Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(4): 2788-2794. **OPEN ACCESS**

Phytochemical analysis, green synthesis of silver and gold nanoparticles, and antibacterial activity of *Eryngium amethystinum*

Taj Ur Rahman[1,*], Yasir Khan[1], Muhammad Aurang Zeb[1], Hammad Khan[1], Wajiha Liaqat[1], Gul Rukh[2], Sheheryar[3], Haroon Khan[4], and Syeda Kainat Izhar[2]

[1]Department of Chemistry, Mohi-Ud-Din Islamic University, AJ&K, **Pakistan**
[2]Department of Chemistry, Abdul Wali Khan University, Mardan, **Pakistan**
[3]Department of Biochemistry and Molecular Biology, Federal University of Ceara, **Brazil**
[4]Department of Weed Science and Botany, The University of Agriculture, Peshawar, **Pakistan**

***Correspondence:** taj_urrehman81@yahoo.co.uk **Received 22-02-2021, Revised: 13-11-2021, Accepted: 15-11-2021 e-Published: 16-11-2021**

The main aim of this research work was to explore crude extracts of *E. amethystinum* phytochemicaly and to synthesize its gold and silver nanoparticles. Phytochemical analysis of methanolic extract indicated the presence of flavonoids, reducing sugar, steroids, terpenoid & glycoside while water extract indicated the presences of alkaloids, reducing sugar, terpenoids, steroids & anthraquinones. Methanolic extract of known concentration were subjected to synthesize silver and gold nanoparticles which were characterized by using well established techniques including UV-Visible spectrophotometric analysis, Fourier-transform infrared spectroscopy, X-ray diffraction and Transmission electron microscopy. The synthesized nanoparticles and crude extracts were further tested against different bacterial strains. The results obtained revealed that this plant is important from medicinal point of view and it needs further phytochemical and biological investigation to explore its hidden medicinal potential.

Keywords: phytochemicals; green synthesis; nanoparticles, antibacterial

INTRODUCTION

The genus *Eryngium* L. is one of the major and most complex genera of the family *Apiaceae* (Calviño et al. 2007) which comprises more than 250 species distributed in Asia, Europe, Australia, North Africa, North, and South America, (Wörz., 2004; Wörz., 2005). Roots of *E. aquaticum* are expectorant, diuretic, diaphoretic, and in large doses emetic. They are used mainly in the treatment of complaints of the sexual organs and kidneys and also as a remedy for snake poison (Coffey, 1993; Moerman, 2009). The root of *E. caucasicum* is nervine and aphrodisiac, whereas the ashes of the plant are used in the treatment of hemorrhoids (Usher, 1974). Infusions of the root and aerial parts of *E. campestre* have shown galactofuge, diaphoretic, antispasmodic, diuretic, expectorant, aromatic, and stimulant properties (Grieve, 1971; Baytop., 1999; Chiej, 1984). Phytochemical studies performed on *Eryngium* species including several secondary metabolites such as phenolics, flavonoids, polyacetylenes steroids, terpenoids, triterpenoid saponins, coumarins, and rosmarinic acid derivatives (Marčetić et al. 2014; Bouzergoune et al. 2016). Antimicrobial, anti-diabetic antioxidant, cytotoxic, antimalarial, anti-mutagenic, and anti-inflammatory activities have been reported from *Eryngium* species (Ural et al. 2014; Benmerache. 2016).

The present work aimed to evaluate the phytochemical analysis and green synthesis of

silver and gold nanoparticles, and biological activity of *E. amethystinum* extracts. And synthesized nanoparticles.

