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GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM PROPOLIS

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ABSTRACT: Development of biologically inspired experimental processes for the synthesis of NPs is evolving into an important branch of nanotechnology. The present study deals with the synthesis of AgNPs using the propolis aqueous extract. The complete reduction of Ag^+ ions was observed after 30 min. of reaction. The colour changes in reaction mixture (light yellow to dark brown colour) was observed during the incubation period, because of the formation of AgNPs in the reaction mixture enables to produce particular colour due to their specific properties (Surface Plasmon Resonance). The formation of AgNPs was confirmed by UV-Vis spectroscopy, XRD pattern, TEM, SEM with EDX, AFM, DLS and TG-DTA. The synthesized silver nanoparticles were predominately spherical in shape, polydispersed and ranged below 100 nm *i.e.* (9 to 30 nm). FT-IR spectroscopy analysis showed that the synthesized AgNPs are capped with biomolecule compounds which are responsible for reduction of Ag^+ ions. The approach of propolis-mediated synthesis appears to be cost efficient, eco-friendly and easy alternative to conventional methods of AgNPs synthesis.

KEYWORDS: Bioreduction, Propolis and AgNPs

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1.INTRODUCTION

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shape and controlled dispersity. With the development of new chemical or physical methods, the concern for environmental contaminations are also heightened as the chemical procedures involved in the synthesis of nanoparticles generate a large amount of

hazardous byproducts. Thus, there is a need for green chemistry that includes a clean, non toxic and environment friendly method of nanoparticles synthesis [1]. As a result, researchers in the field of nanoparticle synthesis and assembly have turned to biological system of inspiration. Many microorganisms, both unicellular and multicellular, are known to produce inorganic materials either intra or extra cellularly often of nanoscale dimension and of exquisite morphology and hierarchical assembly [2]. Some well known examples of microorganisms synthesizing inorganic materials include magnetotatic bacteria for magnetite nanoparticles [3]. Eukaryotic organisms such as fungi may be used to grow nanoparticles of different chemical composition and size. A number of different genera of fungi have been investigated in this effort and it has been shown that fungi are extremely good candidates in the synthesis of AgNPs [4], [5]. Biosynthesis of nanoparticles by plant extracts is currently under exploitation. Use of plants for synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as this eliminates the elaborate process of maintaining cell culture. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extracelluarly using plants or their extracts in a controlled manner according to their size, shape and dispersity [6]. However, there is still need for economic, commercially viable as well as environmentally clean synthesis route to synthesize nanoparticles. A number of approaches are available for the synthesis of AgNPs. Among the various known synthesis methods, plant-mediated nanoparticle synthesis is preferred as it is rapid, cost-effective, eco-friendly, and safe for human therapeutic uses. Innumerous studies from across the world have demonstrated that propolis possess a number of biological activities beneficial for human health, including antimicrobial, cytotoxic and antitumor activities [7], [8], [9], [10], [11], [12], [13], [14], [15], [16], [17], [18], [19], [20]. Here in, to the best of our knowledge, we report for the first time synthesis of AgNPs, reducing the Ag ions by the aqueous extract of propolis. Therefore the present study was aimed to investigate the green synthesis AgNPs of propolis.

2. MATERIALS AND METHODS

2.1. Purchase of Pure Propolis

Commercially available pure Propolis powder (Stakich organic) was purchased from Bloomfield Hills, MI 48303, USA.

2.2. Preparation of Propolis Aqueous Extract

Exactly, 20 g of Propolis powder was mixed with 200 ml of distilled water and kept in a water bath at 60ºC for 10 min. The extract was filtered through Whatman No. 1. Filter paper and the filtered extract were stored at 4ºC for further use.

2.3. Synthesis of AgNPs

Biological synthesis of AgNPs was carried out [21].

2.4. Qualitative Phytochemical Analysis

Preliminary qualitative phytochemical tests were held out for aqueous extract of propolis to identify different phyto-constituents [22].

2.5. Characterization of AgNPs

The synthesized AgNPs were characterized by the following methods:

Synthesis of AgNPs was confirmed by UV-Visible spectroscopy. UV-Visible spectra were recorded as a function of the reaction time on PG Instruments spectroscopy. The studies on size, morphology and composition of the NP were performed by means of Transmission Electron Microscopy (TEM) (PHILIPS TECNAI 10) and Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX) (Carl Zeiss MA15/EVO 18). The purified AgNPs were examined for the presence of biomolecules using FT-IR analysis. Briefly, the spectrum obtained from the dried sample was recorded on FT-IR spectrum (Perkin-Elmer, USA) in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. Particle size analysis was done by dynamic light scattering (DLS) (Zetasizer Ver. 6.20, Malvern, MAL 1062727, UK). Crystalline AgNPs were determined by X-ray diffraction analysis (XRD). Briefly, the biosynthesized AgNPs was laid onto glass substrates on Phillips PW 1830 instrument operating at a voltage of 40 KV and current of 30 MA with Cu Kα1 radiation. Thermal stability of NP was done by thermal gravity-differential thermal analysis (TG-DTA) (NETZSCH STA 409 PC/PG).

