

# Evaluation of Pharmacological Features and Nanoparticle Formation by *Urena lobata*

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## Abstract

*Urena lobata* commonly known as Congo jute or hibiscus burr is a shrub belonging to the family Malvaceae widely distributed throughout India. The plant is utilized by the traditional healers for various ailments such as arthritis, diabetes, cough and malaria. In the present study, phytochemical constituents and antioxidant property of *Urena lobata* was studied in both normal extract and silver nanoparticle form. The antioxidant property of leaf of the plant was also evaluated. We have reported alkaloid, saponin, steroid, terpenoid, tannin, flavonoid and carbohydrate and significant antioxidant activity by DPPH method from this plant. For the first time, silver nanoparticle was successfully synthesized using *Urena lobata* leaf extract in the current investigation and characterized by UV-VIS spectrophotometer and the characteristic surface plasmon resonance peak was identified to be  $\lambda_{424}$  nm. X-ray diffraction (XRD) was employed to ascertain the crystalline nature and purity of the silver nanoparticles which implied the presence of 111, 002, 022, 113, and 222 lattice planes of the cubic structure of metallic silver and Fourier transform infrared spectroscopy (FTIR) was used to determine specific functional groups in silver nanoparticles and the capping agents present in the leaf extract.

**Keywords:** *Urena lobata*, diabetes, saponin, Fourier transform infrared spectroscopy (FTIR).

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## INTRODUCTION

The plant with precious medicinal properties continues to obtain a keen awareness by several scientists for the complete range of biological activities, which range from antibiotics to antitumor. Plants and natural products have long played a crucial role in the treatment of various illnesses either as pure compounds or as extracts. They offer an invaluable source of compounds with a wide variety of chemical structures, biological activities and provide important prototypes of the development of novel drugs. There is a huge range of compounds that can be extracted and characterized by plants. Extraction of bioactive compounds from medicinal plants permits the exhibition of their physiological activity. Malaria is among the most dreaded diseases of the twentieth century and spreading further with the continuance and increasing frequency in the century. *Urena lobata* Linn. (Commonly known as Caesar weed, Congo jute) is a shrub (60–100 cm in length) belonging to the Malvaceae family, widely distributed throughout India. The active constituents of the plant are flavonoids glycoside, alkanes,  $\beta$ -sitosterol stigma-sterol, furocoumarin, imperatorin, mangiferin and quercetin

[1]. Various extracts of the leaves and roots of the plant are used in herbal medicine to treat varied ailments such as cough, malaria, venereal diseases, wounds, toothache, and rheumatism. It also serves as food material for humans as well as animals [2]. It is reported that urenalobosides, a megastigmane glycosides of *U. lobata* inhibits *in vitro* production of nitric oxide in lipopolysaccharide-stimulated RAW 264.7 macrophage cells [3]. Oral administration of aqueous extract *U. lobata* leaves at different doses was found effective to altered the blood glucose and insulin levels and increases the glucagon-like peptide (GLP)-1 levels in diabetic rats [4]. Alcohol root extracts from *U. lobata* possess significant protective effect against *S. aureus pneumonia* and combined therapy of these extracts with Cefazolin sodium have shown additive effects [5]. Clematoside- S a triterpenoid saponin isolated from the leaves of *U. lobata* showed to possess anti-yeast activity. There is an increasing demand for clean, biocompatible, and eco-friendly techniques so many biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported using microorganisms including bacteria, fungi and plants [6, 8]. Plants provide a better platform for nanoparticles synthesis, provide natural capping agents

and also reduces the cost of microorganisms isolation and culture media [7]. Silver nanoparticles have been found to possess both anti-bacterial and anti-inflammatory properties and that is why it has been integrated into many commercially available wound dressings, pharmaceutical preparations, and medical implant coatings, but, there is still need for economic commercially viable as well as environmentally clean synthesis route to synthesize the silver nanoparticles. In the textile industry, silver-embedded fabrics are now used in sporting equipment [9-12]. Various metal nanoparticles synthesis have been reported using fungi like *Fusarium oxysporum*, *Penicillium* sp. and using some bacteria such as *Bacillus subtilis* etc [13-15] but, synthesis of nanoparticles using plant extracts is the most advantageous for green, eco-friendly production

of nanoparticles because plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites [16]. Several medicinally important plants viz. *Oryza sativa*, *Helianthus annuus*, *Saccharum officinarum*, *Sorghum bicolor*, *Zea mays*, *Basella alba*, *Aloe vera*, *Capsicum annum*, *Magnolia kobus*, *Medicago sativa* (Alfalfa), *Camellia sinensis*, *Azadirachta indica*, *Cinamomum camphora*, and *Geranium* sp., marigold flower, *Ziziphora tenuior*, *Abutilon indicum*, *Solanum trilobatum*, *Erythrina indica*, beetroot, *Ocimum tenuiflorum*, *Spirogyra varians*, *Melia dubia*, olive, have been used for synthesis of silver nanoparticles, in the field of pharmaceutical applications and biological industries [17].



