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SYNTHESIS OF VARIABLE SHAPED GOLD NANOPARTICLES IN ONE SOLUTION USING LEAF EXTRACT OF *BAUHINIA VARIEGATA* L.

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We report green, rapid and extracellular synthesis of polyshaped (i.e. triangular, pentagons, hexagonal, and spherical) gold nanoparticles (GNPs) using *Bauhinia variegata* leaf extract (LE). Stable GNPs of various size and shape were synthesized by varying reaction conditions. AFM and TEM analysis revealed 50 nm plates like triangular, hexagonal and polygonal GNPs in addition to spherical ones. Most of NPs synthesis was over in 60 minutes. The size of synthesised GNPs can be varied between 43-145 nm by simply manipulating reaction conditions. Incubation temperature and LE ratio has been found effective in controlling shape of GNPs. Higher temperature (80^{o}C) and LE ratio at 1 mM basic metal ion concentration leads to the synthesis of spherical shaped GNP. Due to variable shape, stability and green method for their synthesis, these GNP could be very useful in targeted drug delivery, biosensor development and other applications where products are in direct human contact.

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Keywords: Gold nanoparticles; *Bauhinia variegata;* leaf extract; polyshaped; green method

1. Introduction

Gold nanoparticles (GNPs) have applications in therapeutics [1, 2], diagnostics and drug delivery [3-6]. GNP has been reported to be synthesized by chemical reduction and physical methods. However, control over size and shape has been a challenge with these methods. GNP with high stability has been demanded in various detection systems involving DNA-GNP conjugates [7]. Therefore, green chemistry based procedures for synthesis of stable GNP of exact size and shape could be of great use. Various green synthesis procedures have been documented in literature. Plant LE mediated synthesis has been preferred over other biological methods due to eco-friendly nature as well as cost and time effectiveness [8, 9]. For NP synthesis, mixing of the reagents in appropriate proportions at well defined conditions is required. But reactants concentration and reaction temperature has been found to determine the ultimate morphology of GNP [10]. These morphologies have own benefits in context of different applications. Polyshaped NPs can easily pass through animal cells due to their differential shape. This property can make them potent future drug delivery agents [11, 12]. Till date exact mechanism involving the synthesis of metallic NPs from plant extract is not fully understood. So, reaction conditions and compositions are used as a tool to vary size and shape of GNP. Size, shape as well as stability of biosynthesized GNP also depend upon the nature of plant used. Different plants have been documented for GNP synthesis [9,13, and 14]. Our recent study has shown good potential of *B. variegata* LE for silver NPs synthesis [15]. Keeping this in view, here we have synthesized stable GNPs using *B. variegata* LE. Fast and eco-friendly green synthesis method developed using *B.*

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variegata LE led to the synthesis of polyshaped GNPs. Importantly, most of the GNP synthesis was over in 60 minutes.

2. Materials and Methods

2.1. Materials

The $HAuCl_4$ was purchased from Sigma-Aldrich, USA. All other chemicals used in the study were of analytical grade. *Bauhinia variegata* leaves were collected from campus of IHBT, Palampur.

2.2. GNP synthesis by B. variegata LE and characterization

B. variegata LE was prepared as described in our previous study for silver NPs synthesis.[15] Briefly fresh *B. variegata* leaves were thoroughly washed, dried, and grinded to make fine powder. The 4 g dry leaf powder was suspended in deionised Milli-Q water, vigorously vortexed and incubated overnight at room temperature. This suspension was centrifuged and the filtered solution of LE was used for further study.

For GNP synthesis, 1 mM aqueous $HAuCl_4$ solution was incubated with 10% (*v/v*) of LE for 4 h at room temperature. The change in colour of solution and increase in absorbance near 550 nm with incubation time indicated the synthesis of GNP. The reaction suspension was centrifuged at 10,000 rpm for 5 min to purify the GNP. The pellet containing GNP was washed thrice with water and stored as lyophilized powder. GNP synthesis was confirmed by UV-visible spectroscopy. The absorption spectra for each reaction mixture were recorded on ND-1000 Nanodrop between 300 to 700 nm. The 2 µl of solution was evaluated for GNP synthesis at different incubation time intervals (0, 30, 60, 90, 120, 240 min).

