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Green Synthesis of Silver Nanoparticles using *Couroupita guianensis* Aubl. and their Characterization

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Abstract: We synthesized silver nanoparticles from the aqueous extracts of leaves, flower petals and fruit pulp of *Couroupita guianensis* Aubl. in this study. *C. guianensis* is a threatened medicinal tree, also known as Cannon ball tree or Nagalingam tree. This plant is used to treat number of ailments such as, cold, intestinal gas formation and stomach ache. The leaf has been found to show antioxidant activity, anthelmintic activity, immune modulator and antinociceptive activity. Fruits are edible and are occasionally eaten, but the smell of the white flesh discourages most people from trying them. Fresh leaves, flower petals and fruit pulp were used to prepare aqueous extracts. The silver nanoparticles were synthesized using silver nitrate ($AgNO_3$) solution at room temperature. The solution color was changed from yellow to dark brown with all the three extracts. The nanoparticle suspension gave maximum UV-Vis absorbance at 415 to 435nm. The FTIR spectra of the leaf aqueous extract was determined to allow identification of possible functional groups responsible for the bioconversion of silver ions to silver nanoparticles (AgNPs).

Key words: Green synthesis, silver nanoparticles, *Couroupita guianensis*, aqueous extracts, characterization.

INTRODUCTION

Biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles. The biological reduction of metal ion to base metal is quite simple, rapid and easily conducted at room temperature. It is an ecofriendly way of synthesis of nonoparticles. The reducing agents involved include the various water soluble plant metabolites (e.g. alkaloids, phenolic compounds, terpenoids) and co-enzymes. Extracts of a diverse range of plant species have been successfully used in synthesizing silver nanoparticles.

Silver is a naturally occurring precious metal, most often as a mineral ore in association with other elements. It has been used in a wide variety of applications as it has some special properties like high electrical and thermal conductivity. Ancient civilizations used this precious metal in medicine, eating utensils, plates, cups, food containers, jewellery, coins, cloths and as a disinfectant for water and human infection[1].

In recent years nanoparticles of silver have been found to exhibit interesting antibacterial activities[2,3]. Presently, the investigation of this phenomenon has regained importance due to the increase of bacterial resistance to antibiotics. Recently, silver nanoparticles exhibiting antimicrobial activity have been synthesized by many researchers using plant extracts[4, 5]. Antibacterial activity of the silver-containing materials can be used, for example, in medicine to reduce infections as well as to prevent bacteria colonization.

Couroupita guianensis Aubl. is a highly medicinal valued plant belonging to the family Lecythidaceae. It is also known as Cannon ball tree and Ayahuma, Kailaspati, Nagalingam etc. The flowers have acquired some religious significance in the country and are offered at Buddhist temples and shrines. This plant is used to treat number of ailments such as, cold, intestinal gas formation and stomach ache. The leaf has been found to show antioxidant activity, anthelmintic activity, immune modulator and antinociceptive activity[6, 7.] The present study is an attempt to synthesize and characterize the silver nanoparticles (SNPs) produced by using the leaf extract of threatened medicinal plant *Couroupita guianensis*, which have been using in traditional medicine without any validation.

MATERIALS AND METHODS

Collection of Plant Materials and Extract Preparation: *Couroupita guianensis* is also known as Cannon ball tree or Nagalingam in different regions. It is a highly medicinal tree species. The plant material was collected from the coastal area of Pondicherry, India. The *Couroupita guianensis* trees were identified with help of Gamble Flora[7], and selected in the Botanical garden, Puducherry and in the campus of Tagore Arts College. Fresh, green and mature leaves, flowers and fruits were harvested during the months of July, 2012 to June, 2013. The leaves and flower petals were thoroughly washed with distilled water and finely cut in small pieces **(Fig. 1)**.

The cannon ball fruits were broken with help of hammer because outer cover is so hard and could not be cut by help of anything. The fresh fruit pulp (white in color which converts into blue to brown within minutes) was collected for the synthesis of nanoparticles. The plant extracts (broth solutions) were prepared by using 5 gm of washed and cut leaves, flower petals and fruit pulp in a 250 ml Erlenmeyer flask with 50 ml of sterile distilled water and then boiling the mixture for 5min. The herbal aqueous extract was collected in separate conical flasks by standard filtration method and stored at 4^oC in a refrigerator.