Fig-1: *Urena lobata* plant in the wild and its flower

## MATERIALS AND METHOD

### Collection of Plant Material

Healthy plant leaves, stem and roots were collected carefully from Malviya Nagar Square, Durg, Chhattisgarh, India. Plant materials were first washed with running tap water and then three-time with distilled water and dried in shade. After drying plant materials were placed in sterile plastic bags in a dark and cool place until use.

### Phytochemical Screening

#### Extraction of Metabolite

Dried leaf, stem, and root of the plant *Urena lobata* was submerged into three different solvents distilled water, methanol, and chloroform and was extracted with the help of soxhlet extraction unit (MSW India) sequentially. Extract of these plant material was then tested for major phytochemical groups like alkaloids, cardiac glycoside, flavonoids, saponin, steroids, tannin, terpenoids [18-20]

#### Test for steroids

0.5ml of the extract was dissolved in 3ml of chloroform. The solution was filtered, 2ml of concentrated sulfuric acid was added and a reddish-

brown colour ring at the interface was obtained as a steroid.

#### Test for saponin

0.5ml of the extract was taken in a test tube then 5ml of distilled water was added to it. The solution was vigorously shaken and stable persistent was observed for the presence of saponin.

#### Test for tannins

0.5ml of extract and 5ml of ed water was taken in a test tube, 1% ferric chloride was added. A deep green, colour appeared which indicated the presence of tannin.

#### Test for flavonoid

0.5ml plant extract and 5ml of distilled water were added to the test tube then it was filtered. 5ml of dilute ammonium solution was added to the filtrate and then concentrated with 0.5ml sulfuric acid. A yellow colour appeared which indicated the presence of flavonoid.

#### Test for alkaloid

0.5ml of the extract was taken and 3ml of methanol was added to it. Then 300 microlitre of acetic

acid ammonium hydroxide was added dropwise. A precipitate appeared which indicated the presence of alkaloid.

#### Test for cardiac glycoside

0.5ml of the extract was treated with 0.2ml glacial acetic acid then dropwise 3.5% ferric chloride was added to the solution. This was layered with 1ml of concentrated sulfuric acid. A reddish-brown ring appeared at the interface which indicated the presence of cardiac glycoside.

#### Test for terpenoids

0.5ml extract was added to the test tube then 2ml of chloroform was mixed to the solution. 3ml of concentrated sulfuric acid was added to form a lower layer. A reddish-brown colour at the interface appeared which indicated the presence of terpenoids.

#### Test for phenol

1ml extract was mixed with 1ml of distilled water and 2drop 10% FeCl<sub>3</sub>. A blue colour appeared for the phenol group.

#### Test for sugar test (molish test)

2ml of extract, 2drop of Alfa- naphthanol and 2ml of concentrated sulfuric acid was added to test the

presence of sugar. The appearance of violet ring confirmed the presence of sugar.

#### Test for protein

1ml extract, 4%NaOH were mixed and then 2 drops of 1%CuSO<sub>4</sub> was added. The appearance of violet colour confirmed the presence of protein.

#### Test for coumarin

1ml of a plant extract with 1ml of dilute sodium hydroxide was exposed with UV light and blue fluoresce was reported as confirmation of coumarin.

#### Evaluation of antioxidant property

The antioxidant activity of *Urena lobata* was examined by DPPH (1, 1-diphenyl -2-picrylhydrazyl) method. For this 0.6mM solution of DPPH was prepared in 100ml of methanol. For test sample, the plant extract (1 ml) with different concentration (0.6, 1.2, 2.5, 5, 10 mg/ml) was taken and 1ml of DPPH was added and final volume of 3ml was makeup with methanol. The tubes were incubated in dark for 30 minutes then absorbance was taken at 570 nm and 1ml (10mg/ml) ascorbic acid and methanol was taken as standard and control respectively.

$$\% \text{ of inhibition of DPPH activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

#### Synthesise of Silver Nanoparticles from Leaf Extract

Fresh leaves of *Urena lobata* washed with distilled water twice followed by Millipore water and left for air-dry overnight. Then 10 gram of plant material was taken with 100ml of double distilled water in 250 ml of Erlenmeyer flask and kept on a water bath for at 80°C for 15 minutes after that plant material was filtered by Whatman filter paper No 1, and then it was reached up to 100ml with double distilled water. For the synthesis of nanoparticles 10ml of aqueous leaf extract and 90ml of 1mM AgNO<sub>3</sub> were mixed and kept on a water bath and incubated for 15 minutes at 80°C, solution turned into yellowish red-brown indicated the nanoparticle formation [17, 21].

