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### PHYTOCHEMICAL ANALYSIS OF CRUDE PLANT EXTRACTS AND LABORATORY SYNTHESIZED GREEN NANOPARTICLES FROM *ACACIA AURICULIFORMIS* A. CUNN

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#### ABSTRACT

Recently the research and analysis of green nanoparticles (NPs) synthesized with plant extracts has been expanded significantly due to their great biological activities. From the variety of agents used for nanoparticles synthesis, silver (Ag) is the most preferred due to its recommended use in medical field as best topical bactericides from ancient times. In the present project, crude bark extracts of *Acacia auriculiformis* A. Cunn. were used for nanoparticles synthesis and comparison of their biochemistry with crude extract was assessed by using biochemistry analytical techniques viz; UV-Visible spectrophotometer (UV-Vis), Fourier transform infra-red spectrophotometer (FTIR), Photoluminescence (PL) and Direct Light Scattering analysis (DLS).

#### KEYWORDS

*Acacia auriculiformis*, Silver nanoparticles, UV-Visible spectrophotometer (UV-Vis), Fourier transform infra-red spectrophotometer (FTIR), Photoluminescence (PL) and Direct Light Scattering analysis (DLS).

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#### INTRODUCTION

In 20<sup>th</sup> century, Nano things come in culture very swiftly. Similar nanoparticles as well as green nanoparticles enter the research field for medicinal and analytical departments of various national and international agencies. Nanoparticles (NPs) can be synthesized by using certain organic agents (mainly carbon) and inorganic (metal ions like silver and gold) agents<sup>1</sup>. Silver (Ag) is the most preferred NPs synthesis agent because of its great bioactivity and

safe nature to humans<sup>2</sup>. For synthesizing stable silver nanoparticles, soluble starch as both the reducing and stabilizing agents can be used<sup>3</sup>. Recently the scientific community also shifted their concern towards ecofriendly, natural and cheaper method of NPs synthesis by using organic agents like microorganisms and plant extracts<sup>4</sup>. Also the use of plant extracts and compounds is most popular for silver nanoparticles (NPs) synthesis due to the gamut of biological activities, easy availability and faster as well as cheaper rate of NP's synthesis with them<sup>5,6</sup>. The nanoparticles had been clinically used for infection, vaccines and renal diseases<sup>7</sup>. The plant extract of petals of herbal species like *Punica granatum*, *Datura metel* and *Aloe vera*<sup>8</sup> and stem extracts of *Svensonia hyderabadensis*<sup>9</sup> had been effectively used for NPs synthesis and investigated for their antimicrobial activities.

Nanoparticles could be synthesized by various approaches like photochemical reactions in reverse micelles, thermal decomposition, sonochemical and microwave assisted process<sup>10,11</sup>. Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics<sup>12</sup>, antimicrobials and therapeutics<sup>13,14</sup> and micro-electronics<sup>15</sup>.

*A. auriculiformis* (also called as Black wattle and Australian Kikkar) is an important medicinal plant and widely distributed member of family Fabaceae and subfamily Mimosoideae. It had reported to be a rich source of polyphenols and tannins<sup>16</sup>. Its anti-helminthic, anti-filarial, anti-insect and microbicidal effects had been well demonstrated<sup>17-22</sup>. Extensive literature and research of the medicinal as well as biological properties of *A. auriculiformis* at cellular stage<sup>23</sup>, drug delivery and diagnostics imaging cancer detection<sup>24</sup> were available in the reports of many scientists. There are no reports available for silver nanoparticles (NPs) synthesis using bioactive extracts of important medicinal plant, *A. auriculiformis*. In the present study, biochemistry of crude extracts (methanol (AAM), acetone (AAA) and water (AAW) extract) of *A. auriculiformis* as well as synthesized nanoparticles was explored.

## MATERIAL AND METHODS

The bark of *A. auriculiformis* was collected from Chandigarh Sec-34 along the road side. The plant species was identified by comparing it with the specimen available in the herbarium (voucher number 6422) of the Guru Nanak Dev University, Amritsar. Utmost care was taken to select the healthy bark.

### Preparation of bark extract

#### Procurement and extraction of plant material

The bark of *A. auriculiformis* was procured and washed with tap water (thrice) and dried in oven at 30°C for overnight and ground to a fine powder with grinder. The extracts of *A. auriculiformis* were prepared by maceration extraction method in which the bark was dissolved in solvent.

#### Protocol for extraction of plant material

Dried bark powder (3Kg) was suspended in 1000ml acetone and kept on shaker for 24hr at room temperature. After 24hr, the suspended solid was filtered off through Whatman No.1 filter paper and filtrate was collected. This procedure was repeated thrice to obtain three filterates of extract. The three filterates were combined and solvent was removed under vacuum using rotary evaporator to obtain solid residue. This light brown coloured dried material was named as "Acetone Extract" of *A. auriculiformis* (AAA). Other extracts i.e. methanol (AAM) and water (AAW) were also obtained with the similar method.

#### Protocol for synthesis of nanoparticles

Take finely powdered extract of the plant. Prepare the stock solution of the plant material with 10mg/ml conc. of extract in 100ml of 7% DMSO. Prepare 1mM AgNO<sub>3</sub> (silver nitrate) solution. Add AgNO<sub>3</sub> solution to extract solution in 9:1. Mix 900ml of AgNO<sub>3</sub> solution to 100ml of plant extracts of test conc. Incubate the solution for 2-3 hrs and filter it. Dry the solution in hot air oven 40-50°C. Grind the dried material into fine powder. Wash the powder with NaCl (sodium chloride). If white precipitates formed, filter it using whatman filter paper. Dry it and powdered the material. Store the material for further experimentation in air tight containers.

### Phytochemical studies

Synthesized nanoparticles and crude bark extracts were sent to different analytical laboratories (Panjab University, Chandigarh and Guru Nanak Dev University, Amritsar Sahib) of Punjab, India for Phytochemical analysis with UV-Vis, FTIR, PL and DLS analysis. The results obtained are summarized here in.

