Original Research Article

Leaf Extracts Silver Nanoparticles of Four Medicinally Important Plants: Used as a Green Manure

ABSTRACT:

Now a day's green synthesis of silver nanoparticles (AgNPs) from plants has an important role in biomedical science, drug discovery and also in biological field. In this investigation, we synthesize AgNPs using the aqueous solution of the leaf extract of 4 indigenous plant samples such as – *Abromaaugusta*,*Barringtoniaacutangula*,*Dilleniaindica*& *Eupatorium odoratum* in room temperature (35±2°C). After that, formation of AgNPs were confirmed by UV-VIS spectrum, thebandswereat (435, 445, 430 & 440nm), respectively. The average size of the AgNPsalsoconfirmed byParticle size analyzer(PAS), the*B. acutangular* species showed the best quality of silver nanoperticles among the other plant extracts. FTIR analysisshowed that,five biomolecule groups like phenols, aromatic, alkyne, alkane and alkene were found in among these plant samples. Our findings suggest that the seed germination percentage, relative seed germination rate, relative shoot & root growth and germination index of the tested plant depends upon concentration gradient of synthesize AgNPs. At 0.6 mg/ml conecentration, the tested plant samples give the best favourable growth condition.

Key Words:Leaf extract;phytochemical;seed germination;silver nano-partical.

1. INTRODUCTION

Nanoparticles are a special group of materials with unique features and extensive applications in diverse fields [1]. Itexhibits completely new or improved properties based on specific characteristics such as size, distribution and morphology. Among the various inorganic metal nanoparticles, silver nanoparticles have received substantial attention for various reasons – silver is an effective antimicrobial agent, exhibits low toxicity [2,3], silver nanoparticles have diverse in vitro and in vivo applications [4,5].Silvernanoparticles i.e. silver particles are of different sizes varying between 1-100 nm in size. Use of plant extract for the synthesisof nanoparticles could be advantageous over other environmentally benign biological processes by eliminating the elaborate process of maintaining cell cultures. Jose-Yacaman and co-workers first reported the formation gold and silver nanoparticles by living plants. Very recently green silver nanoparticles have been synthesized using various natural products like green tea (*Camellia sinensis*) [6] neem(*Azadirachtaindica*) leaf broth [7], starch [8], aloe vera plant extract [9] lemongrass leaves extract [10,11]leguminous shrub (*Sesbaniadrummondii*) [12]etc.

Nanomaterials have also been used for various fundamental and practical applications [13]. Seed germination in *Boswelliaovalifoliolata*[14] and *Pennisetumglaucum*[15]has been shown to be positively affected by treatment with AgNPs.Nanotechnology application to the agriculture and food sectors is relatively recent compared with its use in drug delivery and pharmaceuticals.

Hence the aim of present study is to develop a novel approach for the green synthesis of silver nanoparticles using Indian herbal plantsextracts as a reducing and stabilizing agent.We have carried out a unique protocol for synthesizing of Ag nanoparticles (temperature, time, extract preparation method and storage).The presentstudyhighlights (i) the methodsemployed in the synthesis of Ag

nanoparticles, characterized through UV-VIS absorption, FTIR, DLS analysis, (ii) the effects of silver nanoparticals on seed germination.We investigated the impact of AgNPapplication on the seed germination and seedling growth ofmung bean plants. We also try to make a fertilizer for agricultural land using plant extract containing $AgNO_3$.

2. MATERIALS AND METHOD

2.1.1Collection of plant materials:

In this present investigation four indigenous plant sample i.e.*Abromaaugusta*, *Barringtoniaacutangula, Dilleniaindica& Eupatorium odoratum* leaves were collected from Vidyasagar University campus [23.4320°N & 87.2979°E], Midnapore, West Bengal, India.