Fig. 1: A & B. Fresh shoots and leaves of *Couroupita guianensis* used for extract preparation; C, D & E. Flowers, flower petals and pieces of petals used for the experiment; F, G & H. Mature fruits, broken fruit and fruit pulp used for biogenesis of AgNPs.

Preparation of 1mM Aqueous Solution of Silver Nitrate: 0.017gm of Silver Nitrate ($AgNO_3$) (Himedia, Mumbai) was added to the 100 ml of distilled water and the solution was stirred well continuously until the silver nitrate is dissolved. This 1mM Silver Nitrate solution is stored in brown bottle at 4^oC for further use for the synthesis of Silver Nanoparticles from *Couroupita* leaf, flower and fruit pulp extracts.

Methods of making Nanoparticles using Plant Extracts: Plant mediated nanoparticle synthesis using whole plant extract or by living plant was reported in literature by several researchers[8, 9].

Synthesis of AgNPs: 1mM aqueous solution of Silver nitrate (Himedia, Mumbai) was prepared for synthesis of silver nanoparticles. For the synthesis of AgNPs, two boiling tubes were taken, one containing 10 ml of 1mM $AgNO_3$ solution as control and the second containing 9 ml of 1mM Silver nitrate solution and 1 ml of plant leaf extracts as test solution. These were incubated at room temperature for 1-2 hours. The color change of the leaf extracts from pale yellow to dark brown was checked periodically. The brown color formation indicates that the silver nanoparticles were synthesized from the plant extracts and they were centrifuged at 5000 rpm for 15 minutes in order to obtain the pellet which is used for further study. Supernatant is discarded and the pellet is dissolved in deionized water. The silver nanoparticles were confirmed by color changes and qualitatively characterized by UV-Visible spectrophotometer.

Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The time duration of change in color varies from plant to plant. The pellets were used for characterization or identification of size of the Ag Nanoparticle with the help of FTIR. The same protocol was repeated for three times with regular time intervals.

Characterization of Silver Nanoparticles: To establish the formation of silver nanoparticles, it is necessary to characterize them. The formation of silver nanoparticles is characterized visually with naked eyes by a change in color to yellowish brown.

UV-Vis Spectrophotometeric analysis: AgNPs can be characterized using a UV-VIS spectrophotometer (Systronics Double Beam Spectrophotometer, Model 2202, Systronics Ltd.). UV-VIS absorption spectra of the silver colloids were acquired by using wave length scan between 200nm and 800nm. On an average, a plasmon peak at 410nm implies the formation of approximately 12nm silver nanoparticles. Larger wavelength points to the formation of larger sized nanoparticles.

FTIR Spectral (Fourier Transform Infrared) analysis: Infrared spectroscopy is an important technique in organic chemistry. It is an easy way to identify the presence of certain functional groups in a molecule. Also, one can use the unique collection of absorption bands to confirm the identity of a pure compound or to detect the presence of specific impurities. To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min and the resulting suspension was redispersed in 10ml sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR (Shimadzu,Model- IRAffinity-1, SHIMADZU Corporation, Kyota, Japan).

RESULTS AND DISCUSSION

In the last decade, biosynthesis of metal nanoparticles is a growing need to develop clean, nontoxic chemicals, environmentally benign solvents and renewable materials[10] and hence the focus turned towards "green" chemistry and bioprocesses. Inspiration from nature comes through yeast, fungi, bacteria and plant extracts for the control synthesis of biocompatible metal and semiconductor nanoparticles[11].

Biosynthesis of Silver Nanoparticles: Extracts from plants may act as reducing and capping agents in silver nanoparticles synthesis. The reduction of Ag^+ ions by combinations of biomolecules found in these extracts (e.g. enzymes/proteins, amino acids, polysaccharides, vitamins etc.) is environmentally benign, yet chemically complex[12]. The extract of lower plants (algae, fungi etc.) was also used to synthesize AgNPs at room temperature. Proteins in the extract provide dual function of Ag^+ reduction and shape control in the nanoparticle synthesis. The carboxyl groups in aspartic and/or glutamine residues and the hydroxyl groups in tyrosine residues of the proteins were suggested to be responsible for the Ag^+ ion reduction[13].

