



ISSN NO. 2320-5407

Journal homepage: <http://www.journalijar.com>
Journal DOI: [10.21474/IJAR01](https://doi.org/10.21474/IJAR01)

INTERNATIONAL JOURNAL
OF ADVANCED RESEARCH

RESEARCH ARTICLE

PLANT MEDIATED BIOSYNTHESIS, CHARACTERIZATION AND APPLICATION OF SILVER NANOPARTICLES BY LEAVES EXTRACT OF CUPRESSUS TORULOSA.

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Manuscript Info

Manuscript History:

Received: 12 May 2016
Final Accepted: 19 June 2016
Published Online: July 2016

Key words:

Cupressus torulosa D. Don, Green synthesis, Antibacterial, Dye decolorization, SEM, Silver Nanoparticles (SNP's), TEM, UV analysis, XRD.

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Abstract

In recent nanotechnology engineering could be a burning field for the researchers. Nanotechnology deals with the Nanoparticles having a size of 1-100 nm in one dimension used considerably regarding medical chemistry, atomic physics, and all other known fields. Nanoparticles are used vastly due to its tiny size, orientation, physical properties, that square measure reportedly shown to amend the performance of the other material which is in touch with these little particles. These particles can be ready simply by completely different chemical, physical, and biological approaches. But the biological approach is the most rising approach of preparation, because, this method is easier than the opposite ways, eco-friendly and less time consuming. The Green rapid biogenic synthesis was demonstrated by silver nanoparticles using aqueous extracts of leaves of *Cupressus torulosa* D. Don leaf extract and AgNO₃. Silver was of a selected interest for this technique for its sensible physical and chemical properties. Change in colour determined the formation of nanoparticles. The characteristics of the SNP's were evaluated by UV-visible spectroscopy, Scanning Electron research (SEM), X-Ray Diffraction analysis (XRD), Transmission Electron Microscope (TEM) and therefore the bactericide activity against pathogenic bacteria *Bacillus subtilis*, *Salmonella enterica* and *Pseudomonas aeruginosa* of human health and study concerning the dye decolorization of the dyes Congo red, Orange G and Rhodamine B.

The morphology of the particles formed consists of varied shapes such as spherical, rod, flower-like and hexagonal pattern. The biosynthesis of nanoparticles has received increasing attention because of the growing demand to supply secure, cost-effective and environmentally friendly technologies for nanomaterials synthesis.

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Introduction:-

In modern materials science the field of nanotechnology is one of the most active areas of research. Our environment undergo huge smash up due to swift urbanization and industrialization and a large amount of perilous and superfluous chemicals, gases or substances are released which leads to the growth of advancement in the synthesis processes of nanoparticles. Applications of nanotechnology were highly suitable for biological molecules because of their exclusive properties. The biological molecules undergo highly supervised turnout for making them suitable for the metal nanoparticle synthesis which was found to be reliable and eco-friendly (Harekrishna et al., 2009). Due to its potential application in the synthesis of metal and semiconductor nanoparticles is vast area of research which was implemented in the development of novel technologies. Currently, the uses of environment friendly nanoparticles are in great need that does not produce toxic waste in their synthesis process protocol. To attain this, we are inclined to shift to benign synthesis processes, which happen to be mostly of biological nature. Silver nanoparticles can be synthesized by various approaches.

Silver nanoparticles can be synthesized by a number of approaches. For example, silver ions can be reduced by chemical (Sun et al., 2002), electro-chemical (Yin et al., 2003), Langmuir-Blodgett (Zhang et al., 2006; Swami et al., 2004), phytochemical methods (Callegari et al., 2003), radiation (Dimitrijevic et al., 2001) and biological techniques (Naik et al., 2002). Plant extract plays an important role in the synthesis of nanoparticles as reducing agents and stabilizing agents (Kumar et al., 2009). Plant extracts are easy and convenient alternative to chemical and physical methods used for the synthesis of silver nanoparticles. Various plant extracts used for the synthesis of metallic nanoparticles such as Neem (Shankar et al., 2004), Geranium (Shankar et al., 2003), *Aloe vera* (Chandran et al., 2006), Cinnamomum (Huang et al., 2007), Mushroom (Philip, 2009), *Mangifera indica* (Philip, 2010), *Mangolia kobus* (Song et al., 2009), Pear fruit (Ghodake et al., 2010) and Tulsi (Philip et al., 2011).