Purified GNP was characterized for their morphology using Hitachi S-3400N scanning electron microscope (SEM), Veeco diNanoscope 3D atomic force microscope (AFM) and FEI Techni G^2 200 KV transmission electron microscope (TEM). Clear, well dispersed water suspended GNP solution was placed on to the carbon tape mounted on aluminium stub for SEM imaging. The samples were dried at room temperature in control environment. The images were captured on SEM mode at desired magnification. For AFM analysis, GNP suspension was spread on glass cover slip mounted on AFM stub. Samples were dried at room temperature. The images were obtained in tapping mode using silicon probe cantilever of 115-135 µm length, resonance frequency of 250-292 kHz, spring constant of 20-80 N/m. The scan rate used was 1Hz. A minimum of three images for each sample were obtained with AFM and analysed to assure reproducible results. For TEM analysis, well dispersed GNP suspension was mounted on carbon-coated copper TEM grids by pipeting one drop. Extra suspension was removed using clean blotting paper and the grid was dried prior to measurement. The size and zeta potential/surface charge of NPs was measured by the eletrophoretic mobility of GNP at 25˚C, using a Zeta particle size analyzer (Nano ZS, Malvern).

2.3. Effect of metal ion concentration, incubation temperature and LE ratio on GNP Synthesis

To evaluate the effect of metal ion concentration, GNP synthesis was carried out by incubating 0.5-9 mM $HAuCl_4$ with 10% *B. variegata* LE at room temperature. GNP synthesis was confirmed by UV-visible spectroscopy. Effect of incubation temperature on GNP synthesis was checked by incubating 1 mM $HAuCl_4$ with 10% LE at 40, 60 and 80°C. Similarly, effect of LE ratio was carried out by incubating 1 mM $HAuCl_4$ with 5, 10, 15, 20, 40% *B. variegata* LE. UV-visible spectroscopy and SEM were used for synthesis and initial morphology analysis. Detail size and shape studies were performed on AFM.

2.4. FTIR analysis of GNP

To record FTIR spectra, GNPs were subjected on a Thermo Nicolet 6700 FTIR spectroscope (Thermo, USA). FTIR spectra of GNPs powder in KBr pellets were recorded to identify GNP-associated molecules. To obtain good signal to noise ratio, 256 scans of GNP were taken in the range 400-4000 cm^{-1} and the resolution was kept as 4.0 cm^{-1}.

3. Results and discussion

3.1. Characterization of GNP synthesized by B. variegata LE

It is now well known that different organic components present in various plant extracts direct the synthesis of various sized metallic NPs including GNP [9, 16, and 17**].** In this study, water soluble *B. variegata* LE was found to have the capability to synthesize GNP of various shapes in one solution. Spherical, triangular, hexagonal and polygonal shaped GNPs were synthesized by simply incubating 1 mM $HAuCl_4$ solution with 10% *B. variegata* LE for 4 h. Most of GNP synthesis was over in 60 minutes, however reaction was allowed to complete for 4 h as shown in Fig. 1a.

The shape and size of GNP is mainly determined by the constituents of plant LE, precursor metal ion concentration, reaction temperature and LE ratio [18, 19]. Effect of these parameters on size and shape of *B. variegata* LE synthesized GNP was investigated by SEM, AFM and TEM. In most of earlier plant LE mediated metallic NPs synthesis 1 mM metal ion has been used. In our previous studies on SNP and GNP synthesis using *S. cumini* and *B. variegata* 1 mM metal ion was found to be most effective [9, 14, and 18] Here for GNPs synthesis using *B. variegata* LE, again 1 mM metal ion was found to be most effective. Therefore, 1 mM metal ion was used to see the effect of other parameters on *B. variegata* LE mediated synthesis of GNP. GNP synthesized by incubating 1 mM metal ion with 10% LE was 50 nm in size and polyshaped (Figs. 1b, c, and d).