## RESULTS AND DISCUSSION

### UV-Visible spectrophotometer analysis

The comparison of UV-Vis spectra obtained for three bark extracts (Acetone, Methanol and water extract) and their corresponding nanoparticles (Acetone NPs, Methanol NPs and Water NPs) were given. UV-Visible absorption spectra of crude extracts and their corresponding NPs was shown in Figure No.1, 2 and 3. The spectra showed absorption maxima at 580nm. The peak at 580nm showed the sharp peaks of benzene vapour showing effect on band shape<sup>25</sup>.

The spectra of silver solution containing acetone bark extract showed absorption maxima at 275nm whereas an absorption window from 350nm to 400nm was recorded, which may be assigned to surface Plasmon of various metal nanoparticles<sup>26</sup>.

The spectra of silver solution containing methanol bark extract showed absorption maxima at 275nm whereas an absorption window from 380nm to 420nm was recorded, which may be assigned to surface Plasmon of various metal nanoparticles<sup>27</sup>.

The spectra showed absorption maxima at 280nm. The peak at 280nm showed the quantum dots and metal-silica core shell of metal compounds<sup>28</sup>.

The spectra of silver solution containing water bark extract showed absorption maxima at 275nm whereas an absorption window from 520nm to 700nm was recorded, which may be assigned to surface Plasmon of various metal nanoparticles<sup>27</sup>.

### Photo luminescence Spectroscopy (PL)

A Photoluminescence (PL) spectrum obtained from the crude bark extract and NPs was given in Figure No. 4, 5 and 6. From the spectrum, it was seen that PL excitation intensity peak appeared at 625nm with steady intensity of 1000au till emission peak at 650nm. PL spectrum showed NPs excitation peak at

625nm and emission peak at 670nm with the similar intensity of 1000au for crude extract. During the comparison of spectra of crude extract and NPs, the shift in excitation intensity peak from 630nm to 670nm from crude extract to NPs was recorded (Figure No.4).

A Photoluminescence (PL) spectrum obtained from the crude bark extract was given in Figure No.5. From the spectrum, it was seen that PL excitation intensity peak appeared at 620nm with steady intensity of 1000au till emission peak at 650nm. PL spectrum showed NPs excitation peak at 620nm and emission peak at 670nm with the similar intensity of 1000au for crude extract. During the comparison of spectra of crude extract and NPs, the shift in excitation intensity peak from 650nm to 670nm from crude extract to NPs was recorded. The PL peaks at 610nm to 640nm showed Si=Si binding sites at the surface of oxygen. The Peaks at 650 nm to 730 nm showed Si-nano crystallites with different sizes.

From the spectrum, it was seen that PL excitation intensity peak appeared at 610nm with steady intensity of 1000au till emission peak at 650nm. PL spectrum showed NPs excitation peak at 630nm and emission peak at 670nm with the similar intensity of 1000au for crude extract.

During the comparison of spectra of crude extract and NPs, the shift in excitation intensity peak from 630nm to 670nm from crude extract to NPs was recorded (Figure No.6.).

The PL peaks at 630nm to 650nm showed Si=O binding sites at the surface of oxygen. The Peaks at 630 nm to 730 nm showed Si-nano crystallites with different sizes (Siu and Stokes, 2000). Some Peaks at 610-700 nm shows Si-Si bonds and annealing of Si-NPs<sup>29</sup>.

### Fourier transform Infra Red spectrometer (FTIR)

FTIR spectra analysis for crude extract (Figure No.7A, 8A and 9A) and synthesized nanoparticles (Figure No.7B, 8B and 9B) revealed presence of ten and thirteen absorption peaks in the two spectrums respectively. The peaks assigned for the spectrums were summarized in Table No.1,2 and 3.

With the presence of one extra peaks *i.e.* at 416.10  $\text{cm}^{-1}$ , it may be concluded that the peaks were due to the presence of silver ions in the solution. The peaks between 400 to 500  $\text{cm}^{-1}$  exhibit oxygen bonds by infra- red active vibration modes and peak at 410  $\text{cm}^{-1}$  exhibit triterpene compounds<sup>30</sup>. The NPs showed two highest peaks at 1354.40  $\text{cm}^{-1}$  and 1000.69  $\text{cm}^{-1}$ . The peaks between 1200 to 1350  $\text{cm}^{-1}$  showed C-H bend. The peaks between at 500 to 600  $\text{cm}^{-1}$  also showed C=C-H: C-H bend<sup>31</sup>.

FTIR spectra analysis for crude methanol extract (Figure No.8 a) and synthesized nanoparticles (Figure 8 b) revealed presence of ten and thirteen absorption peaks in the two spectrums respectively. The peaks assigned for the spectrums were summarized in Table No.2.

With the presence of two extra peaks *i.e.* at 423.97  $\text{cm}^{-1}$  and 410.55  $\text{cm}^{-1}$ , it may be concluded that these peaks were due to the presence of silver ions in the solution.

FTIR spectra analysis for crude water extract (Figure No.9 a) and synthesized nanoparticles (Figure No.9 b) revealed presence of ten and thirteen absorption peaks in the two spectrums respectively. The peaks assigned for the spectrums were summarized in Table No.3.

With the presence of two extra peaks *i.e.* at 401.82  $\text{cm}^{-1}$  and 411.18  $\text{cm}^{-1}$ , it may be concluded that these peaks were due to the presence of silver ions in the solution. The peaks between 400 to 500  $\text{cm}^{-1}$

exhibited oxygen bonds by infra red active vibration modes and peak at 410  $\text{cm}^{-1}$  exhibited Triterpene compounds<sup>31</sup>. The NPs showed two highest peaks at 3190.25  $\text{cm}^{-1}$  and 1693.73  $\text{cm}^{-1}$  and these peaks exhibit the carbon-carbon triple bond and having multiple broad peaks<sup>32</sup>.