C. torulosa D. Don is an evergreen tree. *C. torulosa* D. Don is known as Himalayan Cypress. It is a large tree of height up to 45 meter (148 feet) found on limestone terrain in the western Himalaya from 300-2800 meters. The major advantage of using plant extracts for silver nanoparticle synthesis is that they are simply obtainable, safe, and nontoxic in most cases, have a broad variety of metabolites that may aid within the reduction of silver ions, and are faster than microbes in the synthesis. The main mechanism considered for the method is plant-assisted reduction because of phytochemicals. The main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Flavones, organic acids, and quinones are soluble phytochemicals that are accountable for the immediate reduction of the ions (Prabhu and Poulouse, 2012). In mesophytes having well defined and developed metabolic machinery, the process of nano-transformation might be the result because of tautomerization of quinones. The candidate mesophytic genera, *Cyperus* sp. have been reported to contain all the 3 varieties of benzoquinones, namely cyperoquinone (type I), dietchequinone (type II) and remirin (type III) (Thomson, 1976). A very prompt transformation by the plant extract is connotative of a redial tautomerization. No pH shift was either discovered or evoked in the extract, but mild warming and subsequent incubation would possibly have activated the benzoquinone congeners resulting in a particle size reduction additionally as coalescent cluster formation (Jha et al., 2009).

Materials & Methods:-

Binomial name- *Cupressus torulosa*

Scientific classification	
Kingdom	Plantae
Division	Pinophyta
Class	Pinopsida
Order	Pinales
Family	Cupressaceae
Genus	Cupressus
Species	<i>C. torulosa</i>

For the synthesis of silver nanoparticles, leaves samples of *C. torulosa* were collected from the forest of Pauri, Garhwal, Uttarakhand, India. They have been botanically identified in Botanical survey of India, Dehradun. A photograph of plants is shown in Figure 1.



Figure 1:-*Cupressus torulosa* plant

Preparation of leaf extract of *Cupressus torulosa*:-

The leaves were washed with fast flowing water and then separated from its stem. Again it was washed by using distilled water and the water is removed. The sample is kept for drying. 25 grams leaves weigh and washed with distilled water for 5 minutes. Dried and cut into fine pieces. The fine cut leaf pieces were boiled in 500ml flask along with 100ml sterilized distilled water for 15 minutes (Tashi et al. 2016). Then sample extract was filtered successively through by using Whatman filter paper no. 1 (Ajitha et al., 2015). The plant extract was collected and stored at 4°C. Finally, the extract was used for the synthesis of silver nanoparticles.

Synthesis of silver nanoparticles:-

10ml of *C. torulosa D. Don* leaves extract was treated with aqueous solution of 1mM silver nitrate and incubated for 24 hour in dark (Nabikhan et al. 2010). After incubation, maximum absorbance of the sample was measured by using UV-visible spectroscopy. Ultraviolet spectra analysis was performed for leaves extract and the absorption maxima were analyzed at a wavelength of 350-600 nm using on a PerkinElmer Lambda-35 UV spectrometer. Deionized water was used for background correction in UV visible absorption spectra. XRD analysis was carried out on an X-ray diffractometer (X'Pert-PRO).

Characterization of the synthesized silver nanoparticles:-**Scanning Electron Microscope analysis:-**

Detailed analysis of the morphology and distribution of the silver nanoparticles was determined by the help of instruments like Scanning Electron Microscopy using ZEISS machine. About 25µl of the sample pipette out and loaded on a stub provided for SEM analysis. After loading of the samples on the stub, it was fixed in the machine. Images were visualized and collected.

X-Ray Diffraction analysis:-

X-ray diffraction of the sample was carried out by drying the sample to powder form. Then the sample was loaded on to clean glass slide and characterized by X-ray diffraction to indicate the presence of silver nanoparticles.

Transmission Electron Microscope analysis:-

TEM analysis of the sample was carried out by loading sample on to the carbon coated copper grid. In this technique, electrons beam transmitted through an ultra-thin specimen. An image is formed from the interaction. The image is magnified and focused on to an imaging device (Swamy et al., 2014).