3. RESULTS AND DISCUSSION

The phytochemical analysis of aqueous extract revealed the presence of triterpenoids, flavonoids, furanoids, sugars, coumarins, quinines, tannins, phenols and acids. Cardiac Glycosides and flavonoids were absent in the aqueous extract are summarized in the Table 1. Alkaloids are naturally occurring chemical compounds and have a wide range of pharmacological activities including anti-malaria, anti-asthma, anti-cancer, analgesic and anti-bacterial found to be used in traditional and modern medicine [23]. Indian propolis contains aminoacids, ketones, volatile oils and vitamins (ascorbic acid and coumatetralylin isomers) they are beneficial in treating cold, heart diseases and strengthening immune systems in human beings [24]. Presence of much more group of phytochemicals diversity which gives synergic effects in many biological applications [25].Evaluating the synthesis of AgNPs at different hours showed intense elevated brown colour at 30 min. from light yellow color of the aqueous extract. In the present study, supporting the colour change spectral band peaks of corresponding hours confirmed the maximum synthesis at 50 min., showing the UV peak at 420 nm. When the colour change was compared among AgNPs and aqueous extract, there was not that much change in the peak of the latter and the colour also remained same during different time intervals of UV observation. The colour formation in $AgNO_3$ solution indicates the formation of AgNPs with reduction of Ag^+ ions (Fig. 1 and Fig. 2).

Table 1: Qualatative phytochemical analysis of propolis aqueous extract

Phytochemicals	Present(+)/Absent (-)
Acids	+
Alkaloids	-
Coumarins	+
Flavonoids	+
Furanoids	-
Phenols	+
Quinones	+
Saponins	-
Steroids	-
Sugars	+
Tannins	+
Triterpenoids	+

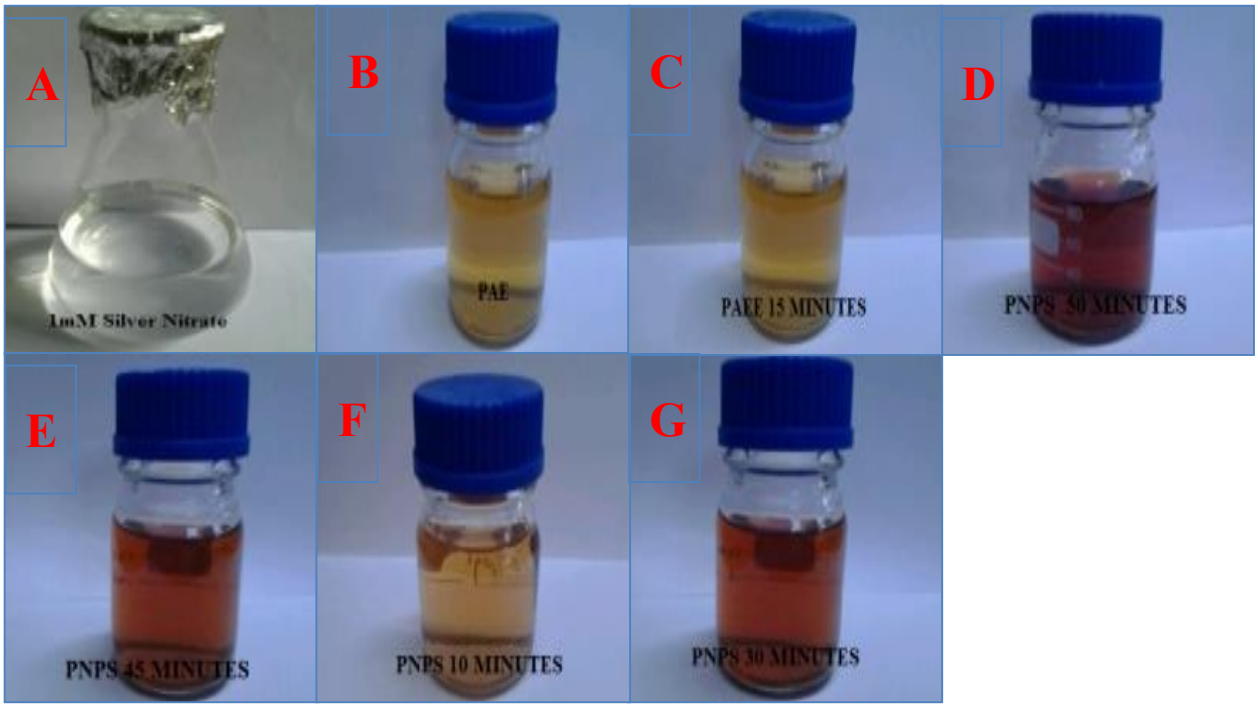

Fig. 1: A- Colour intensity of 1mM silver nitrate solution (colourless), B-C Colour intensity of propolis aqueous extract (light yellow colour), D-G Colour intensity of synthesized AgNPs of propolis at different time intervals (dark brown colour).