#### Direct Light Scattering Analysis (DLS)

The particles size distribution of synthesized silver NPs was evaluated by DLS measurement using a Zetasizer, version 6.32. DLS analysis is a latest technique which works on Brownian movement of electrons when strike to certain objects. With the help of that striking, the size of the target object can be assessed. Biological materials or other chemicals of biological origin can be observed for their size with DLS analysis. The DLS analysis is summed up in Figure No.10,11 and 12 for crude bark extracts of *A. auriculiformis* obtained with Acetone, methanol and water respectively. The NPs synthesized with acetone, methanol and water showed DLS results given in Figure No.13,14 and 15 respectively.

In the current study, silver nanoparticles size is in the range of 25nm to 95nm and the crude extract size range from 55nm to 100nm. It clearly indicated the reduction in particle size (Figure No.10,15).

DLS analysis determined the average particles size distribution profile of synthesized nanoparticles and capping agent enveloped the metallic particles along with the metallic core<sup>33</sup>.

**Table No.1: FTIR spectra analyses for crude acetone extract and silver nanoparticles (NPs)**

S.No	Absorption peaks ( $\text{cm}^{-1}$ )	
	Acetone extract	NPs
1	499.77	<b>1354.40</b>
2	492.91	<b>1000.69</b>
3	480.85	525.22
4	477.54	506.28
5	468.36	492.90
6	458.76	486.99
7	453.34	469.64
8	449.11	463.47
9	437.83	452.00
10	429.54	440.27
11	421.18	430.71
12	409.84	425.05
13	-	416.10

**Table No.2: FTIR spectra analysis for crude methanol extract and silver nanoparticles (AgNPs)**

S.No	Absorption peaks (cm <sup>-1</sup> )	
	Methanol extract	AgNPs
1	511.13	517.80
2	-	<b>500.72</b>
3	495.64	496.51
4	484.55	481.36
5	476.39	470.34
6	462.58	464.63
7	453.09	458.28
8	437.79	448.98
9	421.59	440.74
10	416.63	436.86
11	405.47	432.18
12	-	423.97
13	-	410.55

**Table No.3: FTIR spectra analysis of crude water extract and silver nanoparticles (NPs)**

S.No	Absorption peaks (cm <sup>-1</sup> )	
	NPs	Water extract
1	407.96	401.82
2	411.18	419.96
3	415.24	436.08
4	425.04	454.15
5	432.53	465.09
6	436.25	482.67
7	450.77	489.49
8	461.53	495.96
9	470.22	-
10	483.96	-
11	491.67	-
12	504.36	-
13	1009.34	-
14	1186.80	-
15	1597.61	-
16	1693.73	-
17	3190.25	-

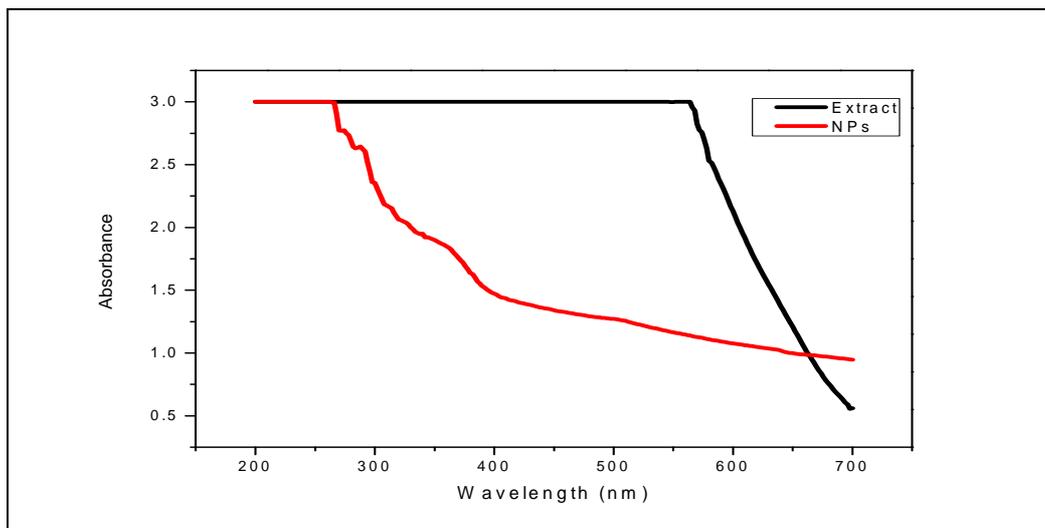


Figure No.1: UV-Visible spectra of Acetone extract of *Acacia auriculiformis* and synthesized NPs

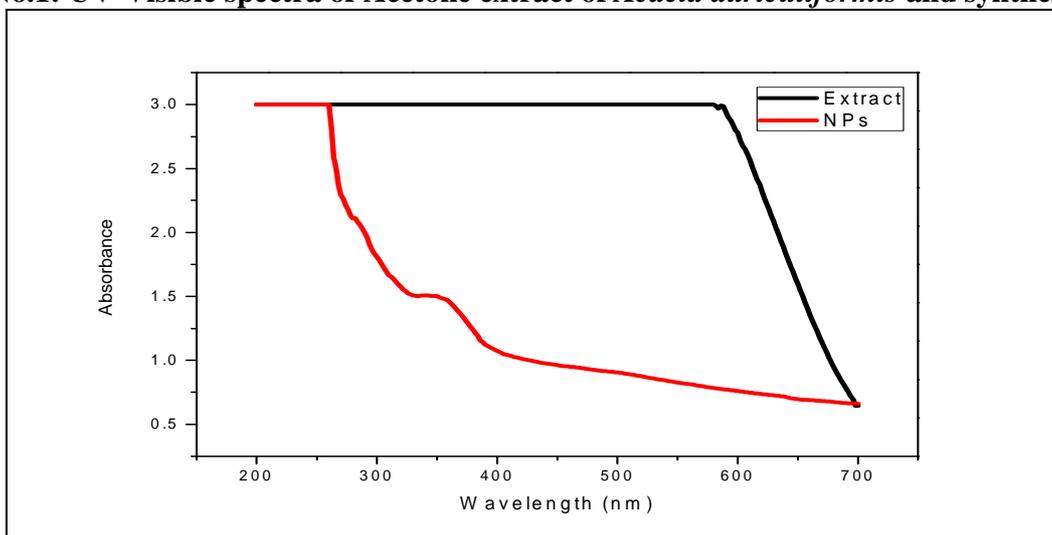


Figure No.2: UV-Visible spectra of Methanol extract of *Acacia auriculiformis* and synthesized NPs