2.1.2.Preparation of plant extracts:

The 20 grams of healthy leaves sample was taken and washed 2-3 times with de-ionized water and surface sterilize for 20-30min. Then kept it in hot air oven at 60°C temperature for 72h for dry, leaves were ground by morter pestle.1gm leaf powder was weighed and mixed with 10ml distilled water. The mixture was poured in a test tube and boiled in water bath for 10-20min till aquas solution colour developed. Then samples were cooled at room temperature and filtred through Whatman filter paper and filtrate used as a plant extract[16].

2.1.3. Synthesis of Silver nanoparticles from plant extracts:

Silver nitrate (AgNO3) was purchased from Sigma-Aldrich, and the aqueous leaf extract solution of four plant samples were used for the bioreduction process. To synthesize silver nanoparticles from *A.augusta*, *B.acutangula, D.indica& E.odoratum,*90ml of 1mM $AgNO_3$ was taken in a 250ml conical flask and 1ml of plant extract was added to it, separately. Then the mixture was stirred on an electrical magnetic bar in dark condition for few hours in normal room temperature (RT). Changes of colour in aquas colloidal solution confirming green synthesis of silver nanoparticles [17].

2.1.4.UV-VIS Spectroscopy

From the bioreduction of silver ions with the four plant leave samples,small amount of aliquot was diluted using distilled water (1:9).The biosynthesized silver nanoparticles were optically measured by the ultraviolet–visible spectrophotometr (UV-VIS) (UV-3600) in 350- 550nm wavelength range[18].

2.1.5.PSA Analysis

Particle size of silver nanoparticles was carried out by PSA analyzer system [Malvern (Nano-ZS90)]. The average distribution of synthesize nanoperticles on the basis of volume, intensity and number weighting was studies comparatively.

2.1.6.FTIR Analysis

The dried plant leaves samples were subjected to FTIR analysis to detect the components after extraction fromthe plant leaves samples and characterization of the substance. The dried sample was mixed with potassium bromide (KBr) crystals and characterized by Fourier-transform infrared spectroscopy (FTIR), (Model no - Spectrum 2). The FTIR spectrum was obtained in the mid IR region of 400-4000cm^{-1} [19]

2.2. Methodology of Seed Germination Test:

2.2.1. Seedling Exposure

The seeds were checked for their viability by suspending them in double distilled water. The seeds which are settled to the bottom were selected for further study. Then surface sterilizations of seeds were done by immersing them into 5% sodium hypochloride solutions for 10 minutes[20]. After this seed were rinsed in double distilled water thrice and then surface sterilization of seeds was done completely. The germinations process was done by added 5 ml of nanoparticle solution into Petridis. 10 sterilized seeds were transferred into each Petridis and kept it for 3hr. In this experiment control seeds (treated with distilled water) were also taken for comparison with the treated ones. After 3hr the soaked seeds were put in prepared pots and placed under shadow condition.

2.2.2. Study of germination and seedling growth equation

This research experiments were conducted as factorial with entirely randomized with three replications on *Vigna radiata* seeds. Different concentration gradients (0.2, 0.6 & 1.0 gm/ml) synthesize nanoperticles were used in this experiment. After seedling, Seed germination percentage (SGP), Relative seed germination Rate (RSGR), Relative root growth (RRG), Relative shoot growth (RSG) and Germination index (GI) were calculated by the bellow given formula [21]$SGP=(G_S/T_S)\times100$, (Eq. (1)); $RSGR=(Sc\ /Ss)\times100$ (Eq. (2)); $RRG=(R_S/R_C)\times100$ (Eq. (3)); $RSG=(S_S/S_C)\times100$ (Eq. (4)) and $GI=(RSG/RRG)\times100$ (Eq. (5)).[Where,S_S= The number of seed germinated in sample; S_C = The number seed germinated in control; R_S = The average root length in sample; R_C = The average root length in control; S_S = The average shoot length in sample; S_C = The average shoot length in control.]After germination root length & shoot length were also recorded through following way, with the help of a thread and scale. Root length was taken from the point below the hypocotyls to the end of the tip of the root. Shoot length was measured from the base of the root-hypocotyl transition zone up to the base of the cotyledons.