Application of silver nanoparticle synthesized by leaves extract of *C. torulosa D. Don*:-**Antibacterial Test:-**

The antibacterial activity of the silver nanoparticles synthesized from leaf extract of *C. torulosa D. Don* was tested by Agar well diffusion method against different human pathogenic bacteria Gram positive *Bacillus subtilis* and Gram negative *Salmonella enterica*, *Pseudomonas aeruginosa* bacteria. Each strain of the human pathogenic bacteria was swabbed uniformly in to the plates having Mueller Hinton Agar media. Wells of equal diameter were made on the plate. Nanoparticles solution was loaded on to each wells of different concentration of 50,100,150 and 200µl using sterile micropipette. Incubate at 32°C for 24 hour. After incubation, the zone of inhibition was measured.

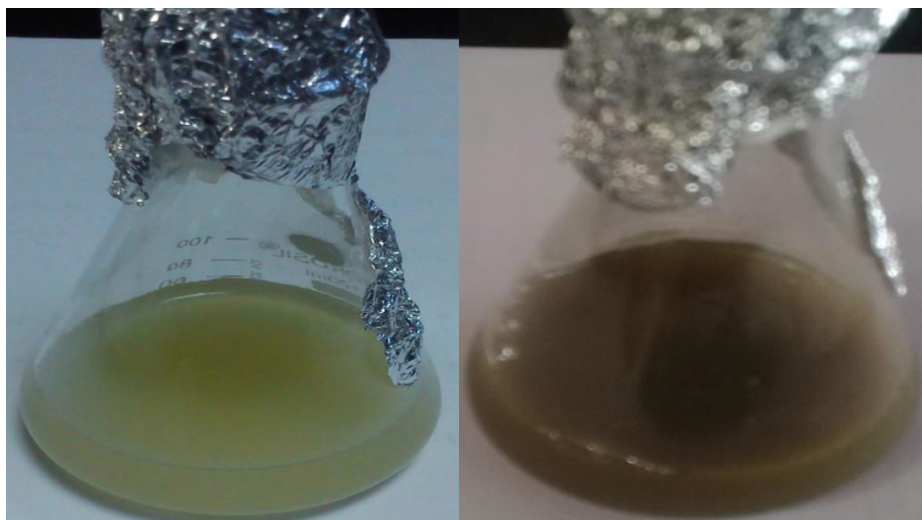
Dye decolourization using silver nanoparticles:-

Decolourize of the dye Congo red, Orange G and Rhodamine B using silver nanoparticles, synthesized from leaf extract of *C. torulosa D. Don*. For decolourization study, 1ppm concentration of Congo red was prepared. One was maintained as blank. Incubate at 32°C for 24 hour. After incubation, samples were withdrawn and analysed spectrophotometrically using UV-Visible spectrophotometer at 498 nm. A similar procedure was applied for the decolourization of Orange G and Rhodamine B at 476 and 550 nm.

Results and Discussions:-**Synthesis of silver nanoparticles from plant extract:-**

Silver nanoparticle resolution has dark brown or dark red in colour (Dipankar et al., 2012; Bindhu and Umadevi, 2015). Leaf extract of *C. torulosa D. Don* showed yellowish green before addition of AgNO₃ however once its treatment with AgNO₃ its colour changes to dark brown that indicated the formation of Ag NP's. Figure 2A indicate the colour of plant extract before the addition of silver nitrate and Figure 2B indicates the colour of synthesized silver nanoparticles in the leaf extract of *C. torulosa D. Don*. This colour amendment is as a result of the property of

quantum confinement that could be a size dependent property of nanoparticles that affects the optical property of the nanoparticles.



(A) Before (B) After

Figure 2:-Colour change of plant extract before after addition of AgNO_3

UV-visible Spectroscopy:-

Leaf extract of *C. torulosa* D. Don exposed to Ag^+ ions showed a distinct and fairly broad absorption at 450nm. Presence of broad resonance indicates an aggregated structure of the silver particles. UV absorption peak of silver nanoparticles range from 400 nm – 450nm (Ramteke et al., 2013). The absorption band in the visible light region is an offshoot of the surface plasmon resonance of silver nanoparticles (Saxena et al., 2010; Mallikarjun et al., 2011; Bonde et al., 2012). The incidence of the height is due to the development of surface Plasmon resonance that happens because of the excitation of the surface plasmons on the outer surface of the silver nanoparticles that gets excited because of the applied electromagnetic attraction field (Naheed et al., 2011).