The green synthesis of silver nanoparticles using leaf, flower petals and fruit pulp extracts of *Couroupita guianensis* was carried out in present investigation. The color was changed in the cell free leaf extract when challenged with 1mM $AgNO_3$ from pale yellow to dark brown (**Fig. 2A,B & C**) within 15 min and attained maximum intensity after 12 hrs with intensity increasing during the period of incubation indicative of the formation of silver nanoparticle. The reaction was completed early (within 30 min) in case of flower petals extract when subjected to 1mM $AgNO_3$ and it did not attain much color changes with time duration as compared to leaf extract (**Fig. 2D,E & F).** In case of cell free extract of fruit pulp the color change was not that much significant as compared to leaf and flower petal extracts (**Fig. 2G, H & I).** Control (without silver ions) showed no change in color of the cell filtrates when incubated under same conditions. Sadowski *et al*.,[14] exploited the fungus, *Penicillium* sp. for the extracellular synthesis of silver nanoparticles the fungal cell filtrate was treated in the dark with Ag^+ ions for the biosynthesis process. The reaction mixture showed color change from colorless to brown which intensified with the increase in incubation period.

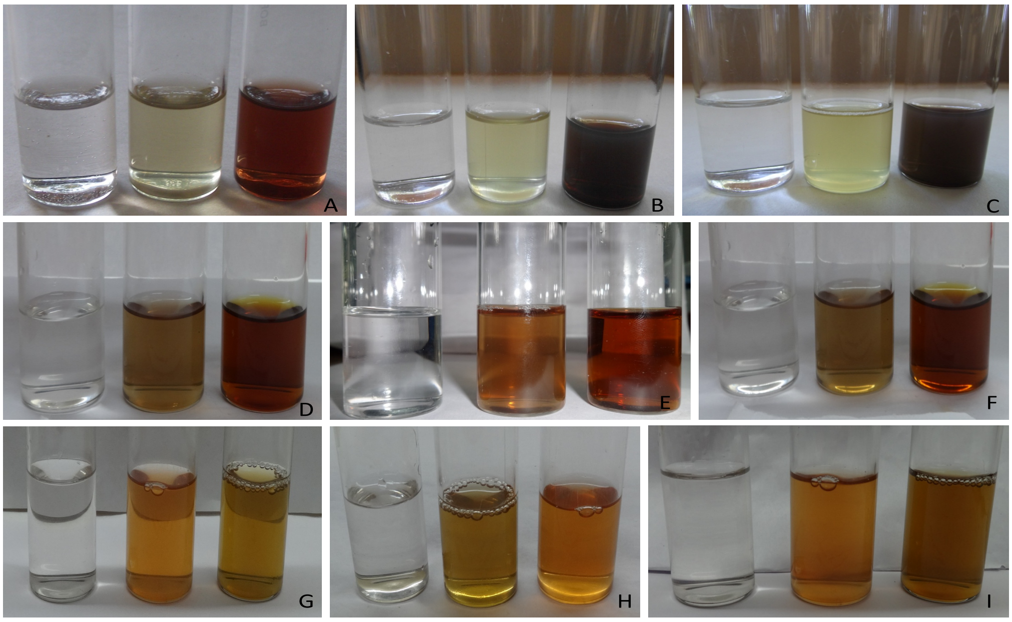

Fig. 2: A, B & C. Changes in the color of the mixture with time when leaf extract challenged with the $AgNO_3$ solution; D, E & F. Changes in the color of the mixture with time when flower petals extract challenged with the $AgNO_3$ solution; G, H & I. Changes in the color of the mixture with time when fruit pulp extract mixed with $AgNO_3$ solution.

Characterization of AgNPs: It is well known that silver nanoparticles exhibit dark brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles[15]. The appearances of yellowish brown color in the reaction vessels suggest the formation of silver nanoparticles (SNPs)[16]. When the cell free extracts were mixed with 1mM $AgNO_3$ the color was changed from pale yellow to dark brown in 15 min and attained maximum intensity after 12 hrs with intensity increasing during the period of incubation indicative of the formation of silver nanoparticle. Shekhawat et al.,[4] reported biogenesis of silver nanoparticles with help of leaf extract of *Turnera ulmifolia*. The color change reaction was completed within 12 hrs in this study.