#### Characterization of NanoParticle By Photometric methods and XRD

Preliminary color transformation into reddish-brown color was observed, spectrophotometric analysis in UV-VIS at range 200 to 600nm wavelength was used to monitor the synthesis of silver nanoparticles. Millipore water was used as a standard for baseline correction. X-Ray Diffraction Analysis was used to determine the nature of silver nanoparticles. The fine ground powdered sample was used for analytical material. The analysis was carried out using Bruker- D4 ENDEAVOR XRD-6000/6100 model with 30kv, 30mA with Cu ka radians at 2θangle 10<sup>0</sup>-90<sup>0</sup>. Debye Sherrer's

equation was used for the determination of the particle size of the silver nanoparticles. For the functional group characterization, the particle was dried for 24 hours and the dried silver nanoparticle was scrapped out for characterization using FTIR spectroscopy. The FTIR spectrum was obtained with BRUKER, ALPHA II, ECO ATR, spectrophotometer.

## RESULT AND DISCUSSION

#### Phytoconstituents

Table-1 showed that Tenine, flavonoid, Steroid, Alkaloid, Saponin, Cardiac glycoside, Terpenoid, Sugar and phenols were found from aqueous leaf extract and only Flavonoid, Steroid, Cardiac glycosides, and Terpenoids were found form methanol leaf extract and only Flavonoid was found from chloroform based leaf extract. From stem Tenine, Steroid, Cardiac glycosides, Terpenoid, Sugar, and phenol were found in aqueous-based extracts, Flavonoid, Steroid, Cardiac Glycosides, Terpenoid, and Sugar were found from the methanol-based extract and only Steroid and Terpenoid were found from chloroform based extract. Similarly, phytochemical analysis of root was performed and Flavonoid, Steroid, Alkaloid, Saponin, Cardiac glycoside, Terpenoid, and sugar were found from the aqueous extract. Steroid, Saponin, Cardiac glycoside, Terpenoid, and sugar from methanol extract, and nothing were found from a

chloroform-based extract of the root of the plant. Conclusively we can state that the leaf part of the plant having maximum secondary metabolite followed by stem and root and the aqueous extract is a more suitable solvent followed by methanol and chloroform for *Urena lobata*. A similar study by Islam et al., [22], reported alkaloid, glycoside, tannins, steroids, saponins, and reducing sugar however they have not detected terpenoids and flavonoids, while Fagbohun et al., [23] detected only terpenoid and saponin from *Urena lobata*.

The various phytochemical compounds detected in many plants are known to have beneficial importance in medicinal sciences. For example, flavonoids which showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities. Plant steroids are identified for its cardiogenic activities and also holds insecticidal and antimicrobial abilities and are used in nutrition, herbal medicine and cosmetics (24-25). Tannins were reported to exhibit antiviral, antibacterial and anti-tumour activities. In another study Gao et al., [26] reported anti-yeast activity in terpenoid of *Urena lobata*. Saponin is a mild cleansing agent, in intracellular histochemical staining and allow access antibody into intracellular proteins. Active substances Stigmasterol,  $\beta$ -Sitosterol, Mangiferin, Gossypetin, Chrysoeriol in leaf extract of *Urena lobata* was also reported by liquid chromatography-mass spectrometry among them mangiferin, stigmasterol and  $\beta$ -sitosterol in was reported strong with inhibitory activity on DPP-IV in-silico [4]. It is also used in various problems like hypercholesterolaemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory, weight loss, etc. and have antifungal properties as well [2, 27]. Glycoside an important phytochemical group was found to have an anti-inflammatory effect [3].

### Antioxidant Property

Various medicinal plants have been reported to have a high antioxidant activity where Phenolics and flavonoids are known as the common antioxidants in plants. As we know that phytochemical metabolite also works as defense material against various stress like water stress, temperature, and infection, etc. It was observed that the factor like geographic location of the plant, types of stress and the solvent system used in the extraction process may act as determining a factor for the distribution of these phytochemicals [24, 25]. Several reports are available that shows the presence of phytochemicals such as quercetin, glycosides, rutin, Kaempferol 3-O- $\beta$  glucopyranoside, Quercetin 3-O-rutinoside, L-tryptophan, and monordicophenoide A, were reported in *Urena lobata* [23, 28-29]. In the present study we have observed dose-dependent 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) induced free radical neutralization ability, and the highest 79.62% DPPH scavenging activity was reported at 10mg/ml concentration of *Urena lobata* plant extract compared to standard ascorbic acid (10mg/ml). Similarly Lissy et al., [30] reported significant superoxide radical

scavenging, hydroxyl radical scavenging, and lipid peroxidation inhibition activity in *Sida retusa*, *Urena lobata* & *Triumfetta rhomboidea* in another study Ali et al., [30] reported significant antioxidant activity in *Urena lobata* fresh leaves. The result of DPPH scavenging activity assay in this study indicates that the plant is potentially active. This indicates that the plant extract has certain compounds that are capable of donating hydrogen to a free radical and which is responsible for the free radical's activity. The ability of this plant extract to scavenge DPPH could also reflect its ability to inhibit the formation of Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defence mechanism [24, 25, 31].