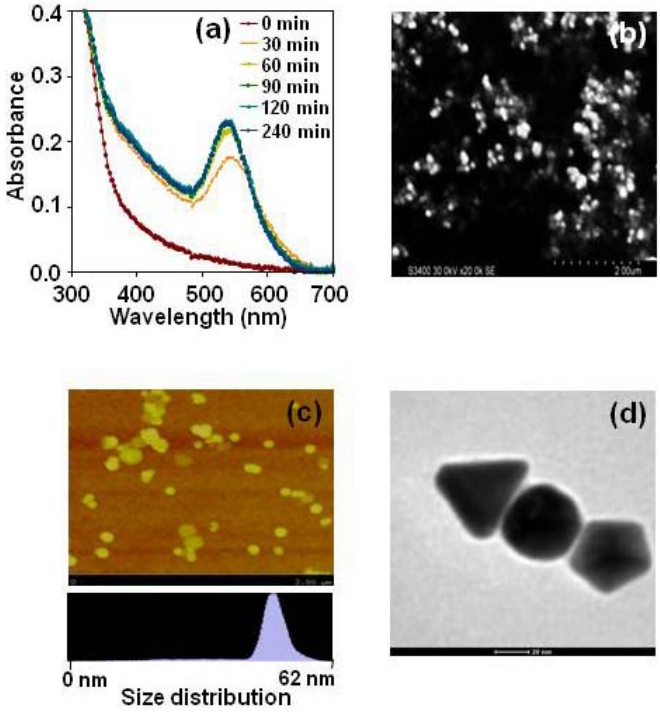

Fig. 1 Characterization of GNP synthesized by Bauhinia variegata LE: (a) UV-visible time scan of GNP synthesized using 10% LE and 1 mM metal ion at room temperature, (b) SEM, (c) AFM and (d) TEM images of GNP.

The spectrum of energy dispersive X-ray spectrometer (EDAX) attached with TEM documented chemical composition of NPs. Strong signals of Au in the spectrum confirmed the presence of gold atoms in the synthesised NPs (Fig. 2a). However some weak signals were also seen. These could be due to atoms of molecules attached to NPs surface [20]. The Cu peak was due to copper grid used in the analysis with TEM (Fig. 2a). Zeta particle size analysis revealed that synthesised GNP were 60 nm in size (Fig. 2b). Such difference in the size of NPs characterized with AFM and zeta particle size analyser is also reported in earlier study [21]. The zeta potential of GNPs was found to be -40 (Fig. 2c). This zeta potential of NPs has been documented for good stability [22]. Hence, indicated that GNPs synthesized using *B. variegata* LE had good stability.