3. RESULTS AND DISCUSSION

The detailed investigation on green synthesis silvernanoperticals by indigenous plant leaves extract such as *A.augusta*, *B.acutangula, D.indica*& *E. odoratum*were reported in this present work. When indigenous plant leaves extract mixed with aqueous solution of silver ions, its reduced to the silver nanoparticles.Thecolour change was observed after incubation,the solution turned from dark brown to faint yellow and then to bright yellow of the reaction mixture, which revealed that a clear indication of the formation of silver nanoparticles[22].

3.1. UV-VIS spectroscopy

The sample was monitored under UV-VIS spectrophotometer for knowing the stability and formation of reduced colloidal silver nanoperticles. In UV-VIS spectra, plant leaves sample i.e. *A. augusta*, *B. acutangula, D. indica*& *E. odoratum* showed maximum absorbance at 435nm, 445nm, 430nm and 440nm, respectively. (Fig.1). Previous researcher established that absorbance at around 430nm for green synthesise silver nanoparticle is a characteristic feature for that noble metal solution [16, 23 &24]. Absorbance in different wave length due to colours, arise from metal nanoperticles due to the resonance properties of surface plasmon in the silver metal.

3.2. PSA analysis

The evidance of size distribution report through particle size analyser revealed that the nanoperticlessynthesize from *B. acutangula*plant leaves extract showed the best quality (smallest size & width)of naoperticles rather than *A. augusta, D. indica*and *E. odoratum.* The particle size range

under the light scattering,synthesized silver nanoparticles were showed that 99.6% of distribution of particles have 16.24 r.nm size & width 3.428 r.nm, in *A.augusta*, 100% of distribution of particles have 22.44 r.nm size & width 6.674 r.nmin*B.acutangula*, 84.7% of distribution of particles have 96.46 r.nm size & width 45.05 r.nm, in *D.indica,*and 100% of distribution of particles have 33.99 r.nm size & width 9.280 r.nmin *E.odoratum*(Fig. 2).

3.3. FTIR analysis

FTIR was carried out to identify the biomolecules that present in leaf extracts of four species. The characterization of bio molecules depends on the absorption bands which were observed at different spectra like- in case of *Abromaaugusta*, band 1611.5 and1595.3cm^{-1}containN-H bending, N=N stretching;N-H bending, C=C stretching, N=N stretching group respectively. In case of *Barringtoniaacutangula*, band 2928.1, 1638, 1561.6, 1488.4, 1396, 1370.5, 1249.5, 1163.5 and 1087cm^{-1} contain C-H stretching; C=N stretching, N-H bending, C=C stretching; N-H bending, C=C stretching, C=C stretching,C-O stretching, C-N vibrations, S=O stretching, C-O stretching, C-N vibrations, S=O stretching, C-N vibrations, S=O stretching, S=O stretching, C=S stretching, C-N vibrations, S=O stretching, C=S stretching, C-N vibrations group, respectively. In case of*Dilleniaindica*, band 3465.6, 3438.6, 2116.8 and 1634.6 cm^{-1}**c**ontain N-H stretching; N- H stretching; C=C stretching; C=N stretching, N-H bending group respectively. In case of *Eupatorium odoratum*, band 1599.9, 1488.4, 1399.2, 1185.8 and 1180.6 cm^{-1}containN-H bending, N=N stretching; C=C stretching, S=O stretching, C-O stretching, C-N vibrations, C=S stretching, S=O stretching, C-N vibrations; C=S stretching, S=O stretching, C-N vibrations group respectively (Fig. 3). Interms of chemical compounds present in plant extracts presented varied resuls between the investigated plant samples. In this present work five types of plant extract chemical compounds were found such as phenols (C_6H_5OH), aromatic ($C_{4r}+2H_{2r+4}$), alkyne (C_nH_{2n-2}), alkane (C_nH_{2n+2}) and alkene (C_nH_{2n}). FTIR analysis showed that phenols, aromatic, alkane and alkene were present in *B. acutangular,* where alkyne and aromatic compounds were present in *D. indica* and *A. augusta,* respectively. But, *E. odorata* species conatian only phenolic and aromatic compounds (Table.1).