UV-Vis spectral analysis: The synthesis of AgNPs had been confirmed by measuring the UV-Vis spectrum of the reaction media. The UV-Vis spectrum of colloidal solutions of AgNPs synthesized from *Couroupita guianensis* leaf extract have absorbance peaks at 415-425 nm regions **(Fig. 3)**, which are identical to the characteristics UV-visible spectrum of metallic silver. The weak absorption peak at shorter wave lengths was due to the presence of several organic compounds which were known to interact with silver ions. Again, the time duration of change in color and reaction completion is varies from plant to plant. *Boswellia ovalifoliolata* synthesized silver nanopartcles within 10 min whereas *Shorea tumbuggaia* and *Svensonia hyderobadensis* took 15 min to synthesize nanoparticles[17].

The UV-visible spectra of the control samples depicted absorption bands at 260-270nm corresponding to proteins, α-NADPH and hydroxyquinoline. The spectra of reaction mixture showed strong surface plasmon resonance at 413nm which intensified with time while, the absence of absorption band at 413nm for the reaction mixture in the absence of enzyme clearly depicted that the reduction of silver involves enzymatic reduction of nitrate to nitrite.

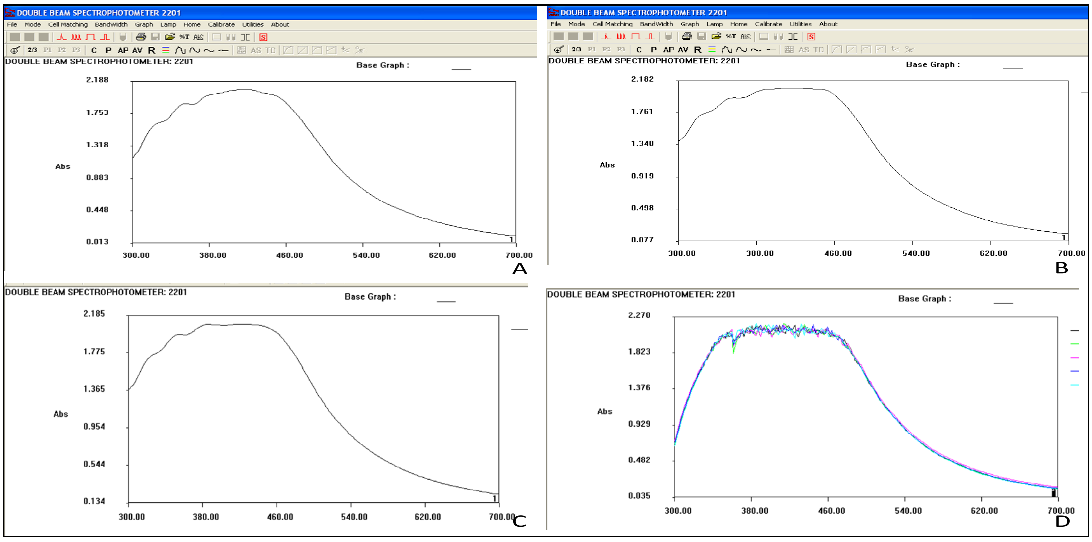

Fig. 3: A, B & C. UV-Visible Spectra of AgNPs synthesized using leaf extract after every 1hr; D. A combined UV-Visible Spectra of AgNPs synthesized using leaf extract after every 1hr.

The *Couroupita guianensis* flower petal extract mediated nanoparticles has also showed absorbance peaks at 418-424nm regions in the spectral analysis as shown in **Fig. 4.** The peaks were stable with time duration also. Thus, indicating that the synthesis of silver requires the reduction of α-NADPH to α-NADP^{+} and the hydroxyquinoline probably acts as an electron shuttle transferring the electron generated during the reduction of nitrate to Ag^{+} ions converting them to Ag^{0}.