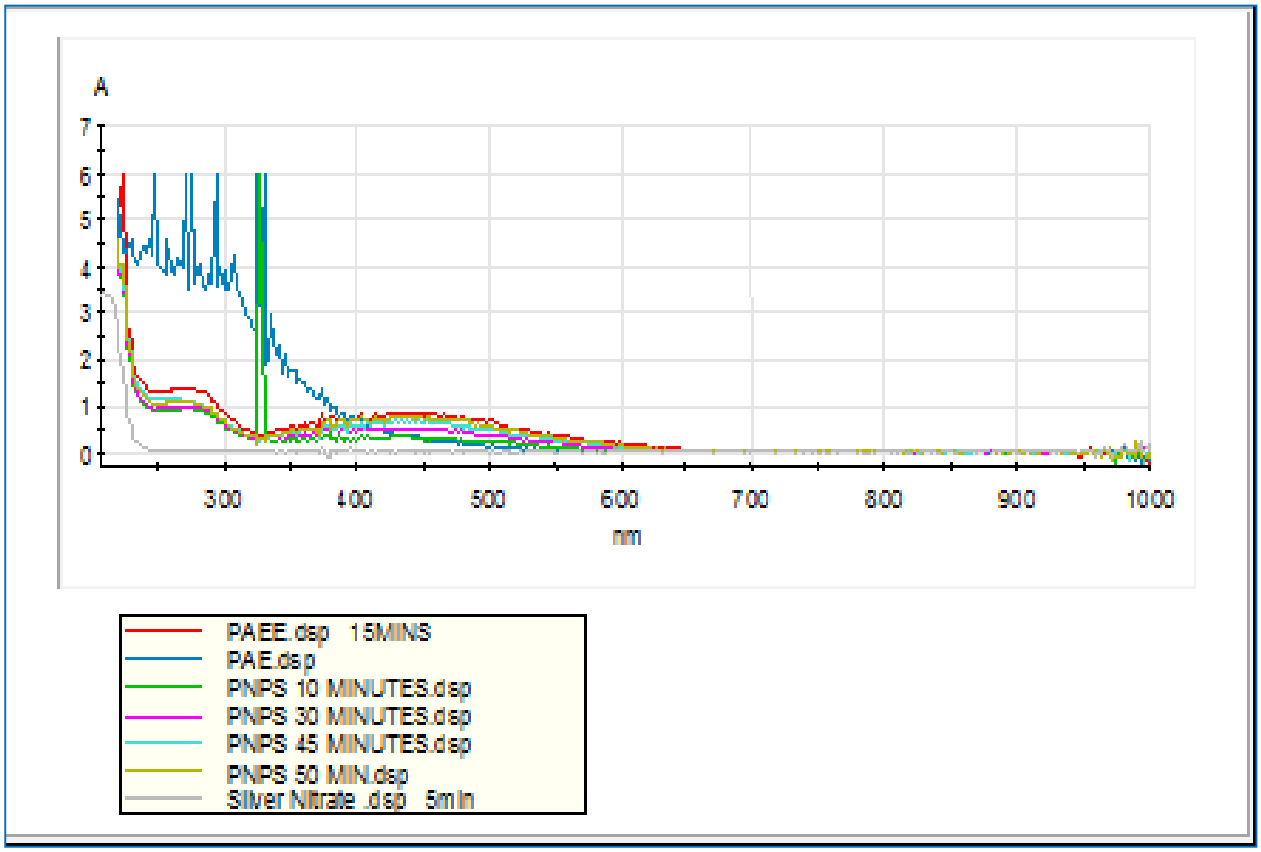

Fig. 2: UV-spectra of synthesized AgNPs of propolis

Therefore, absorption band at 420 nm is merely due to small spherical nanoparticle formation. The colour exhibited by metallic nanoparticles is due to the coherent excitation of all the "free" electrons within the conduction band, leading to an in-phase oscillation which is known as Surface Plasmon Resonance (SPR) [26]. The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium [27]. In many other cases, like AgNPs synthesized plant extracts of *Cinnamomum camphora* [28], *Acacia arabica* [29], *Erythrina indica* [30], *Piper longum* [31], *Origanum vulgare* [32], *Sargassum wightii* [33], *Vitex negundo* [34], [35] *Citrus limon* [36], the absorbance peaks were between 400 and 450 nm. When compared with these plants, AgNPs synthesized from aqueous extract of propolis were active at relatively lower wavelength. According to Rai (2009), [37] the deviation from spherical symmetry leads to a broadening and red shift of longitudinal plasmon resonance along with the appearance of transverse plasmon resonance.The morphology and size of the particles were determined by TEM. The particles are spherical and well dispersed in nature and no agglomeration was noticed. The particle size was below 100 nm *i.e.* (9 to 30 nm) (Fig. 3). SEM image showed high density of AgNPs synthesized by propolis aqueous extract, which was further confirmed by EDX (Fig.4A and B).