4. Application of silver nanoparticles on seed germination and seedling growth of Mungbean seed (*Vigna radiata* L.)

The seed germination test was conducted on mungbean seeds by the use of synthesize silver nanoparticles. We used three different concentrations of silver nanoparticles like 0.2, 0.6 and 1.0 gm/ml to know the impact of seed germination percentage, relative seed germination rate, relative shoot & root growth and germination index.In terms of parsley germination percentage, germination indexes,relative root & shoot growth and seed germination rate were affected significantly by adding synthesize silver nanoperticles concentration. From box&whiskerplotit was revealsthat,themaximum germination percentage was achived by 0.6 gm/ml concentration nanoperticlesin*B. acutangular, A.augusta*,*D.indica*&*E. odoratum*species.Relative seed germination rate and germination index showed some varied results where, *E. odoratum*have maximum RSGP in 1 mg/ml concentrations of synthesize silver nano-perticles, and *D. indica* has highest GI in 1 mg/ml concentrations of synthesize AgNPs, rest plant species were better grown in 0.6 gm/ml concentrations.Relative root growth & Relative shoot growth, both were also better in0.6 mg/ml concentration [25] (Fig.4).

5. CONCLUSION

Silver nanoparticles prepared in this process are fast, suitable, eco-friendly, and can be potentially applied in variety of extracts for preparing different metal nanoparticles. Some biomolecules characterized by FTIR spectroscopy on the basis of bands or peaks like alkenes, phenols, alkyne and aromatic, polysaccharides, carboxylic, aldehyde, acid and amino group. From these molecules we can prepare different kind of drugs against different disease. The present study also included the seed germination and root elongation is a rapid and widely used acute phytotoxicity test owing to

sensitivity, simplicity, low cost and suitability for unstable chemicals. Seed coats, which can have selective permeability, play a very important role in protecting the embryo from harmful external factors. Pollutants as nano-metals could penetrate root system causing obviously root growth inhibition, may not affect seed germination if they cannot pass through seed coats. This may explain that seed germination in our study was not affected by exposure to AgNPs suspension. Exposure to nanomaterials can encourage earlier plant germination and improve plant production.The outcomes of this study are useful for determining the biocompatibility of AgNPs and for identifying potential agricultural applications for nanoparticles in crop improvement and food production.