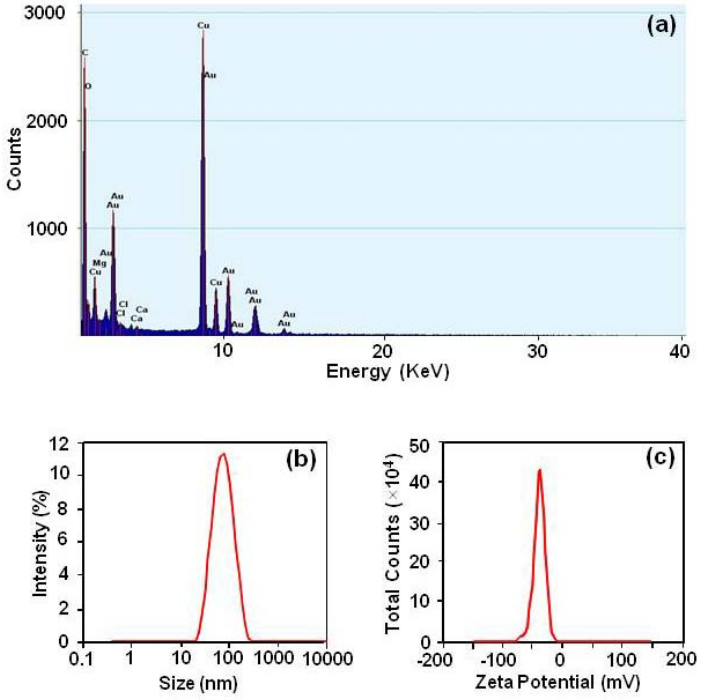

Fig. 2 Chemical composition and zeta potential of GNP synthesized using 10% LE and 1 mM metal ion at room temperature: (a) Energy dispersive X-ray (EDAX) spectrum of GNPs for analysis of chemical composition, (b) size and (c) zeta potential analyzed by zeta particle size analyzer.

3.2 Effect of metal ion concentration on GNP synthesis

In metallic NPs synthesis, precursor metal ions are reduced to small metal that combines to form metallic NPs in the presence of stabilizers from LE [16, 23, and 24]. With increase in incubation time, the reduction of $HAuCl_4$ into GNP was evident from change in the reaction mixture colour and appearance of GNP characteristic absorbance peak at 550 nm. These characteristics have also been documented in earlier studies [14, 25]. With increase in metal ion concentration from 0.5 mM to 1.5 mM in the reaction mixture, there was a quantitative increase in GNP synthesis. Further increase in metal ion concentration to 2 mM led to quantitative decrease in GNP synthesis. However, GNPs synthesized up to 2 mM metal ion concentration were stable. Increase in metal ion concentration above 2 mM caused complete aggregation as observed by disappearance of characteristic peak near 550 nm (Fig. 3a). The size of GNPs synthesized by 10% LE with 0.5-2.0 mM metal ion ($HAuCl_4$) was 46-145 nm (Fig. 3b, c, and e). More than 100 nm sized GNPs were synthesized with or above 1.5 mM metal ion concentrations. These GNPs were variously shaped like triangular, hexagonal and polygonal. Increase in metal ion concentration of the reaction mixture from 2 mM to 9 mM showed no synthesis. However, less than 0.5 mM metal

ion concentration also increased the size of GNPs. Interestingly, use of metal ion concentration above and below 1 mM increased GNPs size as well as level of aggregation.