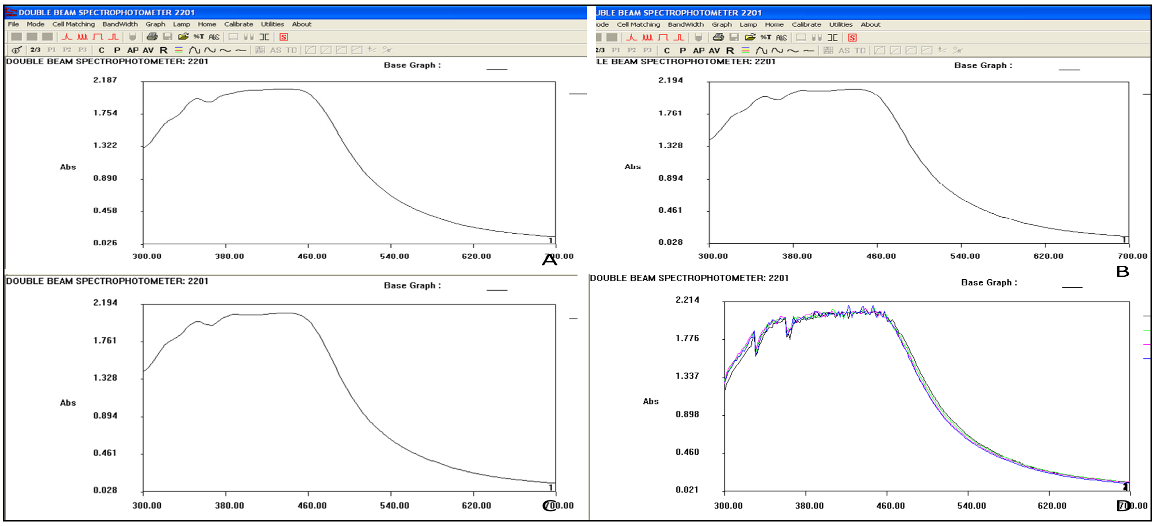

Fig. 4: A,B & C. UV-Visible Spectra of AgNPs synthesized using flower petals extract after every 1hr; D. A combined UV-Visible Spectra of AgNPs synthesized using flower petals extract after every 1hr.

Ramesh *et al.,*[18] reported on reduction of palladium ions by *Glycine max* leaf extract, which was characterized by UV-visible spectroscopic. They concluded that the proteins and some of the amino acids that are exist in soybean leaf extracts were actively involved in the reduction of palladium ions. The color changes with fruit pulp extract of *Couroupita guianensis* was not so prominent and the reaction took long time to complete. The UV-Vis spectral analysis was also followed the same trend as shown in **Fig. 5.** The first two spectral graphs did not show any AgNPs presence but with time duration (After 11 hrs) a very fine peak was observed in this experiment.

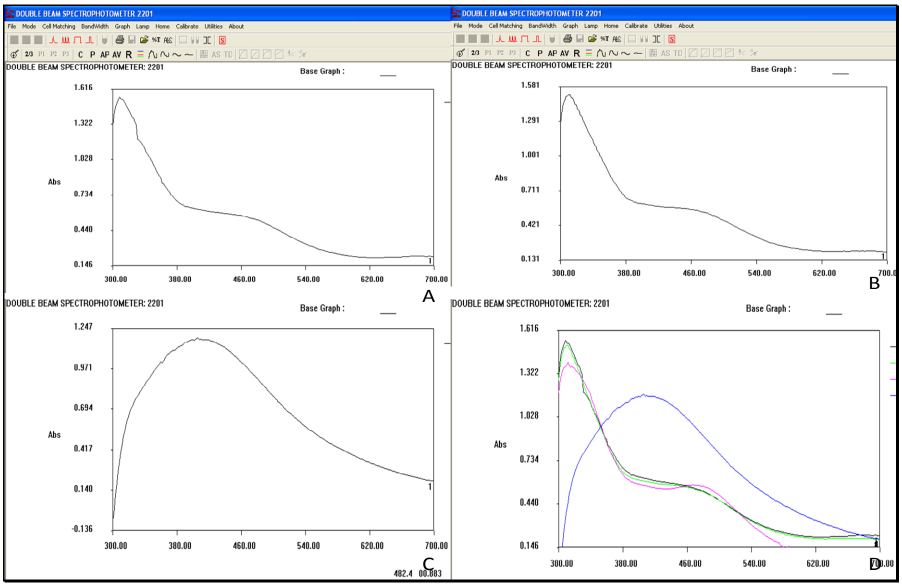

Fig. 5: A, B & C. UV-Visible Spectra of AgNPs synthesized using fruit pulp extract after every 1hr; D. A combined UV-Visible Spectra of AgNPs synthesized using fruit pulp extract after every 1hr.