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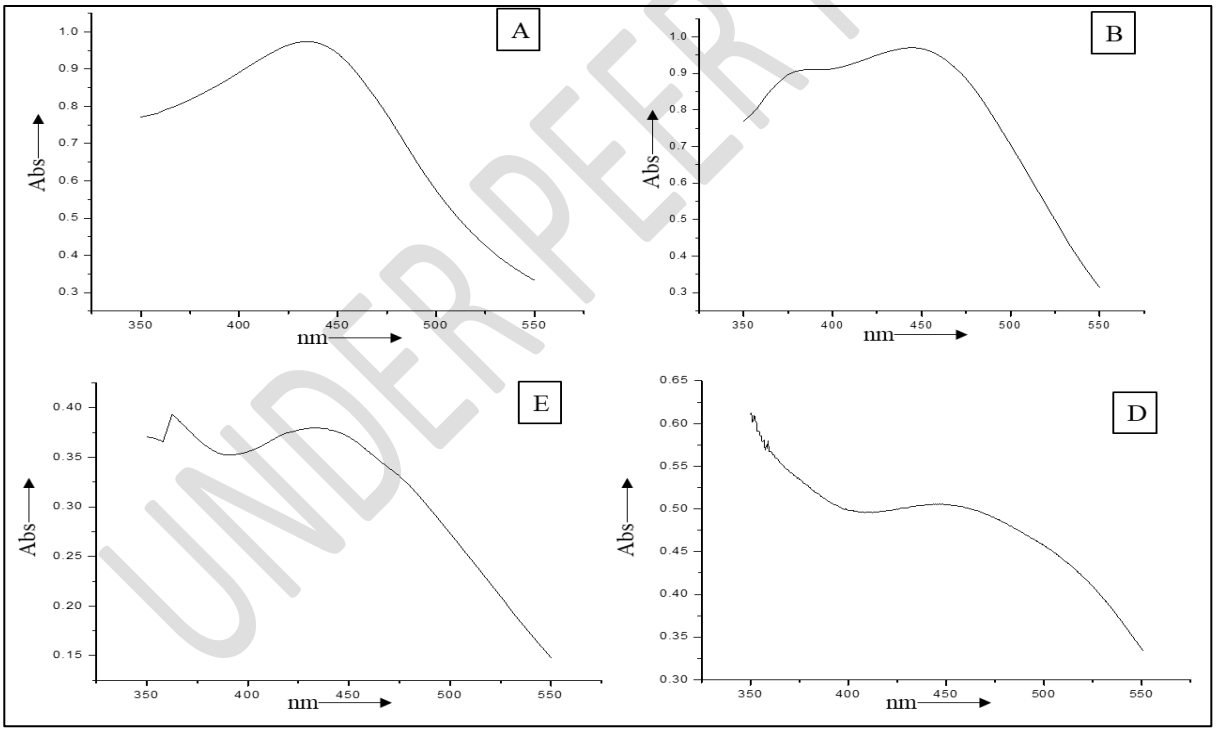

Figure- 1: UV-VIS spectra of reduction of Ag^{+} ions of (A) *Abromaaugusta*(B) *Barringtoniaacutangula* (C) *Dilleniaindica* (D) *Eupatorium odoratum.*