### Nanoparticle Synthesis and Characterization

Silver nanoparticles with their distinctive properties are showing to be a substitute for the improvement of new antibacterial agents. It has got a diversity of uses in the form of wound dressings, coatings for medical devices and impregnated textile fabrics, etc. A detailed study on the biosynthesis of silver nanoparticles by *Urena lobata* was carried out in the present study. Silver ions were found reduced to silver nanoparticles when added to *Urena lobata* plant aqueous extract. It was observed that the color of the solution turned from yellowish to bright yellow and too dark brown after 15 minutes of the reaction, which indicated the formation of silver nanoparticles confirmed by UV-VIS spectrophotometer analysis. The colloidal solution color change from yellowish to bright yellow and, finally, to dark brown, after 1, 24, and 48 h of the reaction was reported, indicating the formation of silver nanoparticles because of excitation of surface plasmon vibrations in synthesized silver nanoparticles [32, 33].

The synthesized silver nanoparticles in the colloidal solution were monitored by UV-VIS spectrophotometer analysis. Figure 9 shows that the absorption spectra of silver nanoparticles formed in the reaction media have an absorbance peak at approx. 424 nm. correspond to a characteristic of these noble metal particles.

Further, the XRD analysis was carried out to confirm the crystalline nature of the particles, and the XRD pattern showed the number of Bragg's reflections that may be indexed based on the structure of silver. To verify the results of UV-vis spectral analysis, the sample of silver ions exposed to *Urena lobata* was examined by XRD. The XRD pattern for silver nanoparticles synthesized using *Urena lobata* and mean particle diameter (d) of silver nanoparticles was calculated from the XRD pattern, according to the line width of the plane, refraction peak, using the following

$$\text{Scherrer equation: } D = \frac{K\lambda}{\beta 1/2 \cos \theta}$$

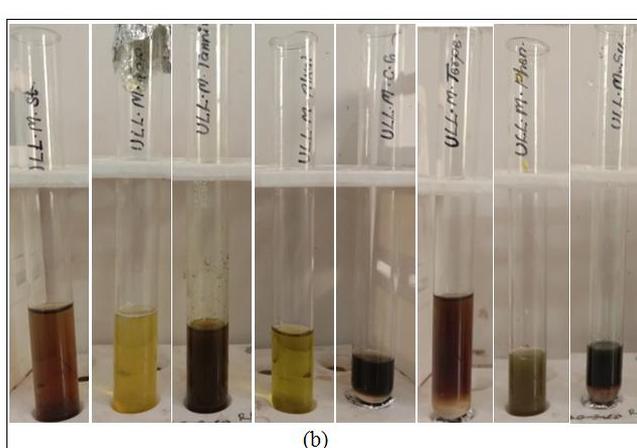
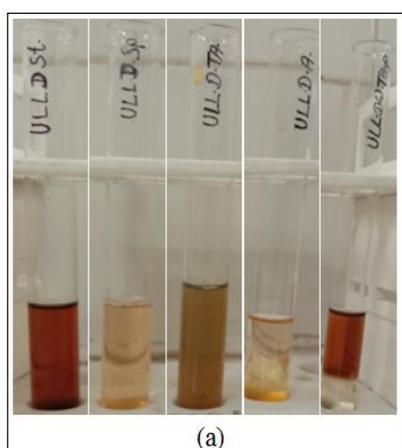
The equation used the reference peak width at angle  $\theta$ , where  $\lambda$  is the X-ray wavelength (1.5418 Å),  $\beta 1/2$  is the width of the XRD peak at half height and  $K$  is a shape factor. The silver nanoparticles synthesized were calculated by the particle size ranges of the silver 28.17. An XRD spectrum of AgNPs formed in our experiments as demonstrated by the peaks  $2\theta$  values of 38.16, 44.28, 64.51, 77.45 and 82.18, corresponding to 111, 002, 022, 113 and 222 respectively, clearly indicated that the AgNPs organized by the reduction of Ag<sup>+</sup> ions by the aqueous extract of *Urena lobata* were crystalline in nature. Various authors have also reported different size of silver nanoparticle viz. 53, 41, 52 and 42 nm, size for *Syzygium cumini*, *Citrus sinensis*, *Solanum tricobatum*, and *Centella asiatica*, respectively [34], 51 nm by olive leaf extract [21], 13.07 nm from *Calliandra haematocephala* leaf extract [33], 12–20 nm. from saffron (*Crocus sativus* L.) wastages [36], 66 - 117 nm using spent tea leaves extract [35]. The FTIR spectrum of Ag NPs synthesized from *Urena lobata* aqueous extract showed the presence of bands at 1635.93 cm<sup>-1</sup>, and 3319.67 cm<sup>-1</sup> for silver nanoparticle and 1636.40 and 3300.67 for aqueous extract. The existence of peaks at 3319.67 and 3300.67 might be due to the AOH stretching of alcohols and