Table 1:-UV-visible spectroscopy data of Ag NP's synthesized from leaf extract of *C. torulosa* D. Don

Wavelength (nm)	Absorbance
350	0.81
400	0.61
450	0.82
500	0.76
550	0.42
600	0.21

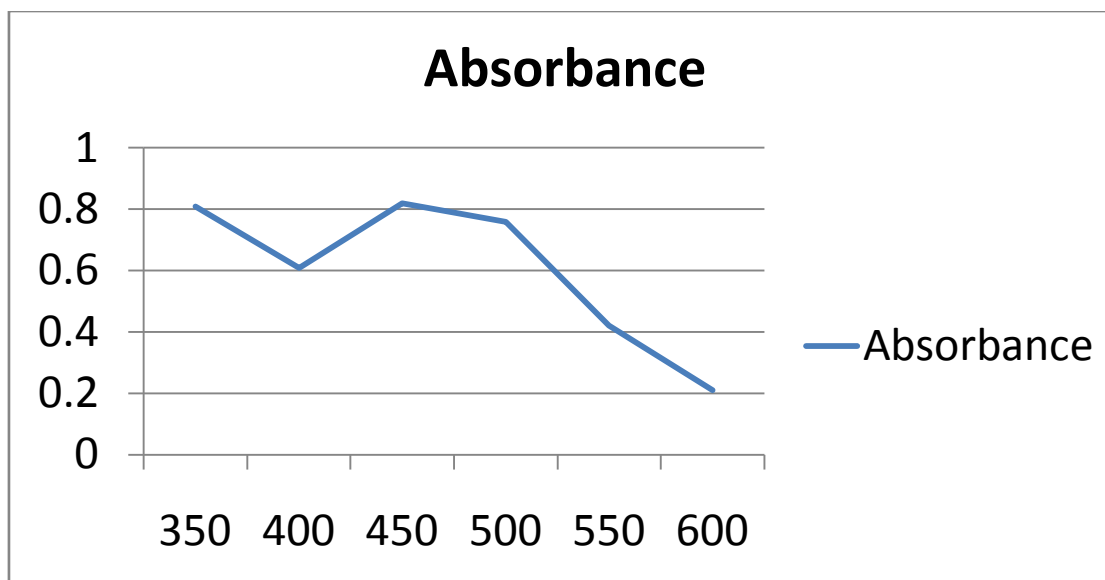


Figure3:-UV-visible absorption peak of leaf extract of *C. torulosa D.Don* synthesized silver nanoparticles

Scanning Electron Microscopy analysis:-

Scanning microscopy (SEM) is employed for morphological characterization at the nanometer to micrometer scale (Schaffer et al., 2009). SEM analysis shows uniformly distributed silver nanoparticles that indicating the stabilization of nanoparticles by capping agents. The silver nanoparticles were spherical in form. The larger silver particles are also as a result of the aggregation of the smaller ones, as a result of the SEM measurements.

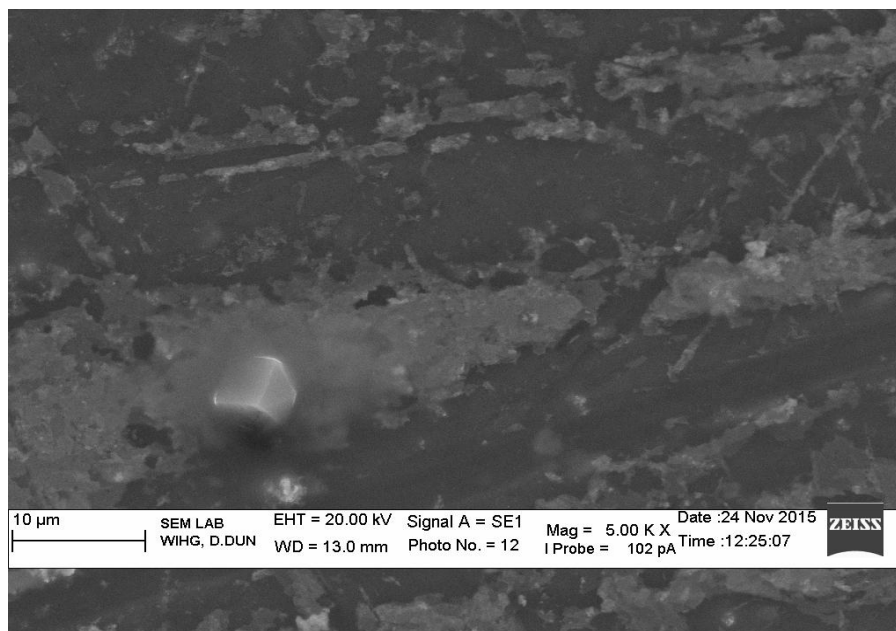


Figure 4:-SEM image of Silver nanoparticles synthesized from leaf extract of *C. torulosa D.Don*