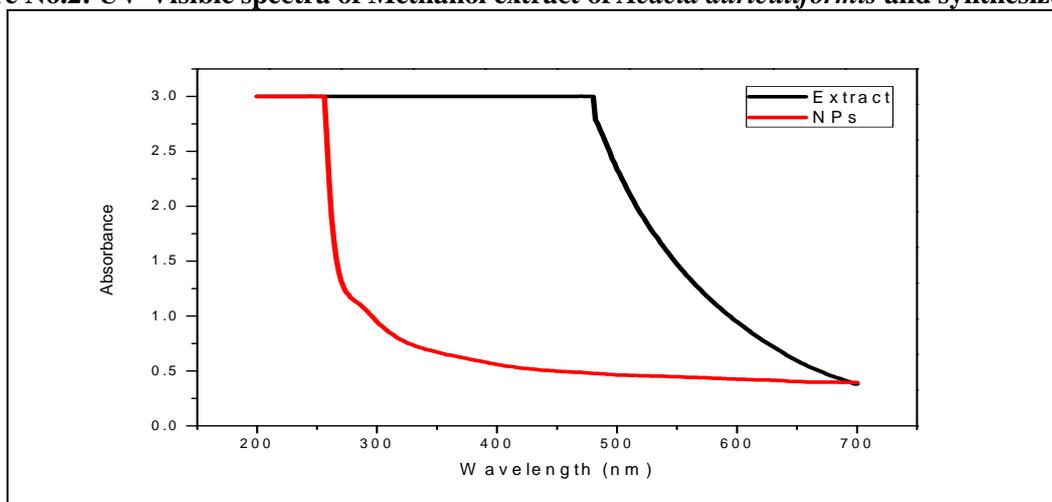


Figure No.3: UV-Visible spectra of Water extract of *Acacia auriculiformis* and synthesized NPs

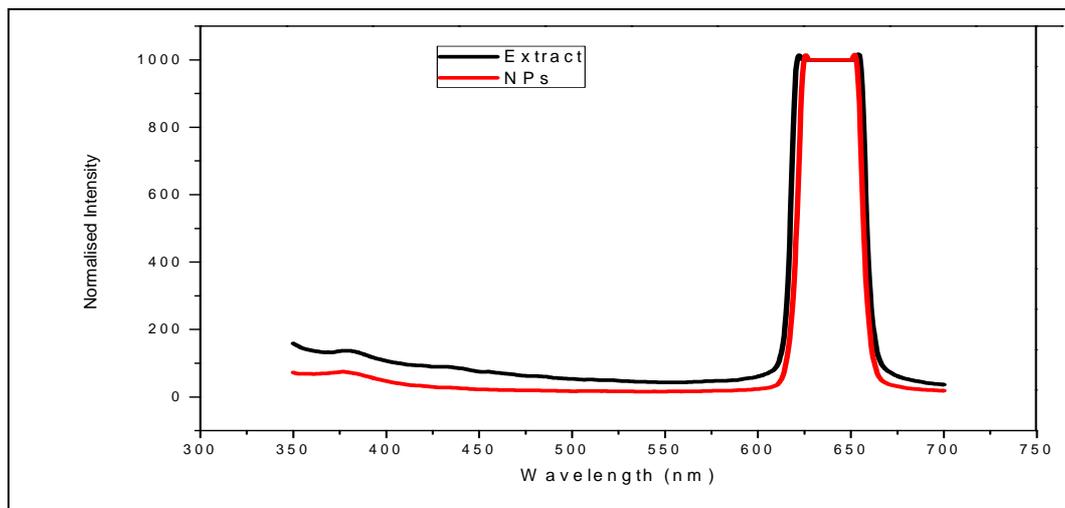


Figure No.4: Photo luminescence spectra of Acetone extract of *Acacia auriculiformis* and synthesized NPs

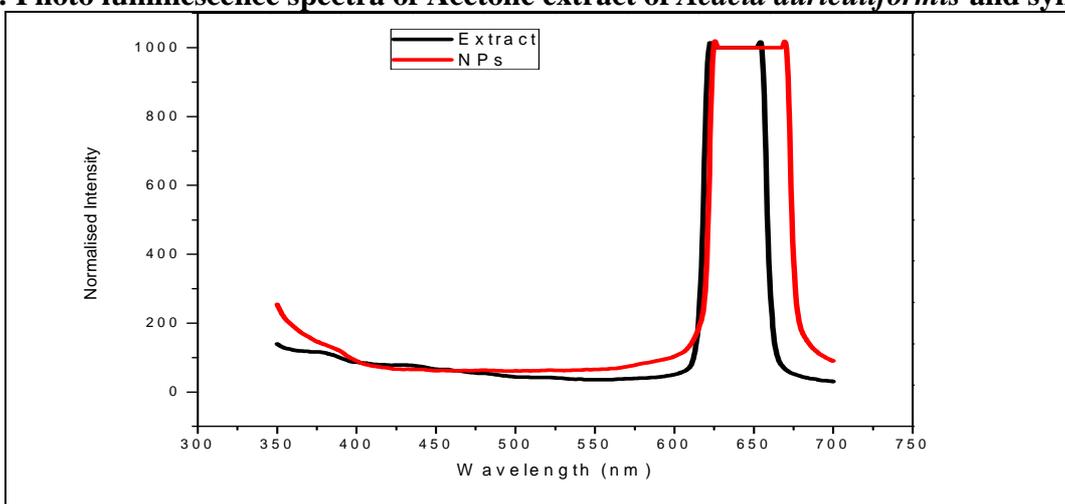


Figure No.5: Photo luminescence spectra of Methanol extract of *Acacia auriculiformis* and synthesized NPs