MATERIALS AND METHODS

2.1. Plant material

E. amethystinum was collected from different areas of District Swabi Khyber Pakhtunkhwa in the month of June 2017 and authenticated by the Department of Botany, University of Peshawar. The vouchers specimens No. Hmd-36 was deposited in the Herbarium, Department of Botany, University of Peshawar, KP, Pakistan. The plant material was washed and dried in shade for fifteen days then chopped and powdered using a grinder and soaked in methanol for 14 days. In this duration the plant materials were separated by process of filtration into two separate flasks. The procedure was repeated three times until a clear crud extract was obtained. The filtrate was evaporated under reduced pressure by using Vacuum Rotary Evaporator keeping the temperature at 40˚C to give crude extracts.

2.2. Phytochemical screenings

The phytochemical screening of both extracts was performed using standard procedures (Wadood et al. 2013).

2.3. Methanolic stock solution

200 mg of methanolic extract was taken and mixed with 200 ml of methanol. The solution was filter in volumetric flask and suspended impurities were removed by filtration and the volumetric flask was kept closed in order to prevent it from fungal attack and evaporation (Logeswari 2015).

2.4. Preparation of 1 mM silver nitrate solution

For the preparation of 1 mM Silver nitrate ($AgNO_3$) 0.169g of $AgNO_3$ was added to 1000 ml of distilled water in a volumetric flask and kept closed and coated by aluminum foil to prevent it from sunlight contact and the container was kept in the refrigerator at 4˚C (Logeswari 2015).

2.5. Preparation of 1 mM gold salt solution

To prepare 1 mM solution of gold salt 0.357g of Auric chloride ($AuCl_3.2H_2O$) was weighed accurately and added to 1000 ml of volumetric flask and makes it 1000 ml solution in de-ionized water the flask was also kept closed and coated with aluminum foil to prevent it from sunlight and stored it in the refrigerator at 4 ˚C (Huang et al. 2007).

2.6. Synthesis of silver nanoparticles

Silver NP's were synthesized by using the following method. Stocks solution of methanol was mixed with salt solutions of silver in a small 200 ml round bottom flask in different ratios such as 1:1, 1:2, 1:3, 1:4, and 1:5. The round bottom flask was connected to the condenser to prevent the escape of vapors and also coated with aluminum foil to prevent the reaction mixture from light. The reaction mixture 1:1, 1:2, 1:3, 1:4, and 1:5 was stirred first at ambient temperature and then warmed up to 40-80˚C for 1-24 hours. The color change was noticed before and after heating. The change in color from transparent to dark brown showed the reduction process and confirmed the green synthesis of silver nanoparticles. The above-given ratios were heated at 30˚C, 40˚C, 50˚C, 60˚C, 70˚C, and 80˚C for 45 minutes up to 24 and 72 hours (Logeswari 2015).

2.7. Synthesis of gold nanoparticles

Gold NP's were synthesized by using the following method. Stocks solution of methanol were mixed with salt solutions of gold in a small 200 ml round bottom flask in different ratios such as 1:1, 1:2, 1:3, 1:4, and 1:5. The round bottom flask was connected to the condenser to prevent the escape of vapors and also coated with aluminum foil to prevent the reaction mixture from light. The reaction mixture 1:1, 1:2, 1:3, 1:4, and 1:5 was stirred first at ambient temperature and then warmed up to 40-80˚C for 1-24 hours. The color change was noticed before and after heating. Change in color from transparent to dark bluish showed the reduction process and confirmed the green synthesis of gold nanoparticles. The above-given ratios were heated at 30˚C, 40˚C, 50˚C, 60˚C, 70˚C, and 80˚C for 45 minutes up to 24 and 72 hours (Huang et al. 2007).

2.8. Characterization of synthesized nanoparticles

The nanoparticles were characterized by using different techniques like UV, FTIR, XRD and TEM (Alaqad and Saleh., 2016).

2.9. Antibacterial activity

The antibacterial potential of methanol and water crude extract of *E. amethystinum* and green synthesized NP's were checked by Ager well diffusion method (Kora et al. 2012).

RESULTS AND DISCUSSION

3.1. Phytochemical screenings

The results of the phytochemical screening of methanolic and water extract of *E. amethystinum* are listed in (Table 1).