XRD analysis:-

The crystalline natures of the synthesized Ag NPs' were characterized using XRD. The XRD pattern obtained for the Ag NP's showed four peaks in the whole spectrum of 2θ values. The XRD patterns obtained from the Ag NP's synthesized by leaf extract of *C. torulosa D.Don*. The XRD peaks at 38.3464° , 44.5435° , 64.6707° and 77.5907° . Silver nanoparticles formed are crystalline in nature is clearly illustrates by XRD patterns (Harekrishna et al., 2009). Size and shape of the silver nanoparticles are responsible for their applications (Sivaraman et al., 2009).

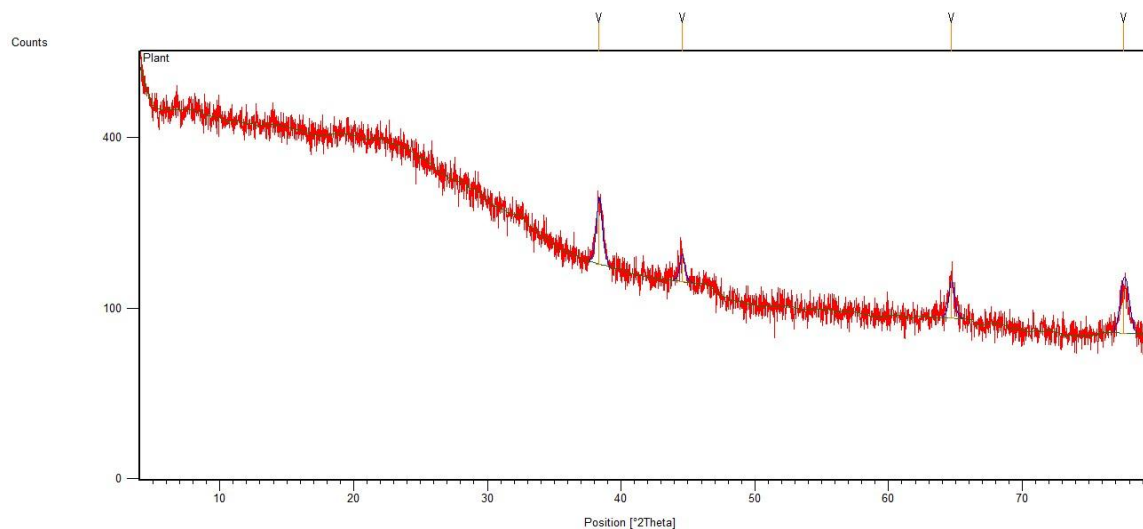


Figure 5:-XRD analysis of silver NP's synthesized from *C. torulosa*D.Don.

TEM analysis:-

Transmission Electron Microscopy (TEM) confirms the formation of nano-crystalline silver nanoparticles. TEM images determine the morphology and size of the synthesized silver nanoparticles (Sadeghi and Gholamhoseinpoor, 2015), shown in figure 6. Particles formed were spherical in shape. TEM image showed that the small particle aggregates are coated with a thin organic layer, which acts as a capping organic agent. Inside the bio-reduced aqueous solution nanoparticles showed good dispersion, even in the macroscopic scale (Kovaris et al., 2012). The particles were monodisperse, with only a few particles of different size (Mittal et al., 2013; Eppler et al., 2000; Shankar et al., 2004).