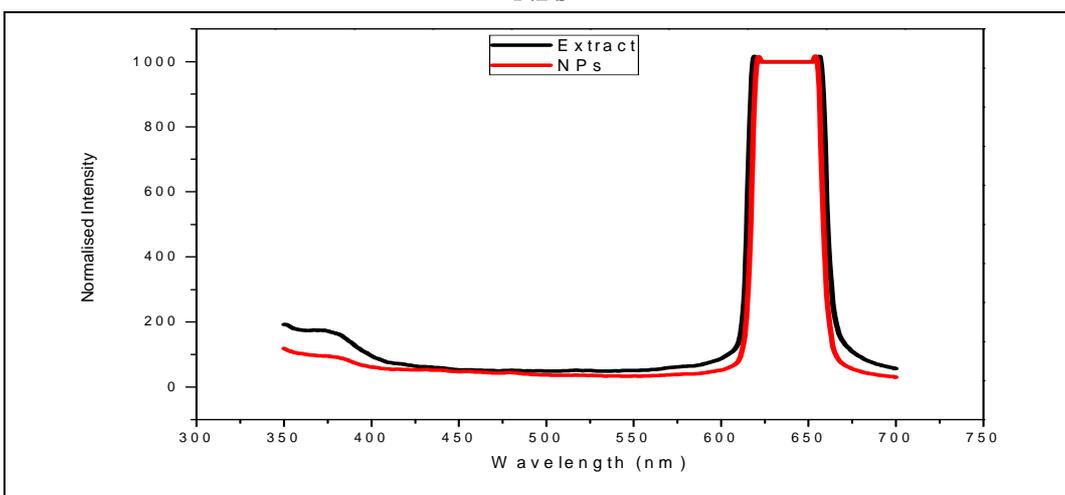


Figure No.6: Photo luminescence spectra of Water extract of *Acacia auriculiformis* and synthesized NPs

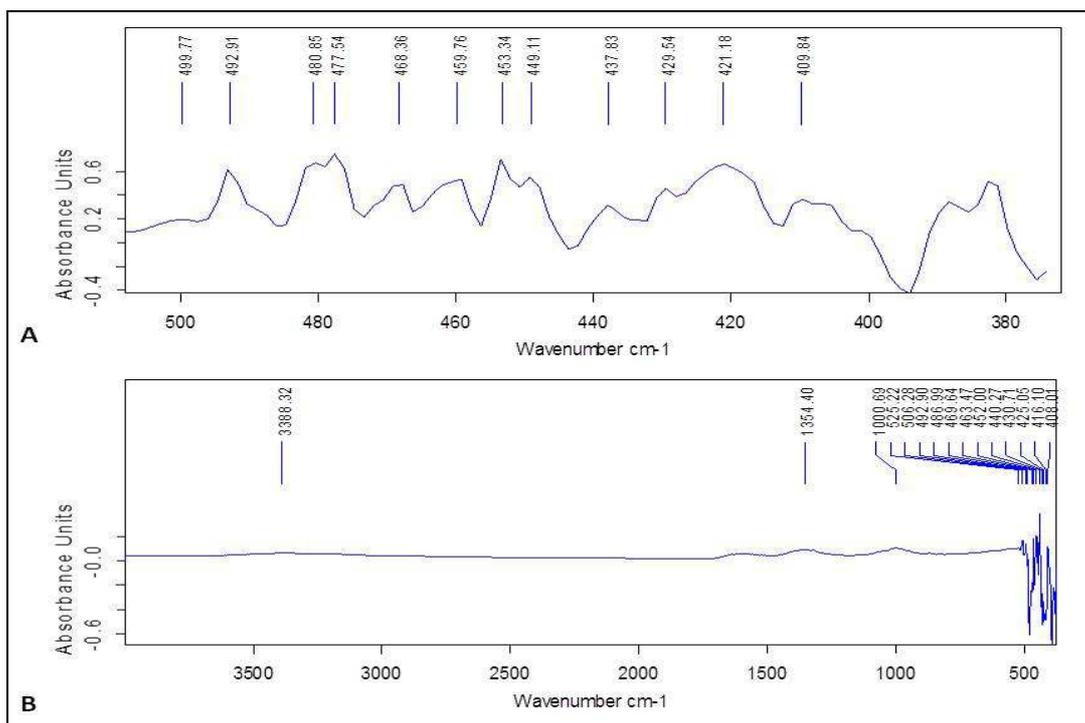


Figure No.7: FTIR spectra of A) acetone extract of *Acacia auriculiformis* and B) synthesized NPs