3.2. UV-Visible spectrophotometric analysis of silver nanoparticles

Synthesis of silver NP's from mixing silver salt solutions with methanolic extract of *E. amethystinum* was observed from color change. Changes in color from transparent to dark brown easily indicated the formation of silver NP's. Synthesis of silver nanoparticles at different ratios such as 1:1, 1:2, 1:3, 1:4, and 1:5 was done, in which efficient synthesis was observed for 1:5 mixture for which UV-Vis spectra was taken which comes in 400-450 nm range which confirms the existence of silver nanoparticles. The reaction mixture 1:5 was also run for a period ranging from 6-24 hours which showed the synthesis of silver nanoparticles was enhanced when the period for reaction mixture was increased maximum absorption occur at 445 nm (Figure 1).

Table 1: Phytochemical screening of *E. amethystinum* crud extracts.

Chemical constituents	Methanolic extract	Water extract
Alkaloids	_	+
Tannins	_	_
Anthraquinones	_	+
Glycoside	+	_
Reducing Sugar	+	+
Saponins	_	_
Flavonoids	+	_
Phlobatannis	_	_
Steroids	+	+
Terpenoids	+	+

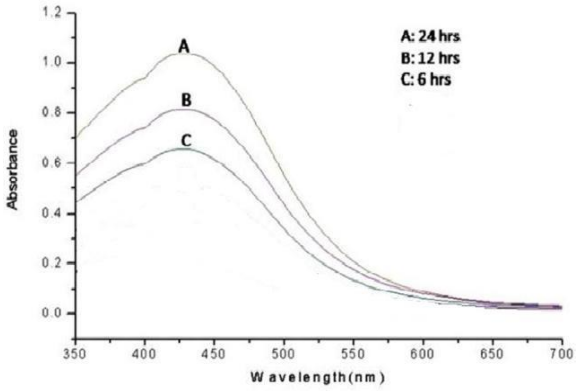

Figure 1: UV spectra of silver nanoparticles

3.3. UV-Visible spectrophotometric analysis of Gold nanoparticles

Synthesis of gold NP's from mixing gold salt solutions with methanolic extract of *E. amethystinum* was observed from color change. Changes in color from transparent into light bluish easily indicated the formation of gold NP's. Synthesis of gold nanoparticles at different ratios such as 1:1, 1:2, 1:3, 1:4, and 1:5 was done, in which efficient synthesis was observed for 1:3 mixture for which UV-Vis spectra was taken which comes in 500-600 nm range which confirms the existence of gold nanoparticles. The reaction mixture 1:3 was also run for a period ranging from 30-90 minutes which showed the synthesis of gold nanoparticles was enhanced when the period for reaction mixture was increased maximum absorption occur at 550 nm (Figure 2).