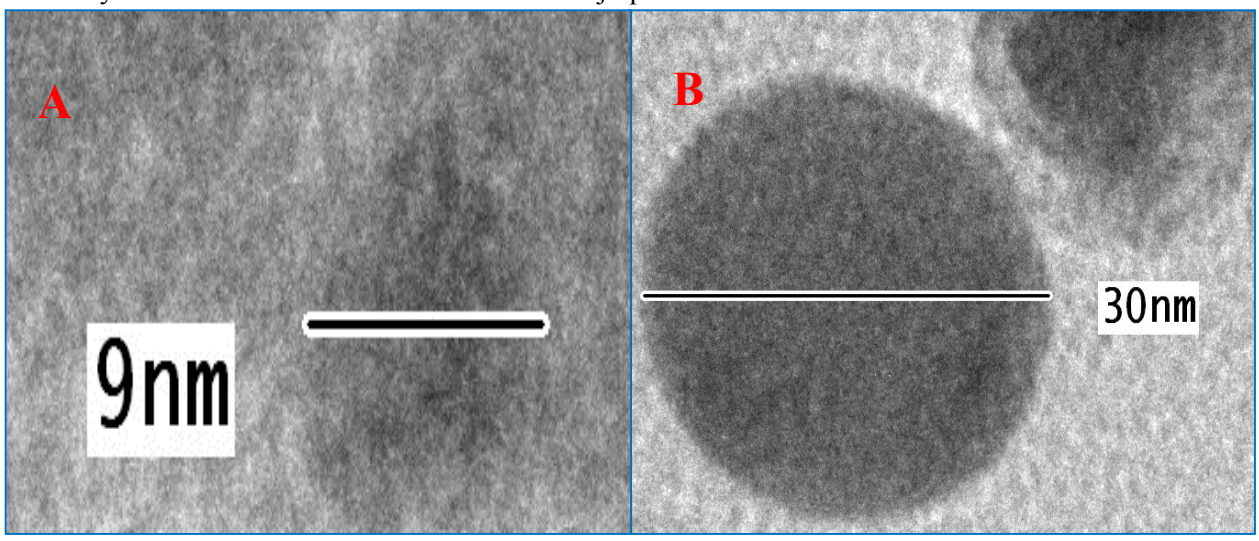

Fig. 3: A and B TEM micrographic images of synthesized AgNPs of propolis

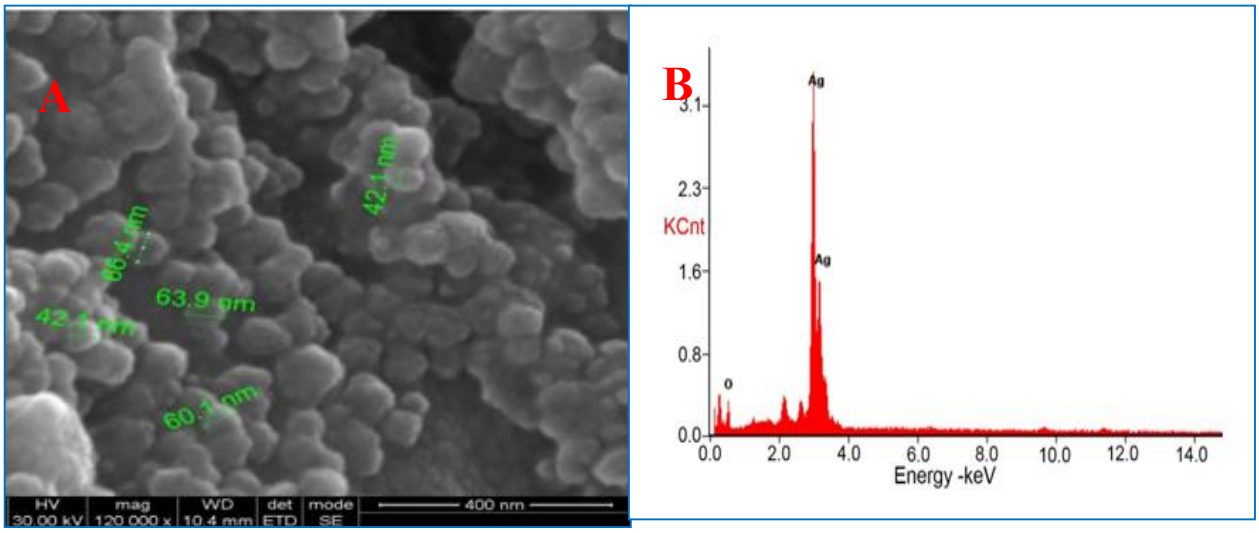

Fig. 4: A- SEM micrographic image of synthesized AgNPs of propolis and B- EDX spectroscopy displays the chemical composition of the AgNPs of propolis

Nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent *i.e.* proteins secreted by plant extracts. The presence of secondary metabolities capping with the AgNPs may be assigned to bio-organic compounds from the plant extracts [38].EDX spectrum confirms the presence of strong elemental signal of the Ag approximately at 3KeV which is typical for the absorption of metallic Ag due to SPR which was observed in biosynthesis of nanocrystals of *Bacillus licheniformis*. From the EDX spectrum, it is pertinent to note that O and Ag elements were present uniformly throughout the sample in homogeneous manner. Identification lines for major emission energies of Ag were displayed in EDX and are in accordance with peaks in the spectrum. Along with Ag peak and O peak were appeared with lower intensity due to the presence of biomolecules of propolis aqueous extract and which are bound to the surface of the AgNPs resulting in the NPs formation [39].Biosynthesized AgNPs were found to be photoluminescent at room temperature in *Aloe vera* and edible mushroom The visible

luminescence of Ag is due to the excitation of electrons from occupied d bands into states above the Fermi level then, electron-photon and hole-photon scattering process takes place results in energy loss and finally photoluminescent radioactive recombination of an electron from an occupied sp band with the hole [40], [41].DLS analysis showed the size distribution of particles with 59.53 width (d.nm), 100.0% intensity and with the polydisperily index (PdI) of 0.165. As expected, the particle size obtained from TEM and DLS scattering is marginally different due to the varying principles used for measurement (Fig.5).