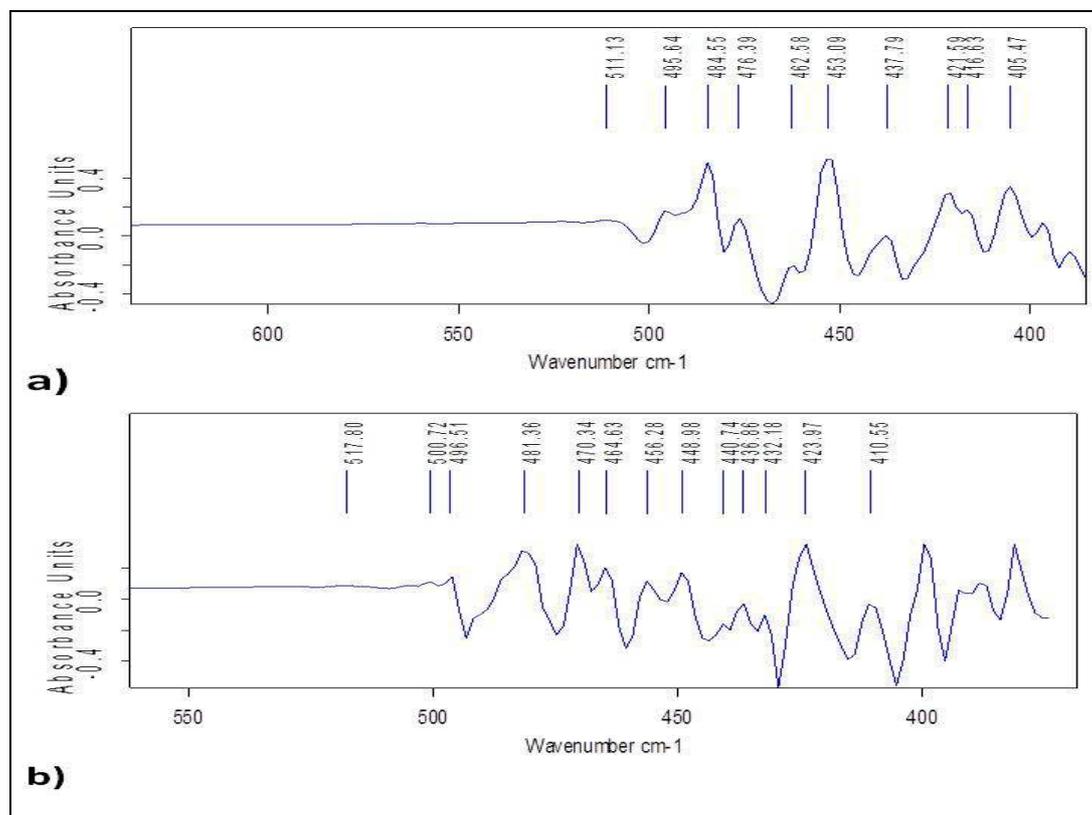


Figure No.8: FTIR spectra of A) methanol extract of *Acacia auriculiformis* and B) synthesized NPs

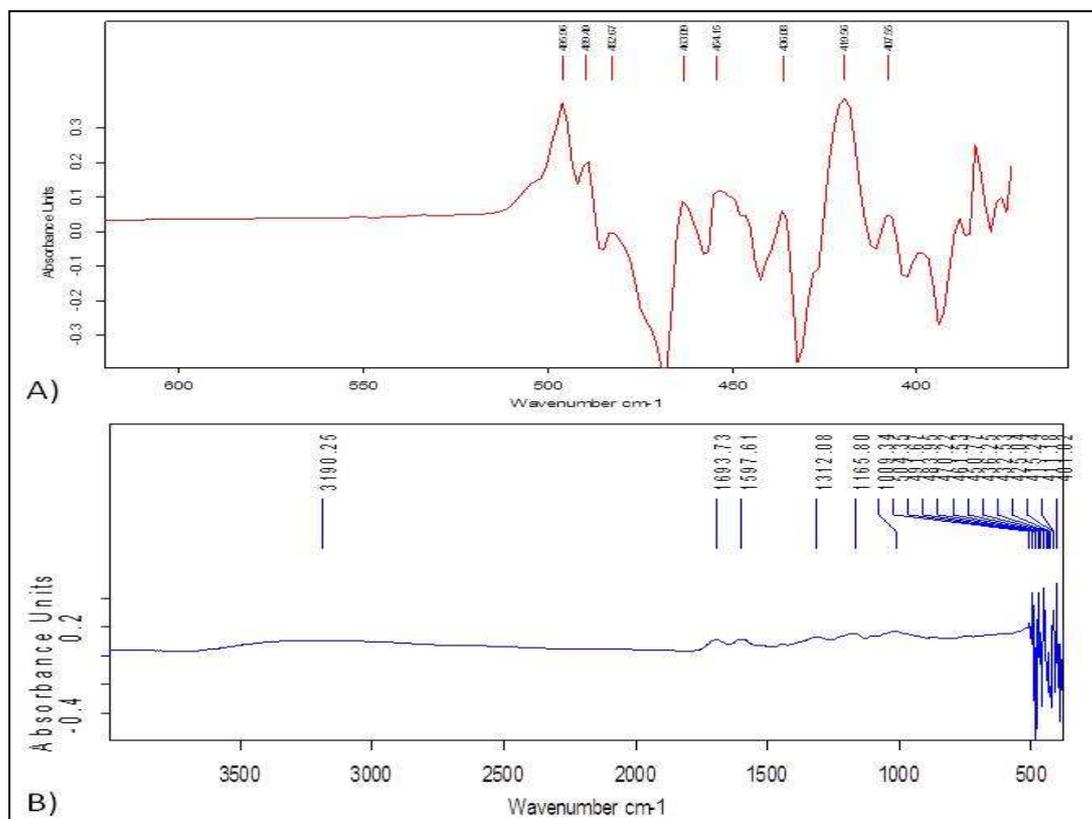


Figure No.9: FTIR spectra of A) Water extract of *Acacia auriculiformis* and B) synthesized NPs