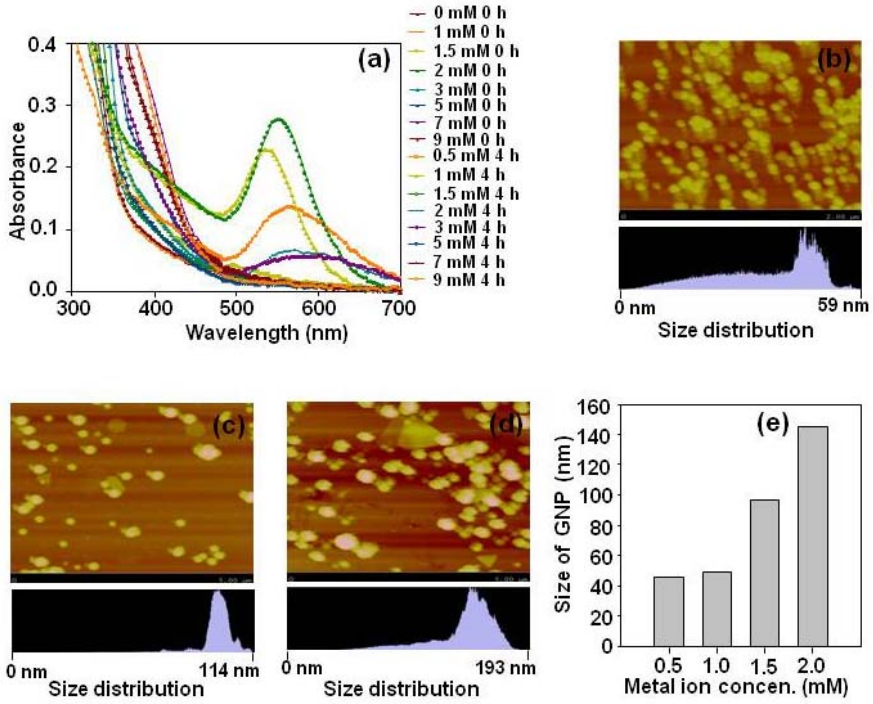

Fig. 3 Effect of varying metal ion concentrations on GNPs synthesis by B variegata LE: (a) UV-visible time scan of GNP synthesized using 10% LE and varying metal ion at room temperature. AFM images of GNP synthesized using: (b) 0.5 mM, (c) 1.5 mM, (d) 2.0 mM metal ion concentrations and (e) bar diagram presenting the size of GNP at different metal ion concentration.

3.3. Effect of incubation temperature on GNP synthesis

Increase in incubation temperature from 40 to 80°C for the reaction mixture also quantitatively increased the GNP synthesis as shown in Fig. 4a. Similar, quantitative increase in GNP synthesis with increase in reaction temperature has also been documented earlier [18]. Such increase has suggested that elevation in incubation temperature of reaction mixture increased the rate of GNP synthesis. However, increase in incubation temperature caused decrease in NPs size. With increase in incubation temperature from room temperature to 80°C, GNP size was decreased from 50 nm to 43 nm (Fig. 4b, c, d, and e). However, this trend differs from that observed with SNP synthesis by the same plant [15]. Decrease in GNP size may be due to increase in rate of reaction upon increase in incubation temperature. This is in agreement with earlier study [24] Song and Kim [24] also reported variations in shape of GNP with reaction temperature and suggested the influence of temperature on nucleation process of metallic nanoparticles. With increase in incubation temperature the reaction rate was enhanced. Hence most of gold ions get involved in the formation of nuclei. As most of gold ions are consumed in nuclei formation chances of secondary reduction of already formed nuclei are less [19, 26]. Therefore, various shaped GNP were observed at room temperature (Figs. 1c, and d) and at 40°C incubation temperature (Fig. 4b). While most of GNP tends to be spherical due to lesser secondary reduction at higher temperatures 60°C (Fig. 4c) and 80°C (Fig. 4d). Results documented that secondary nucleation was favoured at room temperature and up to 40°C.

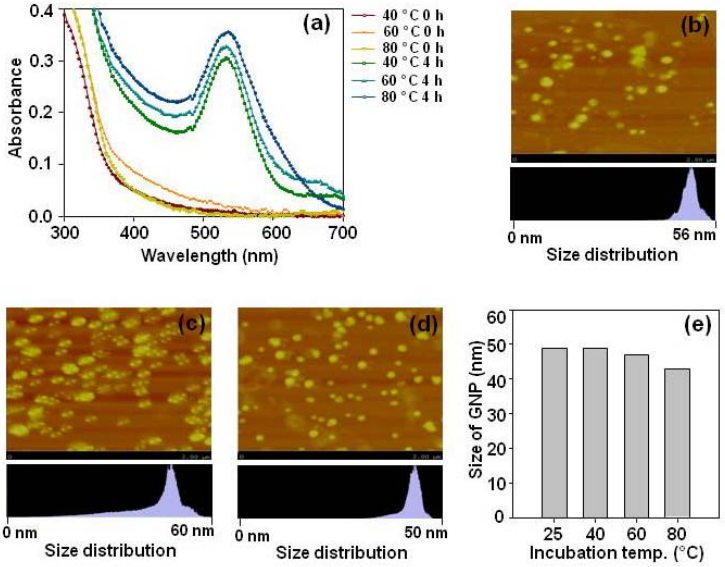

Fig. 4 Effect of varying incubation temperature on GNPs synthesis by B variegata LE: (a) UV-visible time scan of GNP synthesized using 10% LE and 1 mM metal ion at various temperatures. AFM images of GNP synthesized at (b) 40 °C, (c) 60 °C, (d) 80 °C incubation temperature and (e) bar diagram presenting the size of GNP at different incubation temperatures.