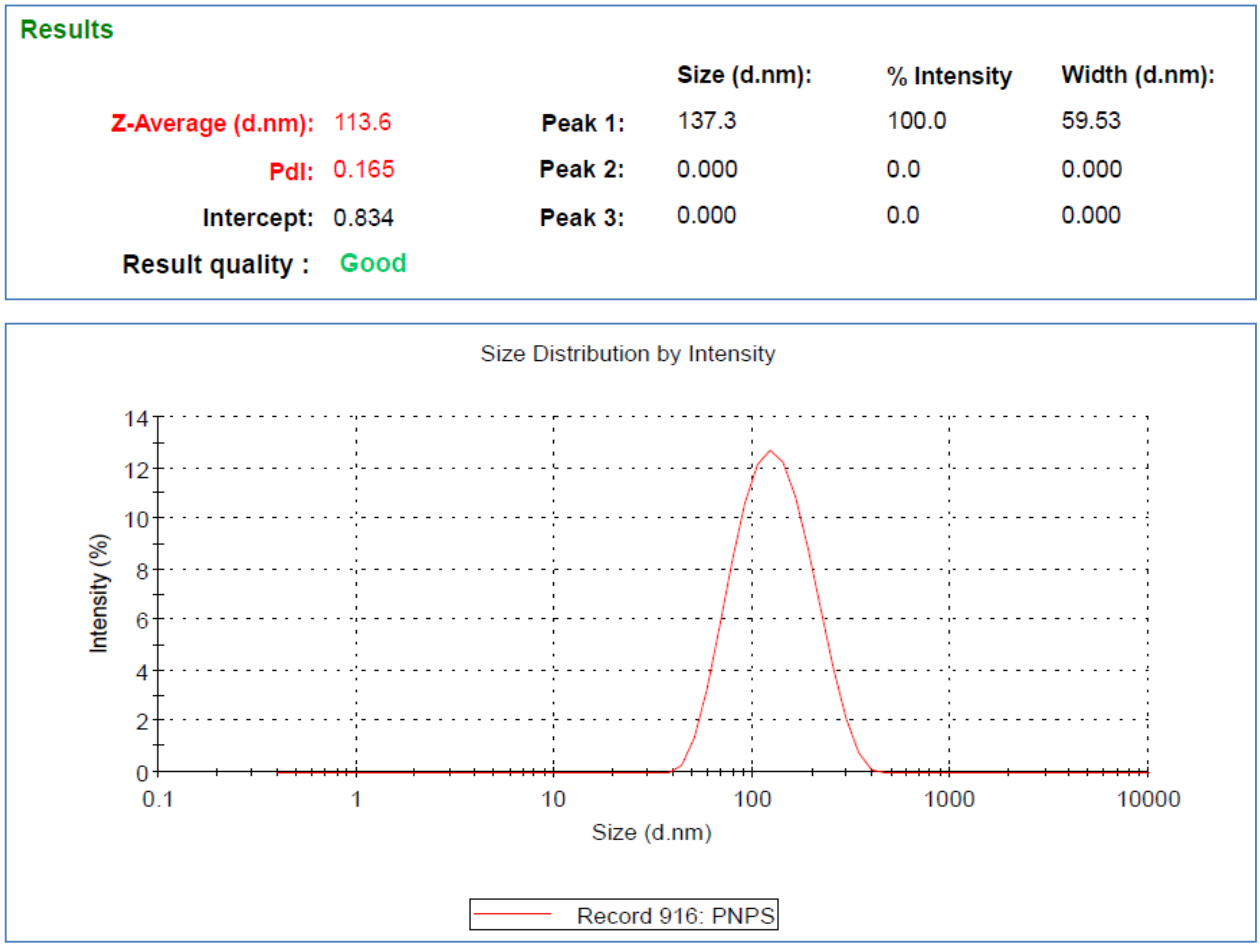

Fig. 5: DLS analysis of synthesized AgNPs of propolis

The AgNPs crystalline structure and size were further characterized by XRD, in which the XRD patterns of AgNPs exhibited several size-dependent features leading to peak position, heights and widths. The XRD spectrum showed the characteristic Bragg peaks at 38°, 44.5°, 64.7° and 77.7° can be indexed to the 111, 200, 220 and 311 reflections of FCC (face centered cubic) structure of metallic Ag, respectively revealing that the synthesized AgNPs are composed of pure Ag. The peak corresponding to 111 is more intense than the other planes suggesting that 111 is the predominant orientation and the synthesized AgNP are crystalline in nature. The XRD results of the present study clearly show that AgNPs formed by the reduction of Ag^{+} ions by propolis aqueous extract are crystalline in nature (Fig. 6).

