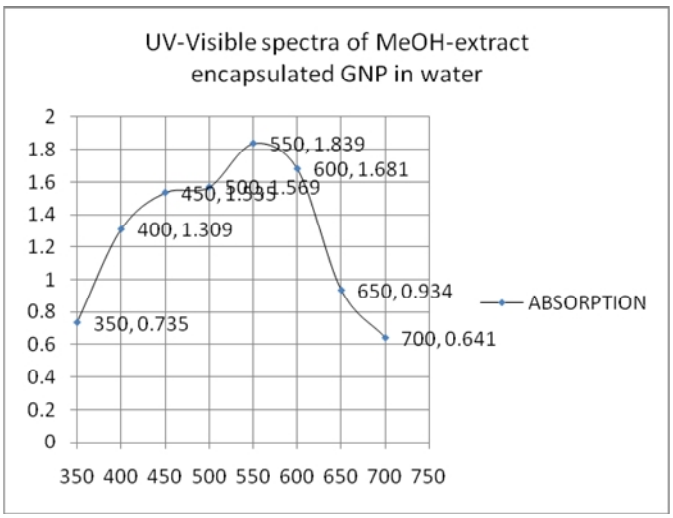

Figure 12.0: UV-Visible spectrum of gold nanoparticles synthesised by using MeOH extract from *Toona ciliata* bark showing the surface plasmon band at 550 nm

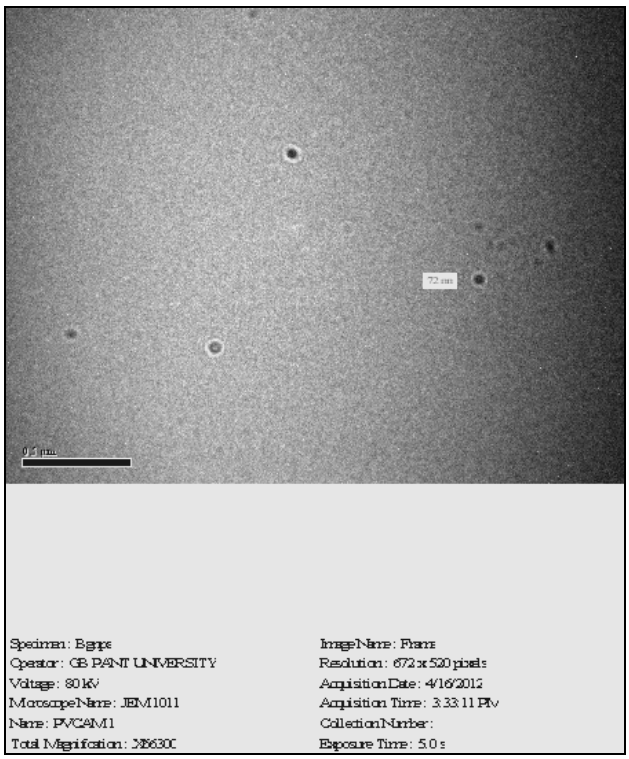

Figure 13.0: TEM micrograph of gold nanoparticles synthesised by using MeOH extract from *Toona ciliata* bark

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