3.4. Effect of LE ratio on GNP synthesis

Use of increased LE ratio from 50 µl (5% LE ratio) to 200 µl (20% LE ratio) LE in the reaction mixture showed a quantitative increase in GNP synthesis. Further increase in LE ratio to 400 µl (or 40% LE ratio) caused aggregation and quantitative decrease in GNP synthesis as shown in Fig. 5a. Earlier studies have also shown quantitative increase in GNP synthesis with increase in LE ratio [18]. The control over NPs synthesis by LE ratio seems to be dependent on procedure of extract preparation and nature of plant used. Interestingly, similar aggregation was also observed in case of SNP synthesised using *B. variegata* LE [15]. The aggregation and dispersion of metallic NPs depends on mixing of metal ions with appropriate amount of reducing agents and stabilizer/capping agents. Use of any of these in inappropriate amount leads to either NPs aggregation or no synthesis [14, 24]. Notably, LE prepared by the method described here leads to stable GNP synthesis up to 200 µl LE (20% LE ratio) and above to this leads to aggregation. With increase in LE ratio in the reaction mixture from 100 µl (10% LE ratio) to 400 µl (40% LE ratio), the shape of GNP tends to be more spherical (Figs. 5b, c, d, and e). Above and below 100 µl LE (or 10% LE ratio) in reaction mixture leads to an increase in size of GNP (Fig. 5f). This shape observation is in agreement with earlier studies [19, 27]. However these studies have shown decrease in size with increase in LE ratio. We strongly believe that the method by which extract was prepared along with other parameters play a crucial role in determining the amount, quality, shape and size of synthesized GNPs.

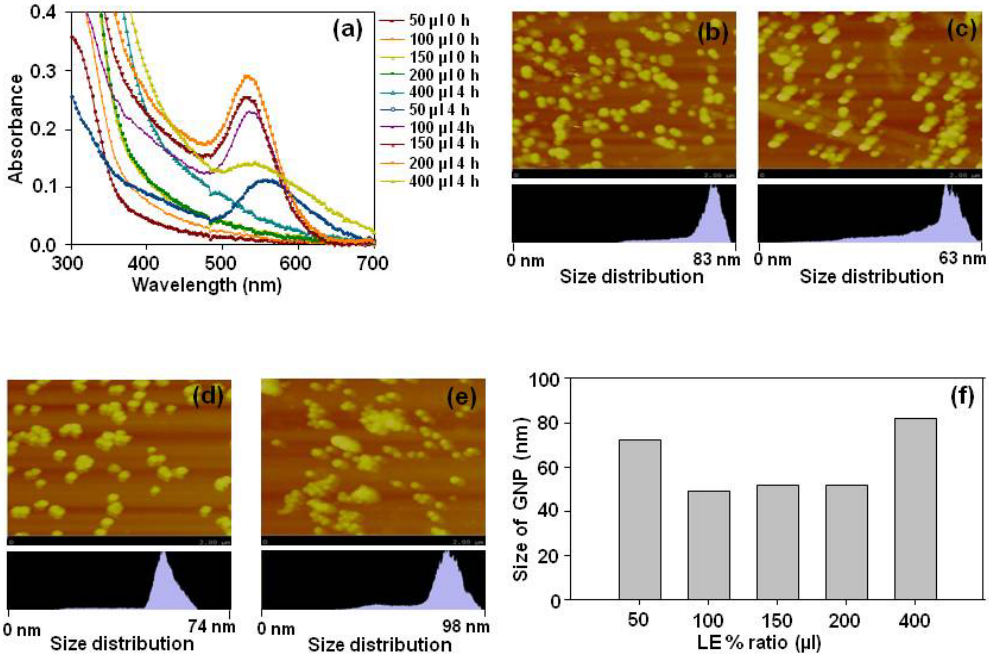

Fig. 5 Effect of varying LE ratio on GNPs synthesis by B variegata LE: (a) UV-visible time scan of GNP synthesized using various LE % ratio and 1 mM metal ion at room temperature. AFM images of GNP synthesized using: (b) 50 µl (5%), (c) 150 µl (15%), (d) 200 µl (20%), (e) 400 µl (40%) B. variegata LE ratio and (f) bar diagram presenting the size of GNP with different LE ratios.