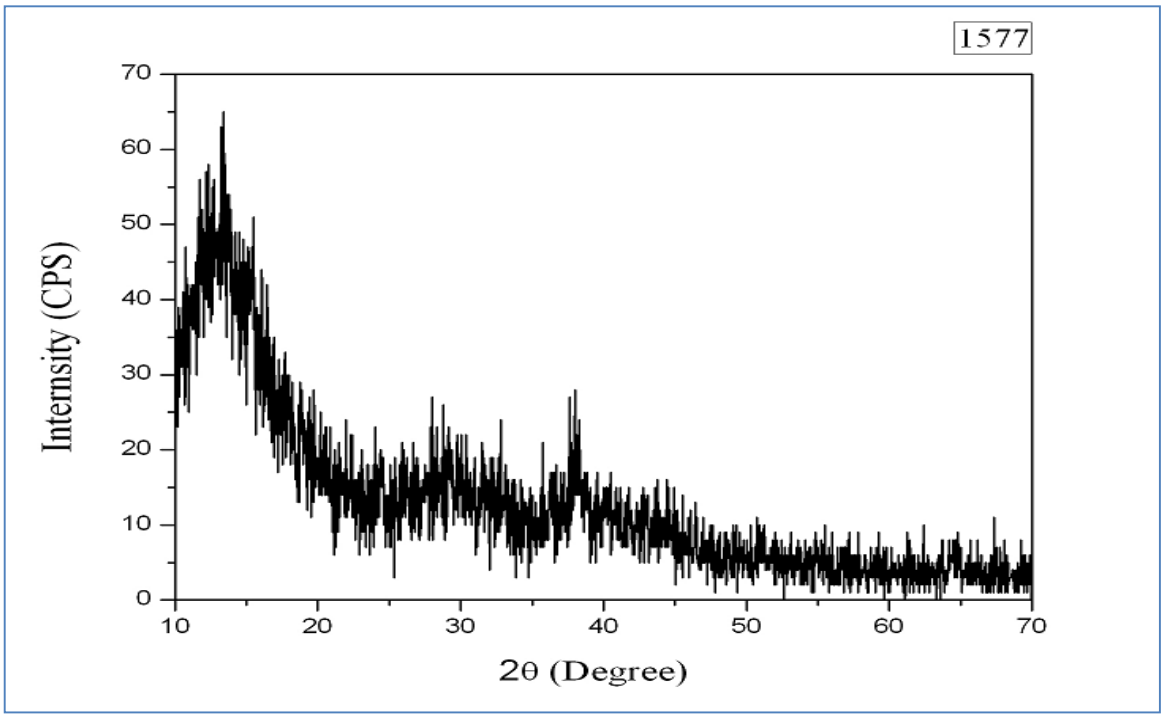

Fig. 6: XRD pattern of synthesized AgNPs of propolis

FT-IR measurement was carried out to identify possible biomolecules responsible for the reduction of Ag^+ ions and capping agent for bio-reduction of AgNPs by propolis aqueous extract. The FT-IR spectrum of the propolis aqueous extract without $AgNO_3$ showed the presence of carboxylic group with the intensive peak at 1716 and 1100 cm^{-1} (Fig.7 and Fig. 8). Most of the FT-IR bands are characteristic of triterpenoids, flavonoids, furanoids, sugars, coumarins, quinines, tannins, phenols and acids present in the aqueous extract of propolis. These records indicate the presence of Ag^+ ions formed due to bioreduction and their possible capping and stabilization as AgNPs. Likewise, results obtained from AFM study represent a clear concept regarding shape of the NPs (Fig.9).