phenols or bending stretching of hydrogen-bonded alcohols and phenols in the leaf extract and was suggested to be due to the stretching of -OH in proteins, enzymes or polysaccharides present in the extract. A strong peak at 1620.09 denoted the bending vibrations of the amide I group and arisen due to a carbonyl stretch in the amide linkages of the proteins and suggested the possible binding of silver nanoparticles with the proteins present in the extract [21, 34]. Synthesized Ag NPs were identified as amide I and arose due to a carbonyl stretch in the amide linkages of the proteins. The results of FTIR showed that the secondary structure of the proteins was unaffected as a result of reaction with the Ag<sup>+</sup> ions or binding with the silver nanoparticles. This result suggests that the biological molecules (i.e. metabolite) prevent clotting and pairing of the nanoparticles and may perform a function for the formation and stabilization of Ag NP in an aqueous medium and seems to have the same functional group as an extract. It is well known that proteins can bind to Ag NP through their free amine groups [17, 33] and, it was suggested that if antioxidants & flavonoid compounds present in the plant extract, are more suitable for the reduction of metal salts like standard antioxidant agents ascorbic acid and gallic acid [36]. Thus by our experimentation, it confirmed that silver nanoparticle of the leaf of *Urena lobata* was successfully synthesized.

**Table-1: Showing phytochemical groups present in *Urena lobata***

Test group	Leaf extract			Stem extract			Root extract		
	D.W.	MeOH	CHCl3	D.W.	MeOH	CHCl3	D.W.	MeOH	CHCl3
Tennis	+	-	-	+	-	-	-	-	-
Flavonoid	+	+	+	-	+	-	+	-	-
Steroid	+	+	-	+	+	+	+	+	-
Alkaloid	+	-	-	-	-	-	+	-	-
Saponin	+	-	-	-	-	-	+	+	-
Cardiac Glycoside	+	+	-	+	+	-	+	+	-
Terpenoid	+	+	-	+	+	+	+	+	-
Sugar	+	-	-	+	+	-	+	+	-
Phenol	+	-	-	+	-	-	-	-	-

D.W. = distill water, MeOH = methanol, CHCl3 = chloroform



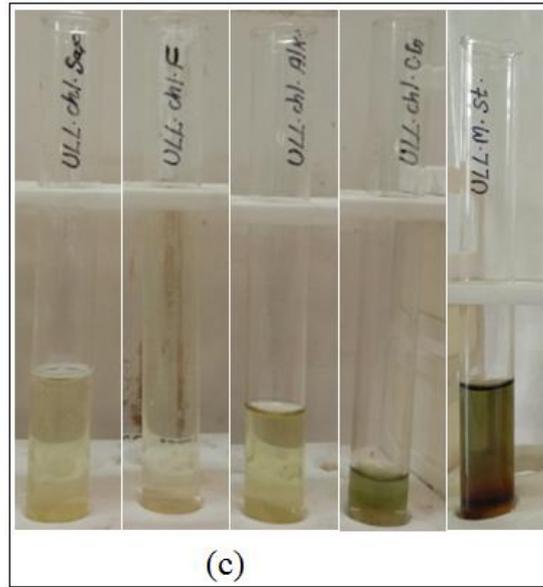


Fig-4: Pictorial representation of phytochemical evaluation of *Urena lobata* leaf extract -(a) in distilled water, (b) in methanol, and (c) in chloroform