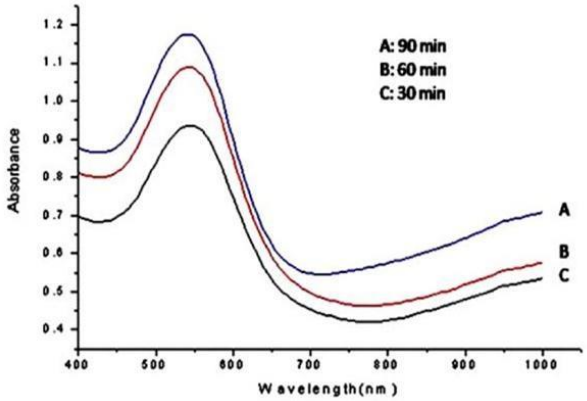

Figure 2: UV spectra of gold nanoparticles

3.4. FTIR analysis of silver nanoparticles

FTIR spectroscopy is an effective technique for the characterization of nanoparticles. Fourier transformation infrared spectroscopy (FTIR) Perkin Elmer spectrometer (Bruker tensor 27) was used for the identification of the green synthesized silver nanoparticles. The data obtained from (Figure 3) revealed a band that appeared at 3400 cm^{-1} corresponds to –OH stretching frequency of phenol and carboxyl group. The spectra at 2145 cm^{-1} show carbonyl stretching ~~starching~~ and be~~i~~nding of amide groups, peak at 1648 cm^{-1} are observed for carbonyl group(C=O) of polyphenols, while the peak at 1050 cm^{-1} is assigned to absorption of C-O-C or -C-N of ether and aliphatic amines while the peak at 736 cm^{-1} indicating the stretching and bending of C-H in the aromatic ring. In FTIR spectra all of the above data shows various groups such as carbonyl, carboxyl, amine,

phenol, and ether group which act as capping and reducing agents in the green synthesis of silver nanoparticles.

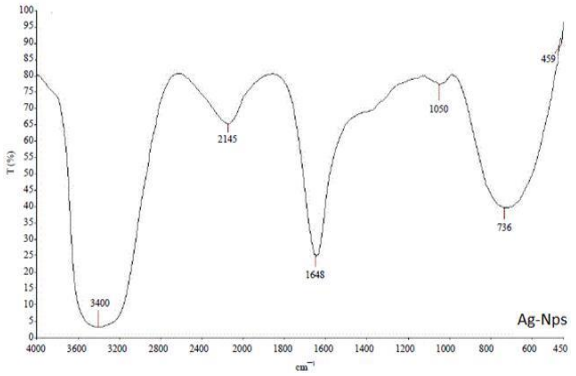

Figure 3: FTIR spectra of silver nanoparticles

3.5. FTIR analysis of gold nanoparticles

FTIR (Bruker tensor 27) spectrometer was also used for the characterization of the green synthesized gold nanoparticles. The data obtained from (Figure 4) revealed a band that appeared at 3440 cm^{-1} corresponds to the –OH stretching frequency of phenol and carboxyl group. The spectra at 2364 cm^{-1} show the stretching and bending frequency of carbon to carbon triple bond in alkynes, peak at 2345 cm^{-1} is observed for carbonyl group (C=O), peak at 2092 cm^{-1} are assigned for nitro group bending, peak at 1637 cm^{-1} indicating the stretching and bending of C=N in imines while the peak at 693 cm^{-1} further confirms the synthesis of gold nanoparticles.

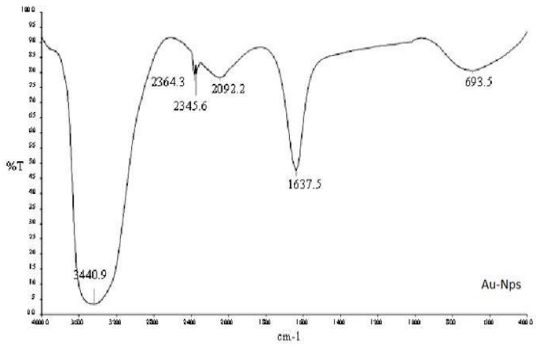

Figure 4: FTIR spectra of gold nanoparticles

3.6. XRD analysis of silver nanoparticles

The X-ray diffraction intense peaks in the XRD spectra (Figure 5) of synthesized silver nanoparticles using the *E. amethystinum* extract were at 111, 200, 220, and 311. The sizes of silver nanoparticles were obtained by using Debye–Scherer's rule $D = K\lambda/(\beta\cos\theta)$, where D illustrated precious stone size, K is the Scherr's constant, λ represent the X-ray wavelength for which 2θ values comes as 38.13°, 44.43°, 64.66°, and 77.66°. The range of λ in this equation is 0.9 and β is the line thickness at half-maximum height. The XRD spectrum recommended that the synthesized silver nanoparticles using the *E. amethystinum* extract are crystalline and having face-centered geometry.