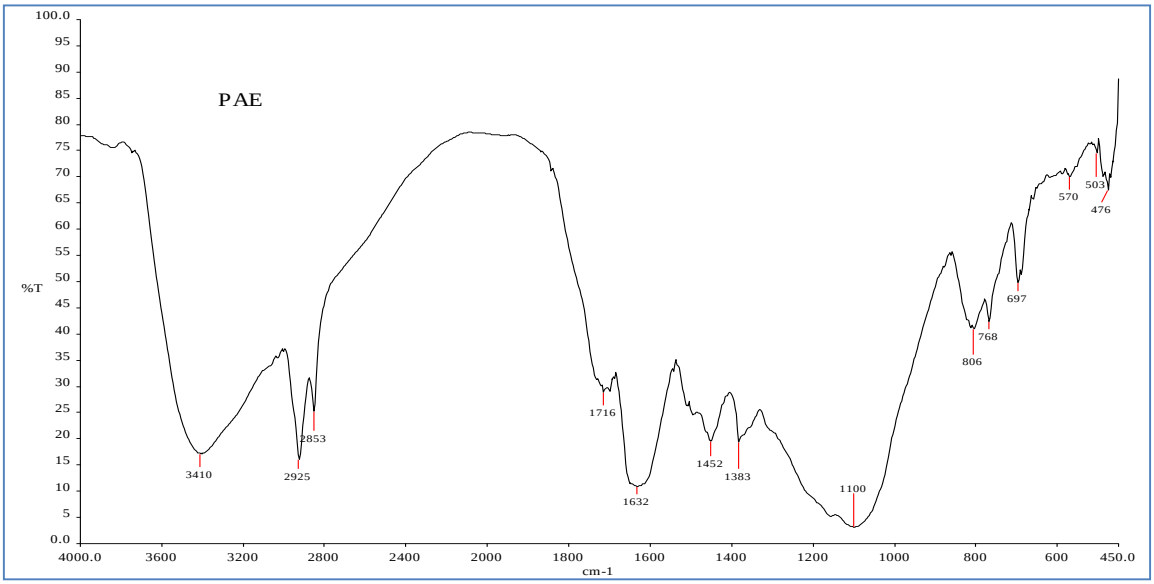

Fig. 7: FT-IR spectra of propolis aqueous extract

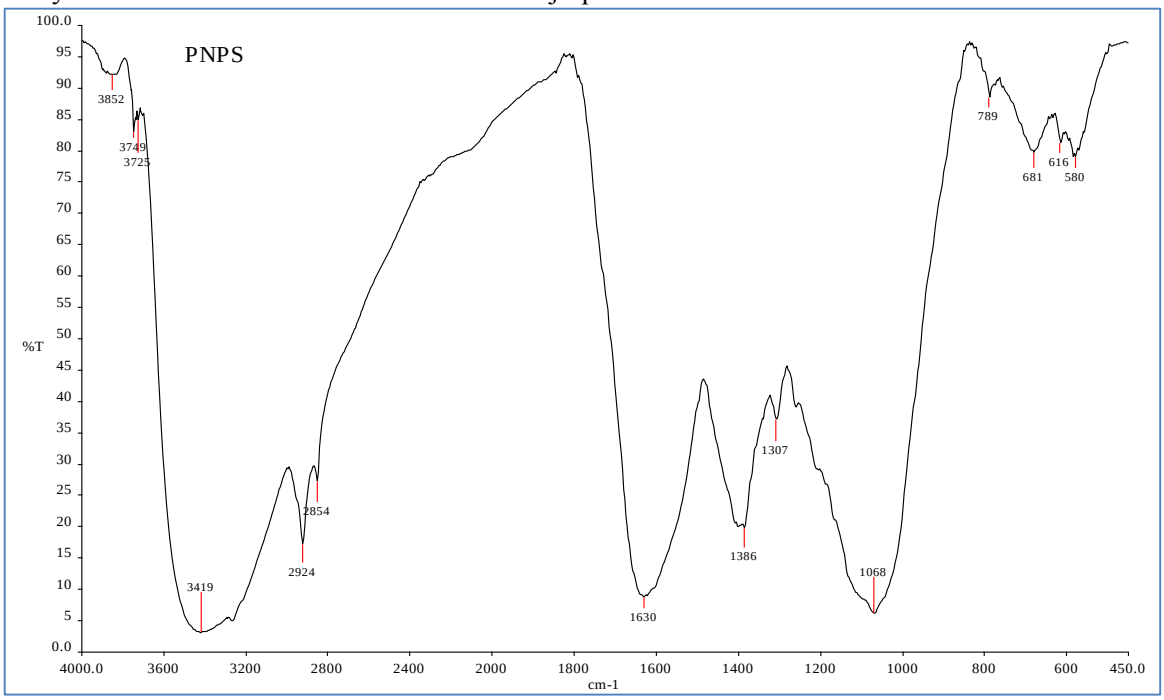

Fig. 8: FT-IR spectra of synthesized AgNPs of propolis

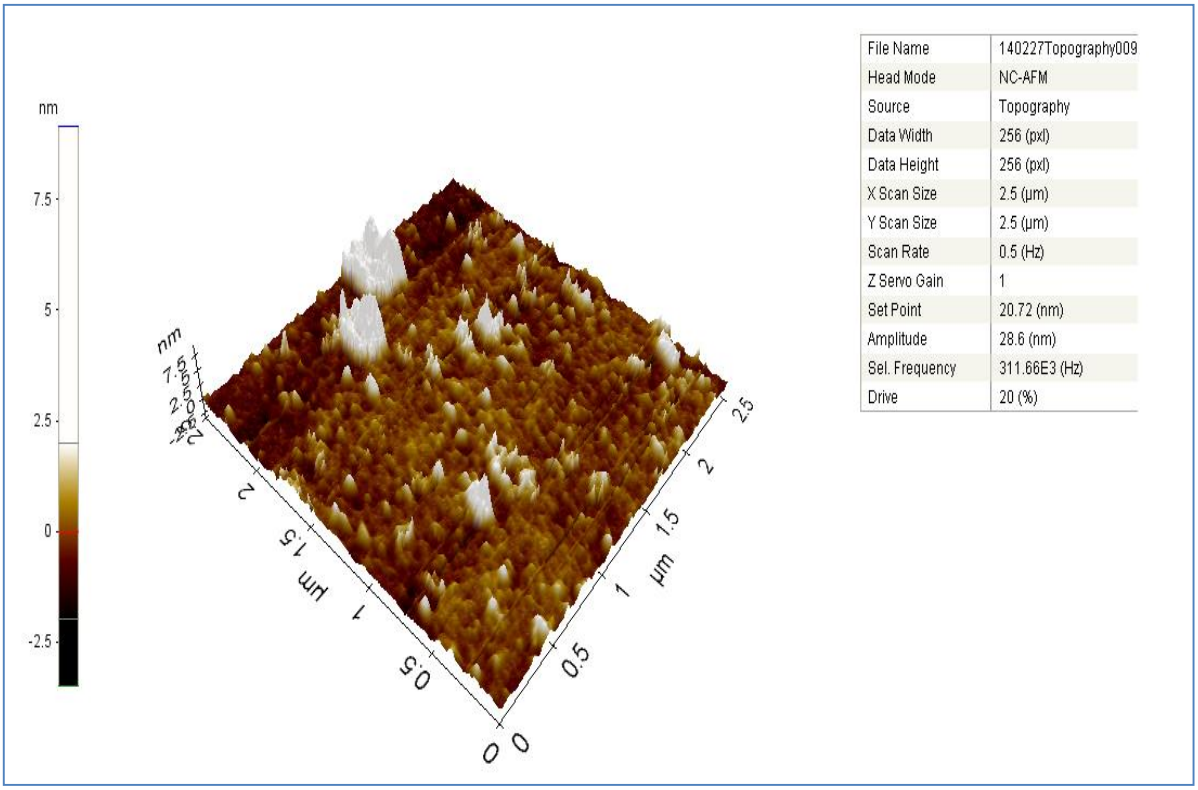

Fig. 9: AFM micrographic image of synthesized AgNPs of propolis

The particles were spherical in shape and well distributed. Gunaskaran and Ponnusamy (2005) [42] stated that phenols, in plane bending vibration of hydroxyl groups are always centered at 1227 cm^{-1}. Similar observations were observed in *Cassia auriculata*[43], *Morganella species*[44], and *Sargassum wightii*[33]. Biomolecules could possibly perform dual functions of formation and stabilization of AgNPs in the aqueous medium [45]. Hence, it may be considered that these