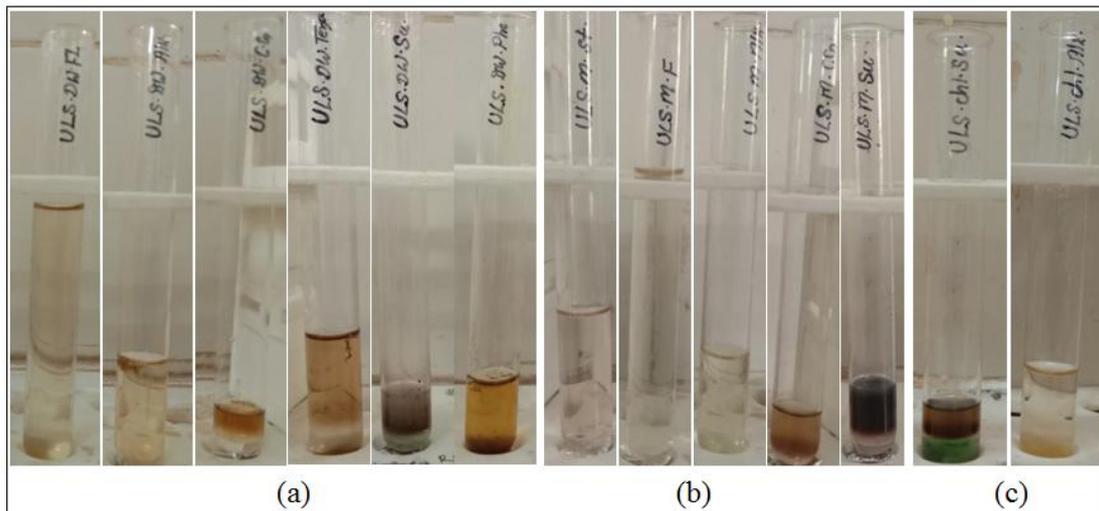


Fig-5: Pictorial representation of phytochemical evaluation of *Urena lobata* stem extract -(a) in distilled water , (b) in methanol, and (c) in chloroform

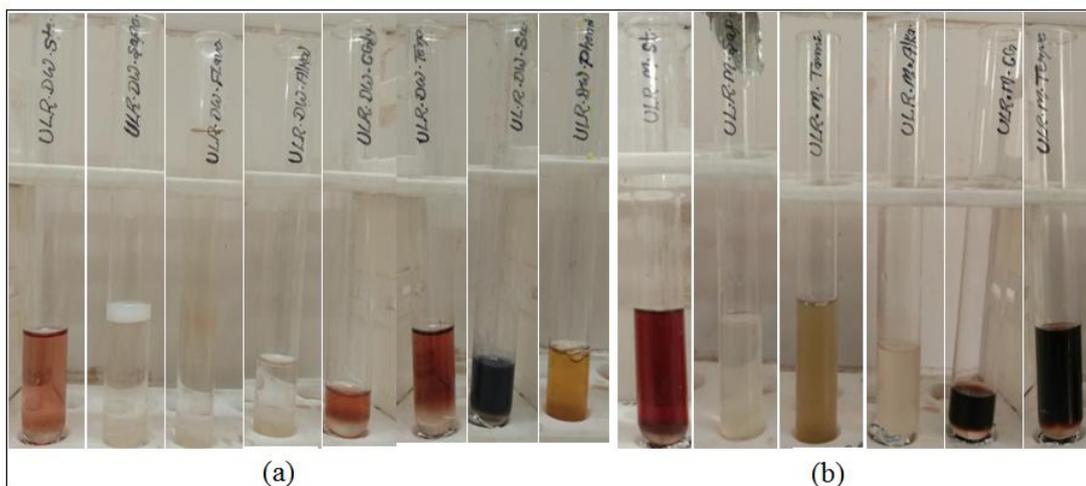
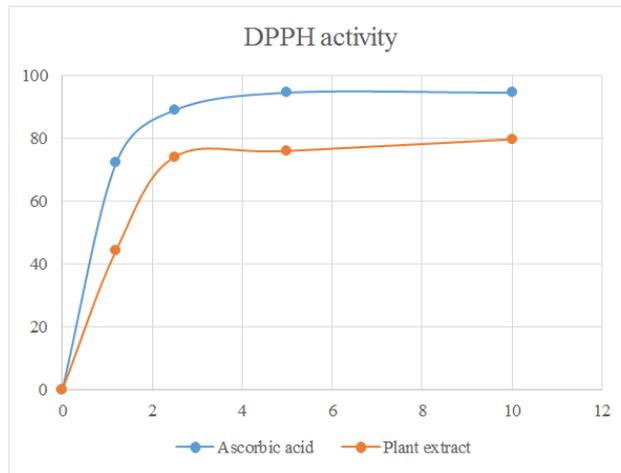


Fig-6: Pictorial representation of phytochemical evaluation of *Urena lobata* root extract - (a) in distilled water extract, (b) in methanol extract and