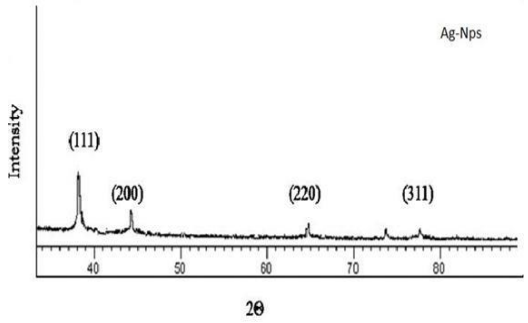

Figure 5: XRD spectra of silver nanoparticles

3.7. XRD spectra of gold nanoparticles

The XRD techniques were also used to conclude the geometrical shape and crystallinity of gold nanoparticles. The X-ray diffraction intense peaks in the XRD spectra (Figure 6) of synthesized gold nanoparticles using the *E. amethystinum* extract were at 111, 200, 220, and 311 for which 2θ values also comes as 38.13°, 44.43°, 64.66°, and 77.66° which concluded that the synthesized gold nanoparticles using the *E. amethystinum* extract are also crystalline and having FCC geometry.

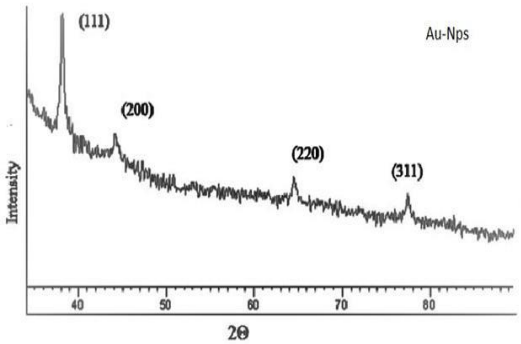

Figure 6: XRD spectra of gold nanoparticles

3.8. TEM analysis of silver nanoparticles

Shape, size, and morphologies of synthesized silver NP's were examined with transmission electron microscopy and are shown in (Figure 7) the obtained result showed that the synthesized silver NP's had spherical and hexagonal shapes and lies in the size range of 100 nm. TEM result further confirmed the existence of the synthesized silver nanoparticles. The images were taken at 100,000 magnifications at 100 kV.

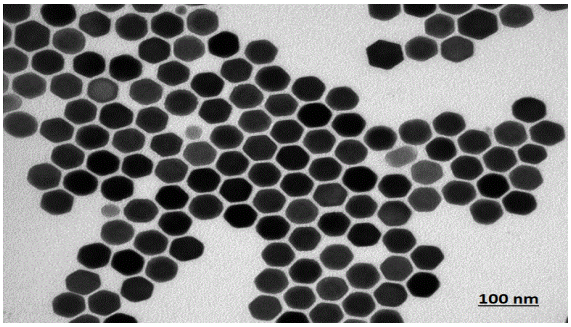

Figure 7: TEM image of silver nanoparticles

3.9. TEM analysis of gold nanoparticles

Shape, size, and morphologies of synthesized gold NP's were also examined with transmission electron microscopy and are shown in (Figure 8) the obtained result showed that the synthesized gold NP's had also a spherical shape and lies in a size range of 100 nm. TEM result further confirmed the existence of the synthesized gold nanoparticles. This image was also taken at 100,000 magnifications at 100 kV.