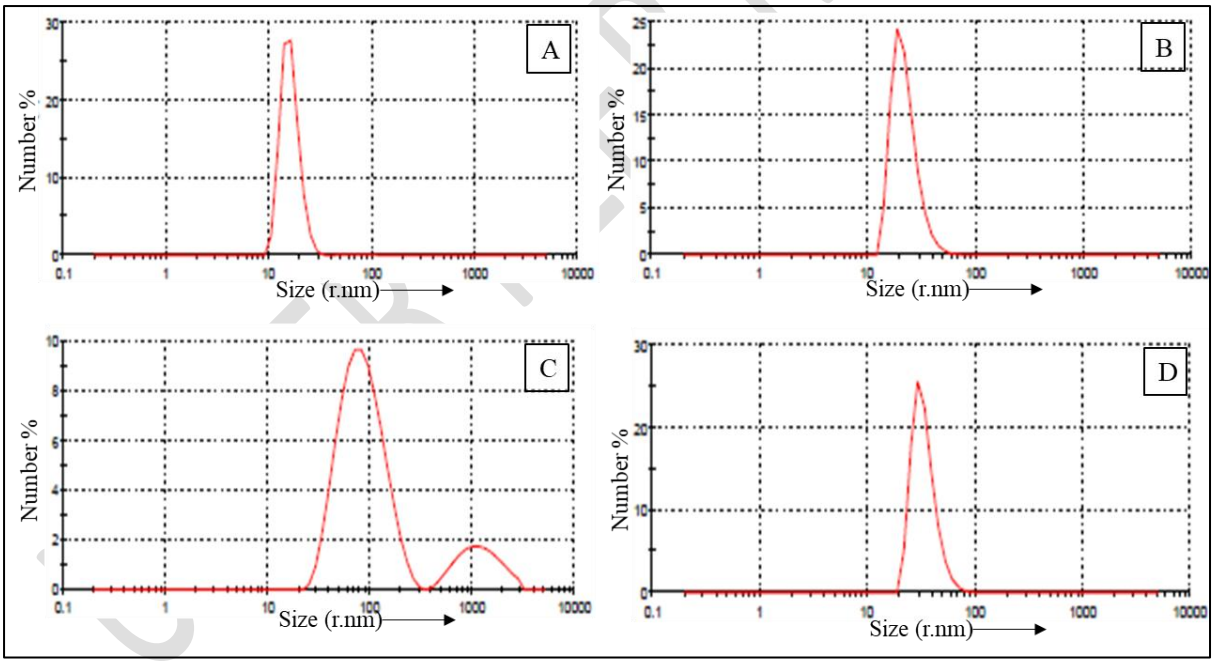

Figure- 2:PerticalSizeanalyser (PSA) image of synthesized silver nanoparticles of (A) *Abromaaugusta*(B) *Barringtoniaacutangula* (C) *Dilleniaindica* (D) *Eupatorium odoratum.*