**Table-2: Showing percentage DPPH antioxidant activity**

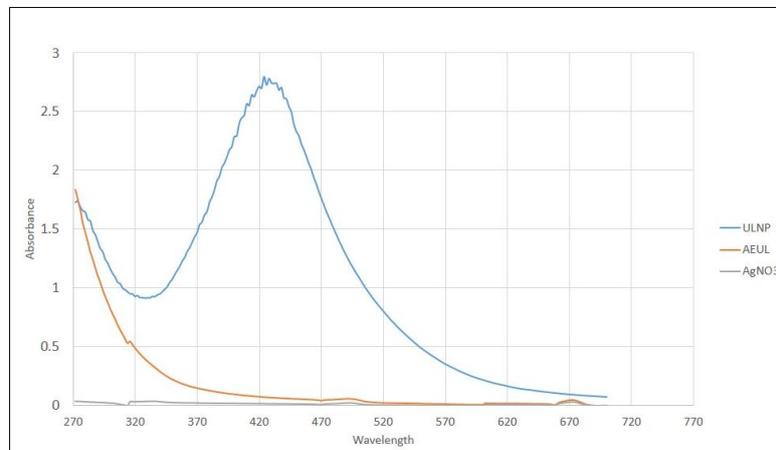
Sr. No	Test Sample	Concentration	Absorbance (at 517 nm)	Percentage activity
1	Control	-	0.160	0.000
2	Ascorbic acid	10.0mg/ml	0.010	94.44
3	Ascarbic acid	5.0 mg/ml	0.010	94.44
4	Ascarbic acid	2.5 mg/ml	0.020	88.88
5	Ascarbic acid	1.2 mg/ml	0.050	72.22
6	<i>Urena lobata</i>	10.0mg/ml	0.037	79.63
7	<i>Urena lobata</i>	5.0 mg/ml	0.043	75.92
8	<i>Urena lobata</i>	2.5 mg/ml	0.047	74.07
9	<i>Urena lobata</i>	1.2 mg/ml	0.100	44.44



**Fig-7: Graphical representation of Antioxidant activity of *Urena lobata***



**Fig-8: Showing visible indication of nanoparticle synthesis**



**Fig-9: Showing comparative absorption spectra for aqueous extract of *Urena lobata* leaf (AEUL), AgNO<sub>3</sub>, and Ag Nanoparticle (ULNP) in the UV-VIS range**

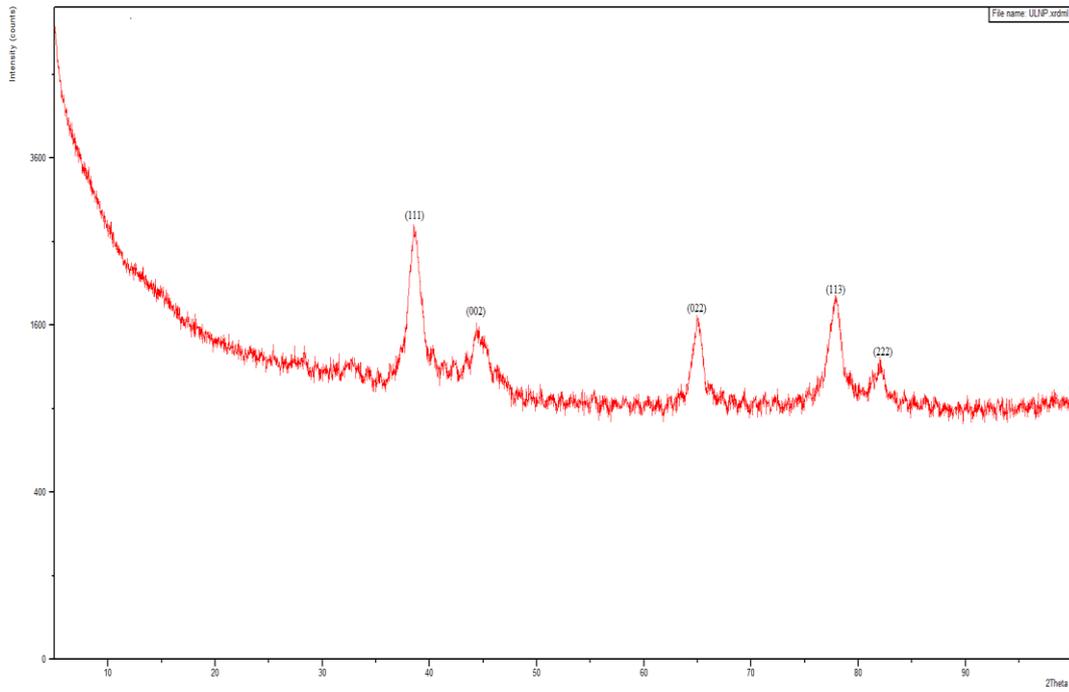
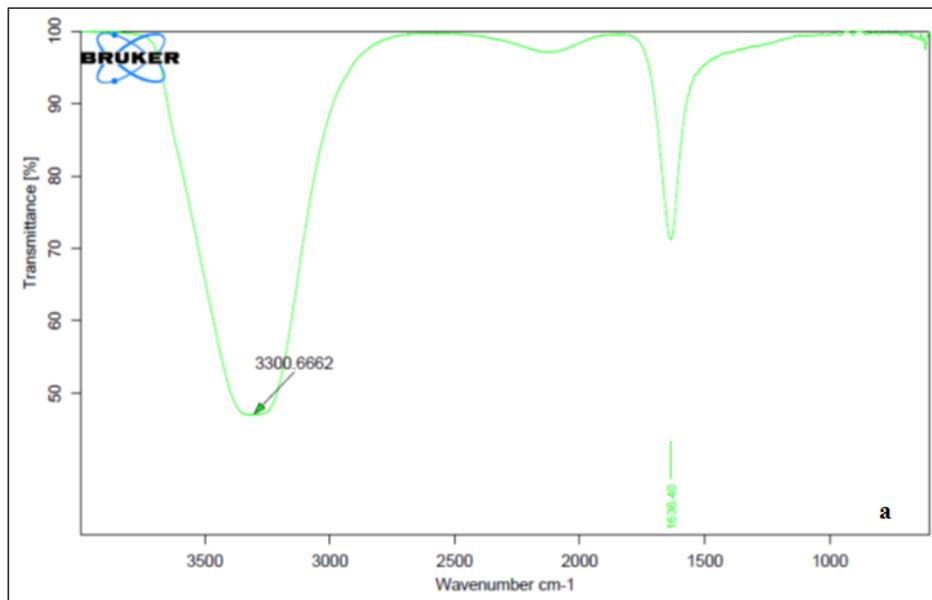