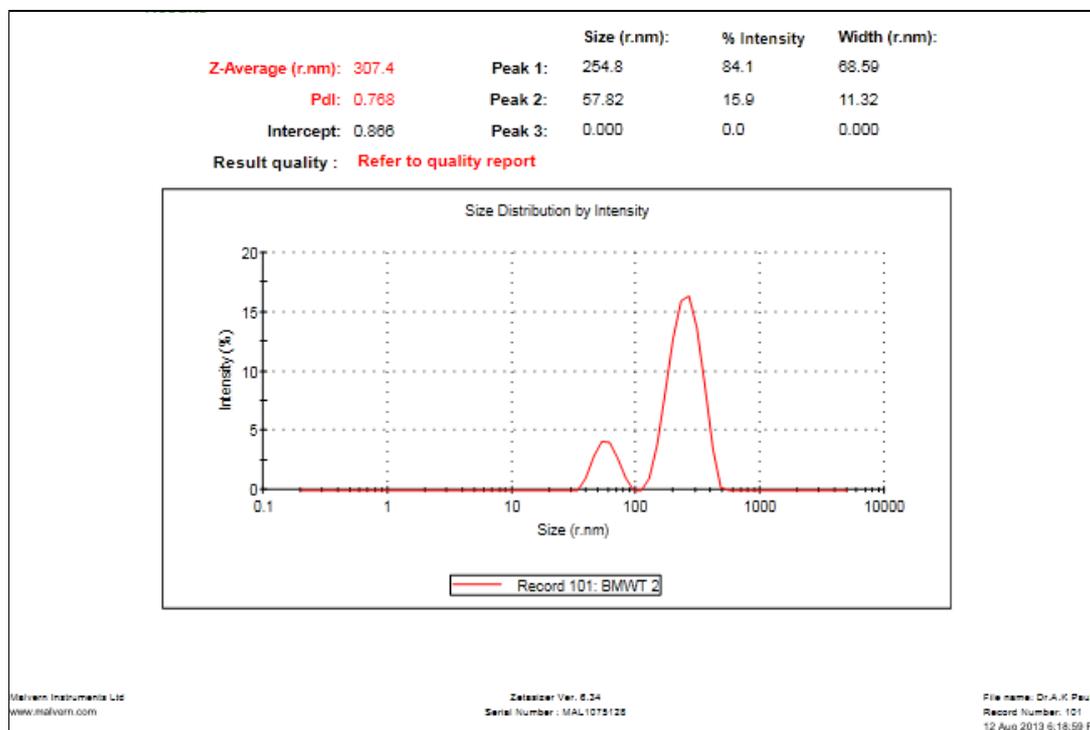


Figure No.10: DLS analysis of crude acetone extract of *A. auriculiformis*

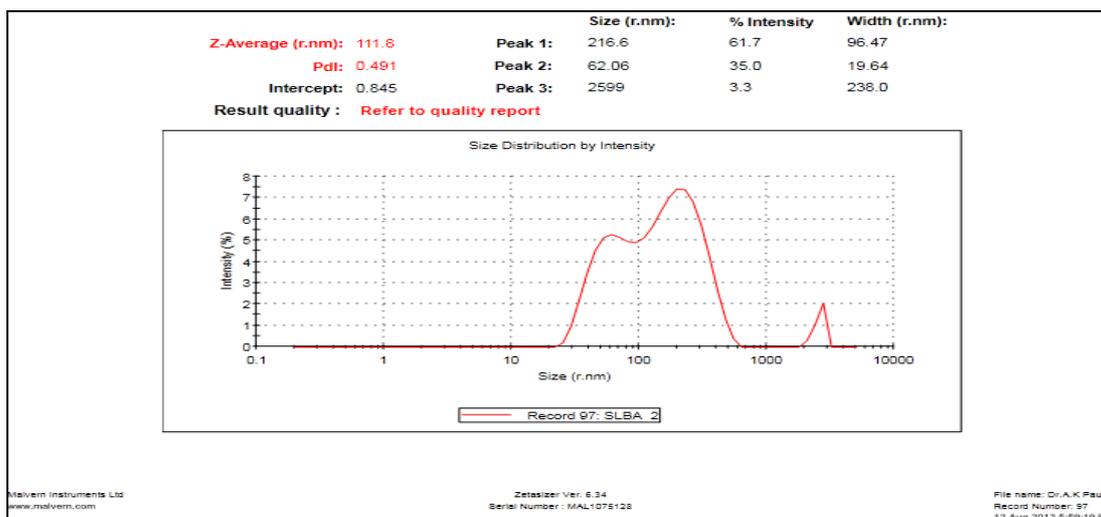


Figure No.11: DLS analysis of crude methanol extract of *A. auriculiformis*

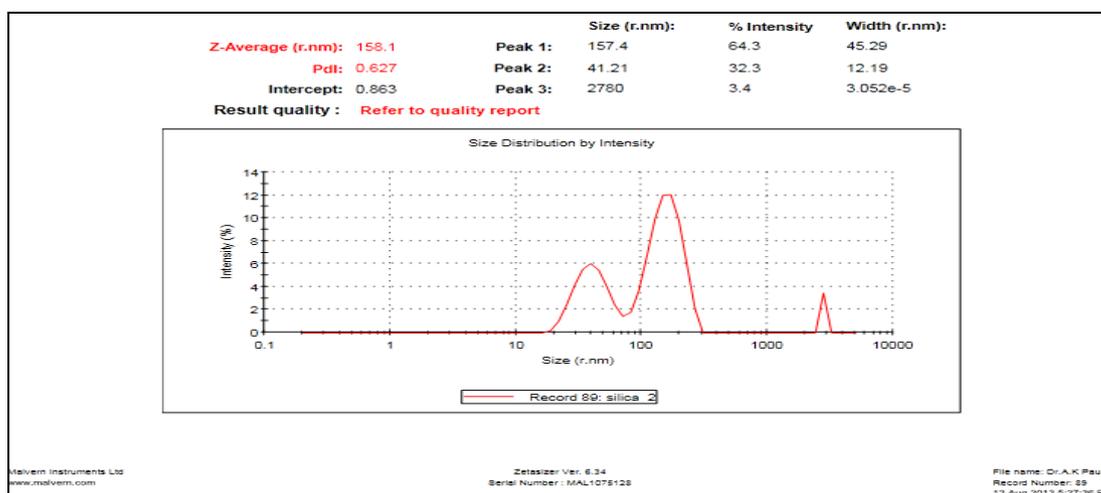


Figure No.12: DLS analysis of crude water extract of *A. auriculiformis*

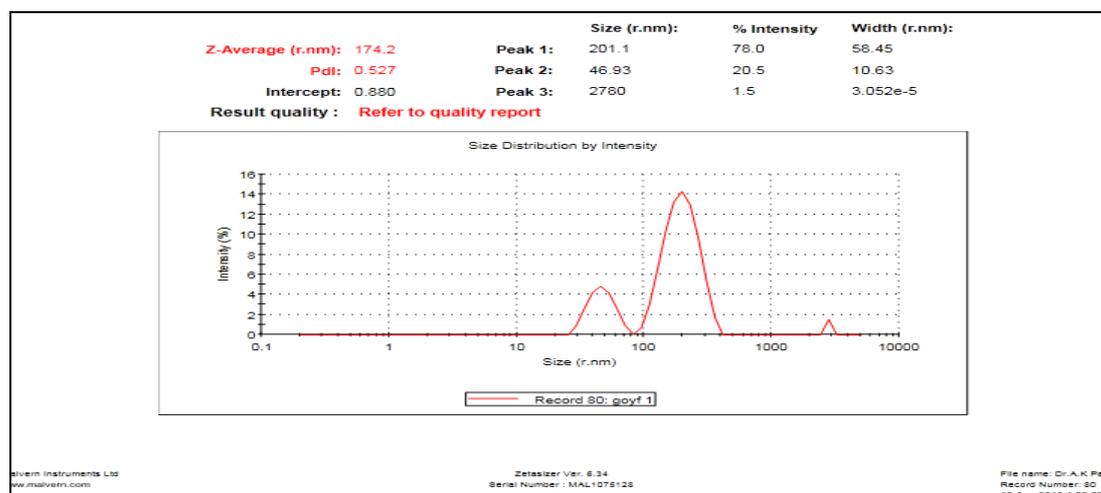


Figure No.13: DLS analysis of synthesized NPs with acetone extract of *A. auriculiformis*

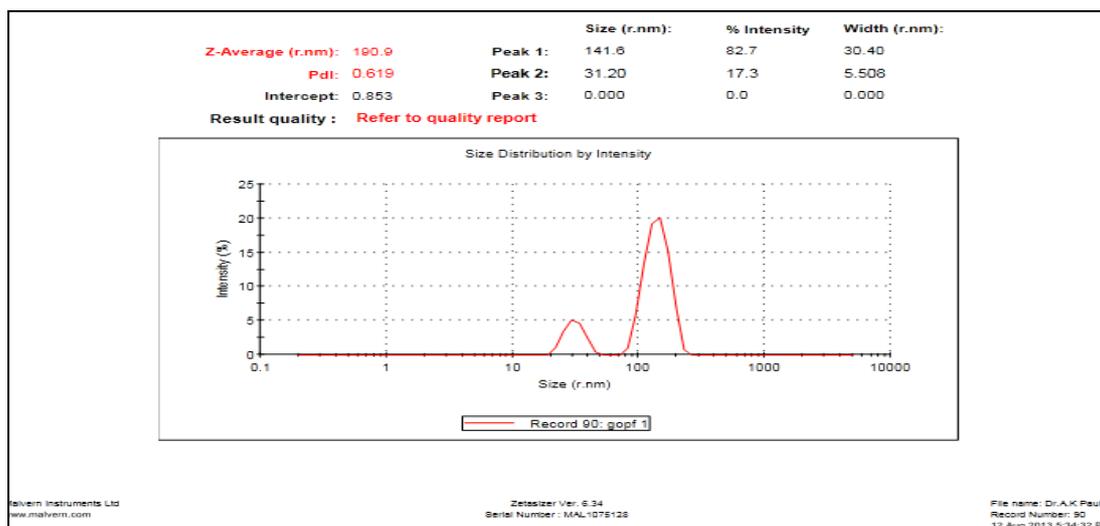


Figure No.14: DLS analysis of synthesized NPs with methanol extract of *A. auriculiformis*

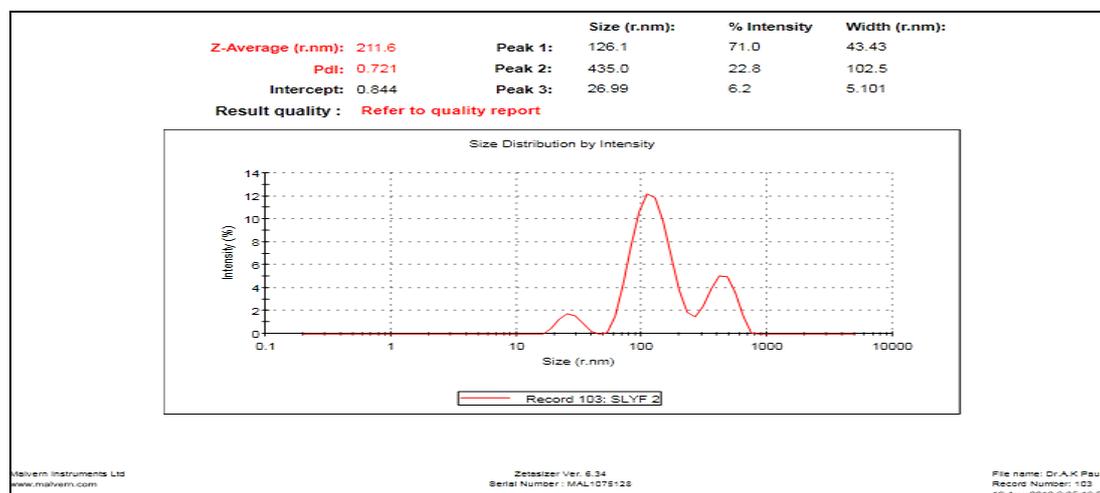


Figure No.15: DLS analysis of synthesized NPs with water extract of *A. auriculiformis*

## CONCLUSION

The present paper gives an idea about the green and ecofriendly synthesis method for silver nanoparticles. All the characterization techniques clearly reveal the reduction in particles size of crude extract with the addition of silver and the reaction resulted in silver nanoparticles synthesis. The biomedical applications of silver nanoparticles will be more pronounced when the ecofriendly and natural agents of synthesis take the place of conventional chemical agents.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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