FTIR analysis: A hypothetical mechanism for the synthesis of silver nanoparticles was corroborated according to the FTIR study of silver nanoparticles. The FTIR spectra of silver nanoparticles depicted intense peak at 1119 cm^{-1} corresponding to stretching vibrations of Ag-N bonds and two broad bands at 1405 and 1642 cm^{-1} attributed to symmetric and asymmetric C=O stretching vibration of CO_2. Selective enhancement of these Raman bands indicated that C=O bonds and Ag-N bonds lie perpendicular to the nanosilver surface and gets associated with the formation of a cap over nanoparticles. Also, the symmetric and asymmetric stretching bonds of CO_2 significantly broaden due to distortion of the respective bond angles and bond lengths which further support in the encapsulation of silver nanoparticles. The band at 240 cm^{-1} confirmed the formation of a chemical bond between silver nanoparticles and the nitrogen of amino groups[19].

FTIR analysis was used for the characterization of the extract and the resulting nanoparticles. FTIR absorption spectra of water soluble extract after reduction of Ag ions are shown in **Fig. 6.** Absorbance bands in the figure are observed in the region of 500–4000 cm^{-1} are 1119, 1364, 1405, 1642 1734cm^{-1}. These absorbance bands are known to be associated with the stretching vibrations for –C C–C O, –C C– [(in-ring) aromatic], –C–C– [(in-ring) aromatic], C–O (esters, ethers) and C–O (polyols), respectively[19]. In particular, the 1119 cm-1 band arises most probably from the C–O group of polyols such as hydroxyflavones and catechins. The nanoparticles synthesized using *Couroupita guianensis* leaf extract was only investigated for FTIR study in present investigation. Ramesh et al.,[18] investigated on reduction of palladium ions by soybean (*Glycine max*) leaf extract and which was examined by UV-visible spectroscopic technique. They concluded that the proteins and some of the amino acids that are exist in soybean leaf extracts were actively involved in the reduction of palladium ions. Further it was confirmed by Fourier Transformations Infrared Spectroscopic analysis. A phytoapthogen *F. solani* (USM-3799) was harnessed for the extracellular biosynthesis of silver nanoparticles by Ingle et al.,[20]. The biosynthesized nanoparticles were characterized using FTIR. The FTIR spectra of the silver nanoparticles depicted presence of functional groups like C-N, C-O-C, amide linkages and –COO-. These functional were found to play an important role in the capping of nanoparticles and their further stability in aqueous solution.

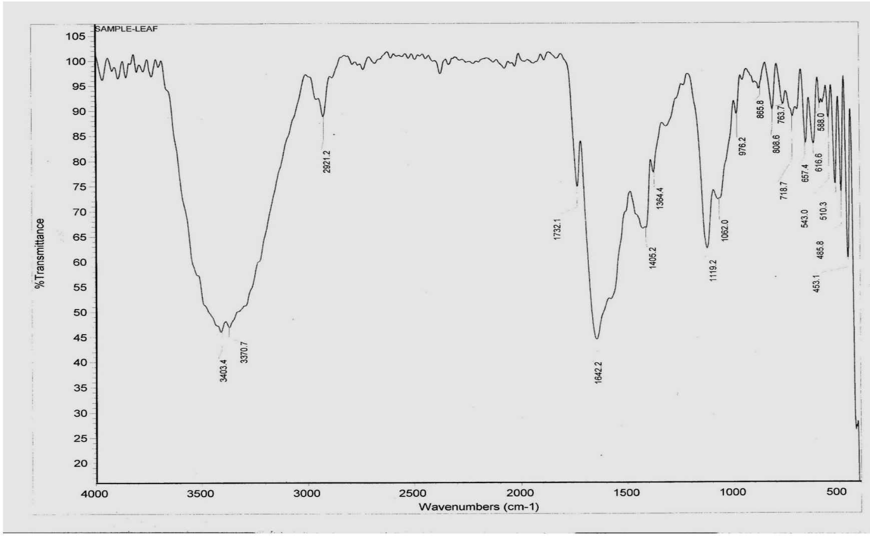

Fig. 6: FTIR spectral graph of AgNPs synthesized using leaf extracts.

CONCLUSION

In conclusion, the bio-reduction of aqueous Ag^+ ions by the leaf, flower petal and fruit pulp extracts of the *Couroupita guianensis* plant has been demonstrated. In the present study we found that fruits can also be a good source for synthesis of silver nanoparticles. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). There is no report describing synthesis of silver nanoparticles using *Couroupita guianensis* plant parts, it is first time reported during present investigation that to by use of flower petals and fruit extract.

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