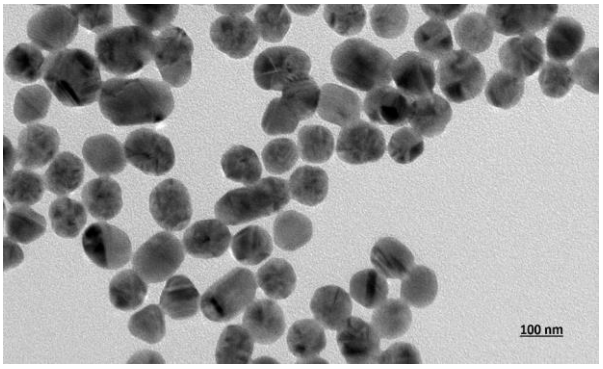

Figure 8: TEM image of gold nanoparticles

3.10. Antibacterial activity of crude extracts and synthesized nanoparticles

Antibacterial potential of crude *E. amethystinum* extract of methanol, water, and synthesized gold and silver nanoparticles were checked by using the agar well diffusion method. For this anti-microbial assay agar medium of different bacterial strains such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus cereus, Salmonella typhi,* and *Methicillin-resistant Staphylococcus aureus* were made in agar plate then *E. amethystinum* extract of methanol, water, and synthesized gold nanoparticles along with standard *Ampicillin* was poured into an agar plate.

The antimicrobials present in the crude extract of methanol, water, and synthesized gold and silver nanoparticles diffused into freshly prepared bacterial strains and inhibited their growth. The resulted zone of inhibition was circular because there was a uniform flow of growth. The inhibited zone diameter was measured in millimeters which are shown in (Table 2), to get an accurate result each *E. amethystinum* extract and synthesized gold and silver nanoparticles were treated for the same bacterial strain thrice and the result was obtained by taking their mean.

The antibacterial potential of the methanolic extract, water extract, and synthesized silver and gold nanoparticles are given in above (Table 2) and are also expressed in graphical form (Figure 9).

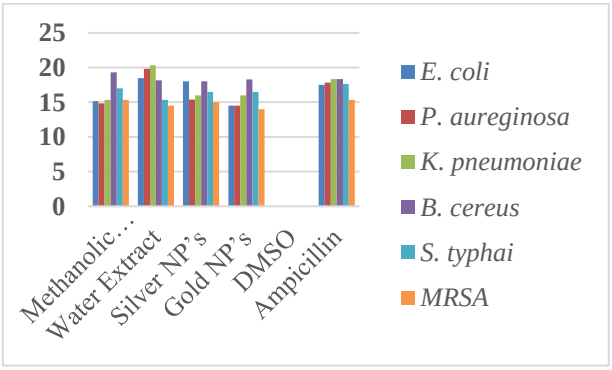

Figure 9: Graphical representation of the antibacterial activity of crude extracts, and synthesized silver and gold nanoparticles.

Methanolic extract from the above data showed good inhibition 19.33 mm against *B. cereus* while crude extract of water showed inhibition 18.5 mm, 19.83 mm and 20.33 mm against *E. coli*, *P.aureginosa,* and *K. pneumonia.* On the other hand, silver nanoparticles showed inhibition of 18 mm each against *E. coli* and *B. cereus* while gold nanoparticles showed inhibition of 18.3 mm against *B. cereus.* In this bioassay standard, Ampiciline was used as positive control and DMSO was used as a negative control.

Table 2: Antibacterial activity of crude extracts and, synthesized silver and gold nanoparticles.

Sample	*E. coli*	*P. aureginosa*	*K. pneumoniae*	*B. cereus*	*S. typhai*	*MRSA*
Methanolic extract	15.16	14.83	15.33	**19.33**	17	15.33
Water extract	18.5	19.83	20.33	18.16	15.33	14.5
Silver NP's	18	15.4	16	18	16.5	15
Gold NP's	14.5	14.5	16	18.3	16.5	14
DMSO	0	0	0	0	0	0
Ampicillin	17.5	17.83	18.33	18.33	17.67	15.33

CONCLUSION

In the current study, phytochemical screenings, and synthesis of gold and silver NP's was carried out using extract of the medicinally important plant *E. amethystinum*. The synthesized NP's were characterized by various characterization techniques. The crude extracts and synthesized nanoparticles were tested against different bacterial strains. The results of antibacterial activity revealed that the crude extracts and synthesized nanoparticles possess significant antibacterial potential. However, further in vivo experiments must be carried out to explore its hidden medicinal importance.