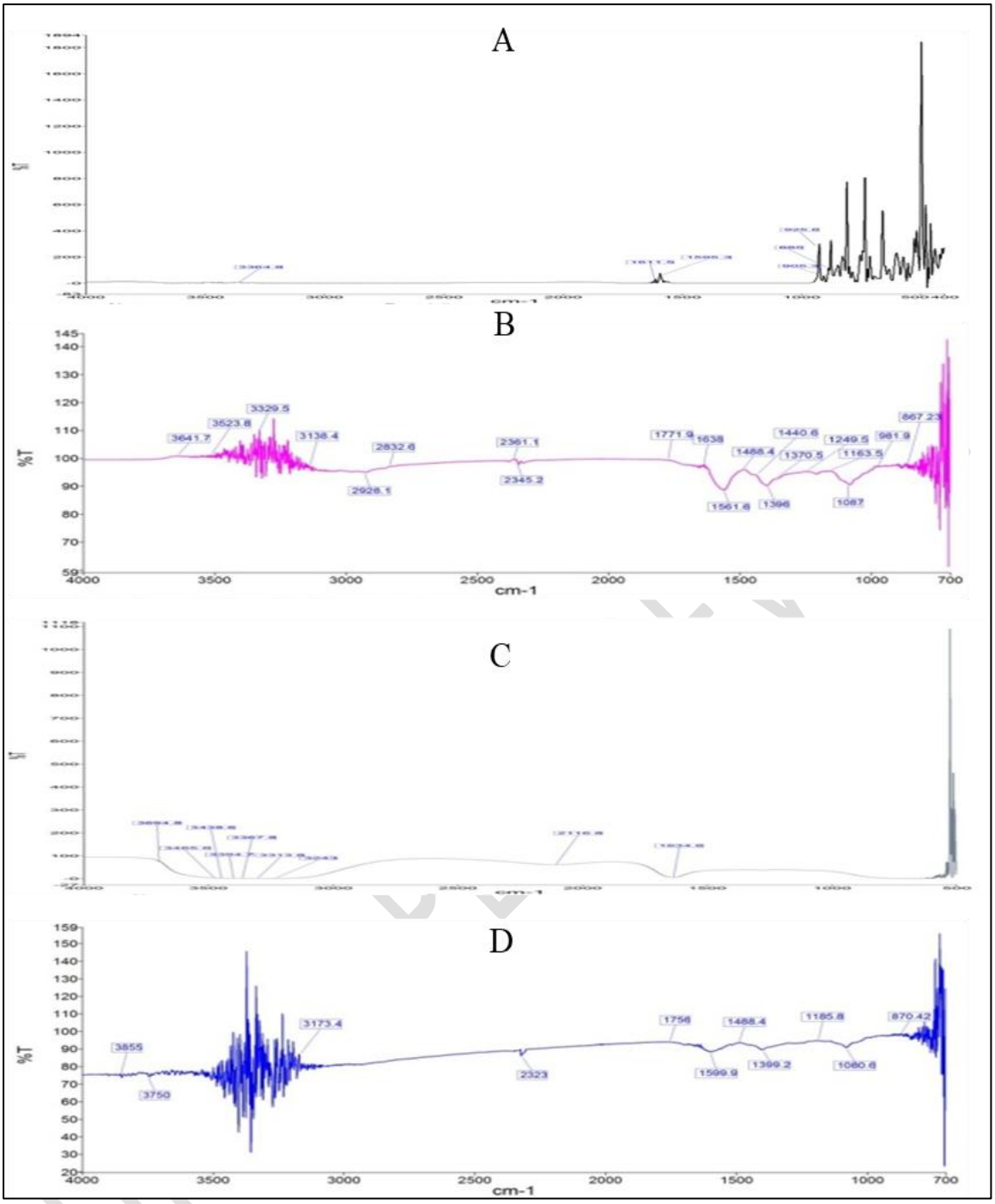

Figure-3:FTIR images of leaf extracts of (A) *Abromaaugusta*(B) *Barringtoniaacutangula* (C) *Dilleniaindica* (D) *Eupatorium odoratum*.

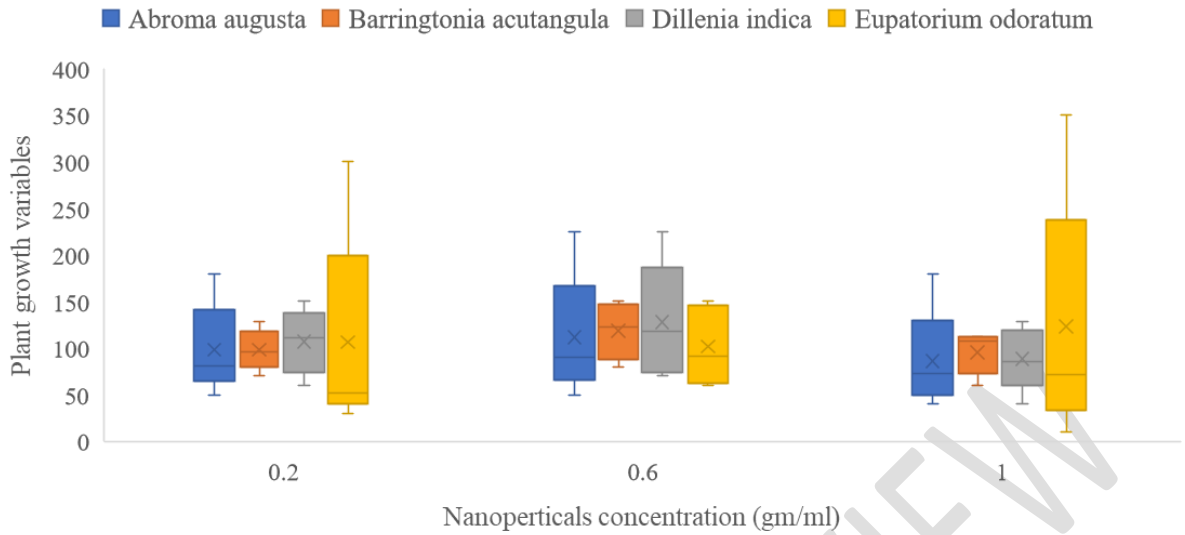

Figure- 4: Box & whisker plot of different plant growth variables with synthesize silver nanoperticles.

Table-1: Different phytochemical group present in extracted plant leaf samples.

Chemicalcompounds	Structure	*E.odorata*	*D. indica*	*B. acutangula*	*A. augusta*
Phenols	C_6H_5OH	+	-	+	-
Aromatic	$C_{4r}+2H_{2r+4}$	+	-	+	+
Alkyne	C_nH_{2n-2}	-	+	-	-
Alkane	C_nH_{2n+2}	-	-	+	-
Alkene	C_nH_{2n}	-	-	+	-