biomolecules apart from reduction process, found to form a layer covering the AgNPs to avoid agglomeration and thereby AgNPs stabilization.The TG-DTA curve of $AgNO_3$ and biosynthesized AgNPs was 23.652 mg and final mass left out after the experiment was only 64.53% (599.4°C) indicating the presence of metallic nature of $AgNO_3$, whereas AgNPs showed 35.47% (599.4°C) of the initial mass at a temperature of about 600°C indicating that bulk decomposition occurred in the sample. The biosynthesized AgNPs reached 98°C and the weight loss was up to 35%, which is basically due to vapour or water being released. The TG-DTA analysis of AgNPs revealed that NPs were thermally stable at least at 90°C in a nitrogen atmosphere (Fig.10).

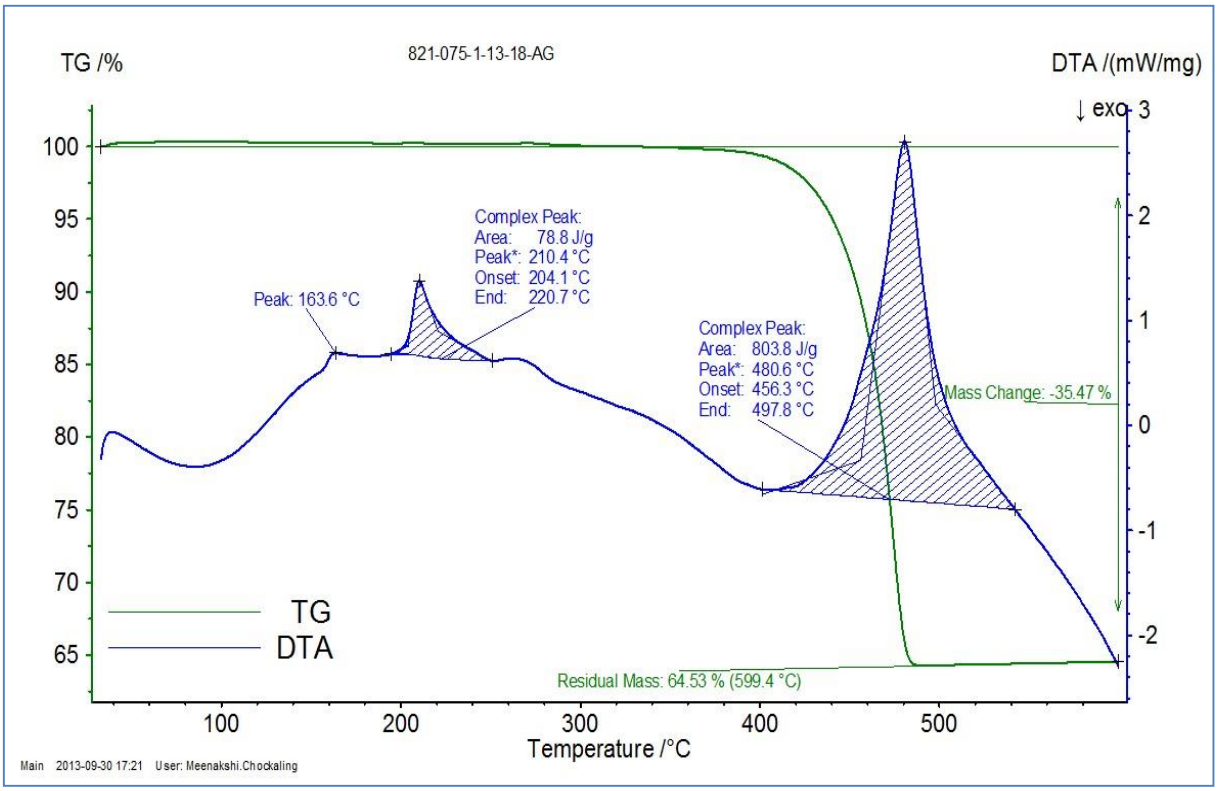

Fig. 10: TG-DTA analysis of synthesized AgNPs of propolis

Peak at around 98°C is attributed to desorption of water and the exothermic peak at 280°C confirms the release of residual chemisorbed water and the release of organic residues [46]. Weight loss of the AgNPs could be due to the thermal degradation of the organic substance as well the organic substance capping around the nanoparticles [47]. Similar observations were observed in the *Lactobacillus plantarum* exopolysaccharide [48].

4. CONCLUSION

In conclusion, the bioreduction of aqueous Ag ions by the aqueous extract of the propolishas been demonstrated. The reduction of the metal ions through propolis aqueous extract leading to the formation of AgNPs extracellularly and the synthesized nanoparticles are quite stable in solution. The control of shape and size of AgNPs seems to be easy with the use of propolis aqueous extract. The synthetic methods based on naturally occurring biomaterials provide an alternative means for

obtaining the nanoparticles. Use of propolis in synthesis of nanoparticles is quite novel leading to truly 'green chemistry' route. This green chemistry approach towards the synthesis of nanoparticles has many advantages such as, process scaling up, economic viability and safe way to produce nanoparticles and this bioreduced AgNPs can used as a therapeutic agent to cure many dreadful diseases of human beings.

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6. CONFLICT OF INTEREST

The authors declare no conflicts of interest

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