3.5. FTIR analysis identified GNP associated molecules

B. variegata is rich in glycosides cardiac, saponins, sugars, tannins, flavanoids and insulin like proteins [28, 29]. During GNP synthesis, some of these molecules are involved in synthesis and stabilization. The molecules present in LE get attached or adsorbed to NPs surface. FTIR is used to identify such molecules [9, 14]. To identify GNP associated molecules, FTIR spectrum of GNP synthesized by incubating 1 mM metal ion with 10% LE was recorded (Fig. 6). The peaks at 615-667, ~876, 897-1162, 1644, 1259, 3401, 2918, 1432 and 1315-1380 cm^{-1} corresponds to C-H bending in alkynes, acidic pectins [30], carbohydrates [31], C=O stretching and contribution from C-N stretching or C=C groups/aromatic rings [18, 31], amide III [32], OH stretching alcohol/phenols or carbohydrates, C-H stretching in saponins [33], C-H bend/COO^- symmetric stretching due to the acidic group of polygalacturonic acid [30], and OH deformation vibrations in the aromatic ring/phenol [33-35] respectively. FTIR interpretation suggests the involvement of mainly saponins and carbohydrate compounds in synthesis and stabilization of GNPs by *B. variegata*. Capping of GNP with molecules of LE can make them non-toxic and useful for applications such as contrasting agents in bio-imaging [26, 36].

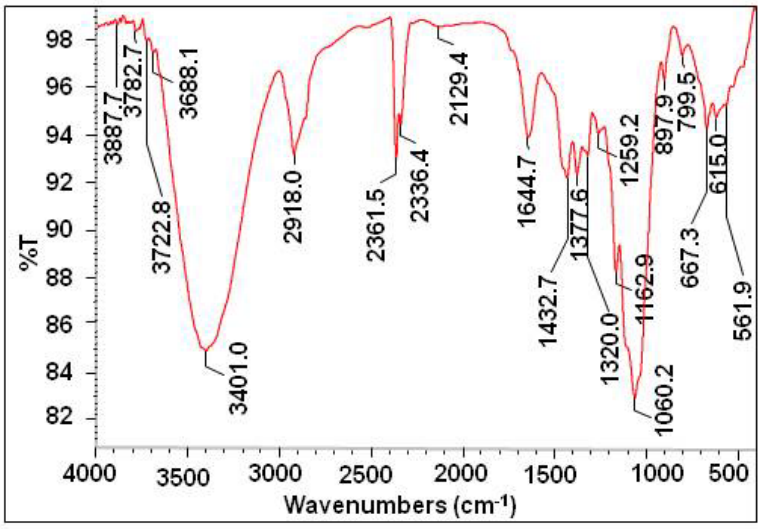

Fig. 6 FTIR spectrum of B. variegata LE synthesized GNP. Peaks at ~876-1162, 1644, 3401, 2918, 1432 and 1315-1380 cm^{-1} correspond to carbohydrates, C=O stretching and contribution from C-N stretching or C=C groups/aromatic rings, OH stretching, C-H stretching in saponins, C-H bend/COO symmetric stretching due to the acidic group of polygalacturonic acid, OH deformation vibrations in the aromatic ring, respectively. All these peaks together indicate the involvement of mainly saponins and carbohydrate in GNP synthesis.

4. Conclusion

Synthesis of GNP of various size and shapes is of prime importance for various nanotechnology based applications. Medicinally important *B. variegata* plant has shown capability to synthesize various shape and sized stable GNP in very less time. Attachment/adsorption of medicinally important molecules present in extract like saponins and carbohydrates will enhance the quality of GNP. Furthermore, simply varying reaction conditions size of GNPs was easily varied from 43-145 nm. Hence, GNPs synthesised using *B. variegata* LE can find applications in various fields like medicine, drug delivery and diagnostics.

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