CONFLICT OF INTEREST

The authors declared that the present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The authors are grateful to the Department of Chemistry, Mohi-Ud-Din Islamic University, AJ&K, Pakistan for providing lab facilities.

AUTHOR CONTRIBUTIONS

All the authors contributed equally.

REFERENCES

Alaqad, K., & Saleh, T. (2016). Gold and silver nanoparticles: synthesis methods, characterization routes and applications towards drugs. J. Environ. Anal. Toxicol,Vol, 6, 525-2161.

Baytop, T. (1999) Therapy with Medicinal Plants in Turkey-Past and Present. 2nd ed.. Nobel Tip Basimevi, Istanbul.

Benmerache A, Magid AA, Berrehal D, Kabouche A, Voutquenne-Nazabadioko L, Messaili S, Abedini A, Harakat D, Kabouche Z. Chemical composition, antibacterial, antioxidant and tyrosinase inhibitory activities of glycosides from aerial parts of Eryngium tricuspidatum L. Phytochem Lett. 2016; 18: 23–28.

Bouzergoune F, Ciavatta ML, Bitam F, Carbone M, Aberkane MC, Gavagnin, M. Phytochemical study of Eryngium triquetrum: isolation of polyacetylenes and lignans. Planta Med. 2016; 82: 1438–1445.

Calviño CI, Martínez SG, Downie SR. (2007) The evolutionary history of Eryngium (Apiaceae, Saniculoideae): rapid radiations, long distance dispersals, and hybridizations. Molecular Phylogenetics and Evolution, 46, 1129-1150.

Chiej R. (1984) Encyclopaedia of Medicinal Plants. MacDonald, Edinburgh.

Coffey, T. (1993). The history and folklore of North American wildflowers. Facts on File Limited. Grieve M. (1971) A Modern Herbal. Dover Publications, Inc., Minola, N.Y.

Huang, J., Li, Q., Sun, D., Lu, Y., Su, Y., Yang, X., & Hong, J. (2007). Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. Nanotechnology,Vol, 18, 105104.

Kora, J., Beedu, R., & Jayaraman, A. (2012). Size-controlled green synthesis of silver nanoparticles mediated by gum ghatti (Anogeissus latifolia) and its biological activity. Organic and medicinal chemistry letters,Vol, 2, 17.

Logeswari, P., Silambarasan, S., & Abraham, J. (2015). Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. Journal of Saudi

Chemical Society,Vol, 19, 311-317.
Marčetić MD, Petrović SD, Milenković MT, Niketić MS. Composition, antimicrobial and antioxidant activity of the extracts of Eryngium palmatum Pančić and Vis. (Apiaceae). Cent Eur J Biol. 2014; 9: 149–155.Moerman DE. (2009) Native American Medicinal Plants: An Ethnobotanical Dictionary. Timber Press, Portland, OR.
Ural IO, Kayalar H, Durmuskahya C, Cavus I, Ozbilgin A. In vivo antimalarial activity of methanol and water extracts of Eryngium thorifolium Boiss (Apiaceae Family) against P. berghei in infected mice. Trop J Pharm Res. 2014; 13: 1313–1317.
Usher G. (1974) A Dictionary of Plants Used by Man. Macmillan Pub. Co., New York.
Wadood, A., Ghufran, M., Jamal, B., Naeem, M., Khan, A., & Ghaffar, R. (2013). Phytochemical analysis of medicinal plants occurring in local area of Mardan. Biochem Anal Biochem, Vol, 2, 1-4.
Wörz A. (2004) On the distribution and relationships of the South-West Asian species of Eryngium L. (Apiaceae-Saniculoideae). Turkish Journal of Botany, 28, 85-92.
Wörz A. (2005) A new subgeneric classification of the genus Eryngium L. (Apiaceae, Saniculoideae). Botanische Jahrbücher fur Systematik, Pflanzengeschichte und Pflanzengeographie, 126, 253-259.