Fig-10: Showing XRD graph of nanoparticle synthesized by *Urenal lobat* extract with five different peaks between 35<sup>o</sup>-85<sup>o</sup>  $\theta$  angle; the number above the peaks are hkl number for that respective peak

Table-3: Showing peak list, hkl,  $\theta$  angle and their peak intensity for respective peak

Peak No.	h	k	l	d [Å]	2Theta[deg]	I [%]
1	1	1	1	2.34404	38.370	100.0
2	0	0	2	2.03000	44.600	47.5
3	0	2	2	1.43543	64.910	27.7
4	1	1	3	1.22414	77.991	30.9
5	2	2	2	1.17202	82.180	8.9



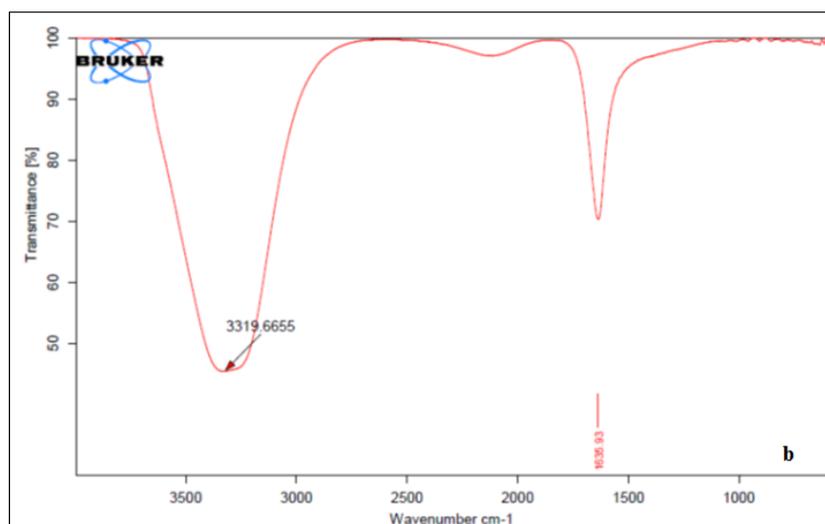


Fig-11: Represent the FTIR spectrum of (a) aqueous extract, and (b) Synthesized silver nanoparticle

## CONCLUSION

Based on the above findings we can conclude that various significant phytoconstituents in *Urena lobata* have been reported viz. – Tenin, Flavonoids, Steroid, Alkaloid, Saponin, Cardiac glycoside, Terpenoid, Sugar and Phenol. The leaf part having more secondary metabolite than stem and root respectively and better extraction can be done in the aqueous extract in comparison to methanol and chloroform respectively.

The plants having a good antioxidant ability as we confirmed DPPH induced free radical neutralization ability up to 79.62% and thus suitable for pharmaceutical application.

We have successfully synthesized silver nanoparticle with the leaf of *Urena lobata* and confirmed by UV-VIS spectrophotometry, FTIR and XRD characterization.

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