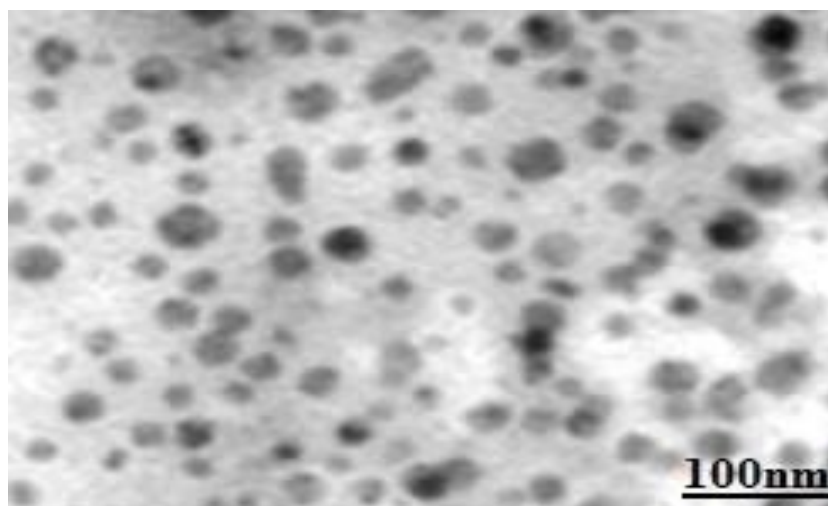


Figure 6:-TEM image of silver NP's synthesized from *C. torulosa* D.Don.

Antibacterial Test:-

Antimicrobial activity of the synthesized AgNP's from the leaf extracts of *C. torulosa*D.Don testing was done against three pathogenic bacteria *Bacillus subtilis*, *Salmonella entrica* and *Pseudomonas aeruginosa* by following the procedure of Sondi et al., (2003) that showed promising medicinal drug activity against all the pathogens. The zone of inhibition (Table 2) of Ag NP's against *Bacillus subtilis* was found to be 5mm for 50 μ l, 6mm for 100 μ l, 6mm for 150 μ l and 3mm for 200 μ l whereas *Salmonella entrica* showed 6mm for 50 μ l, 7mm for 100 μ l, 8mm for 150 μ l and 10mm for 200 μ l. Ag NP's showed zone of inhibition of against *Pseudomonas aeruginosa* as 4mm for 50 μ l, 6mm for 100 μ l, 6mm for 150 μ l and 6mm for 200 μ l.

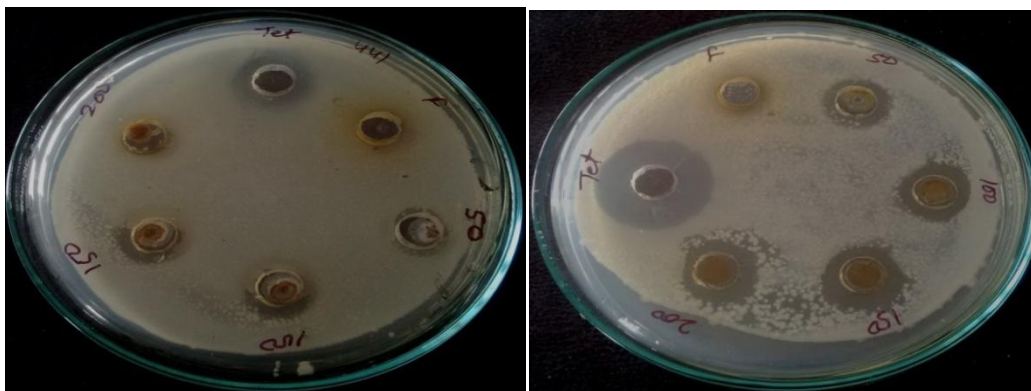
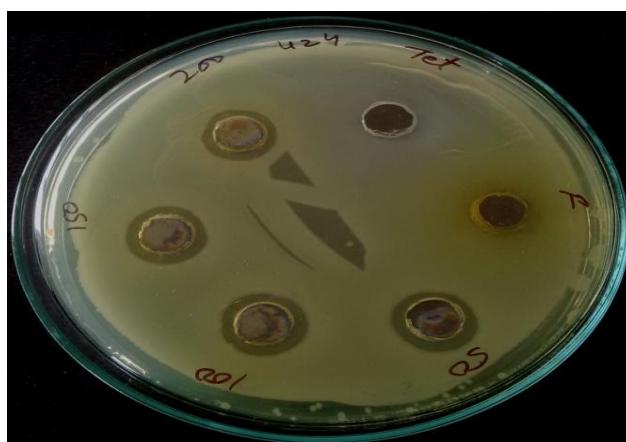


Figure 7:-

Antibacterial test against *Bacillus subtilis* and *Salmonella enterica*

Figure 9:-Antibacterial test against *Pseudomonas aeruginosa*Table 2:-Zone of inhibition (mm) of silver nanoparticles synthesized from *C. torulosa* D.Don.

S.No	Human Pathogen	Antibiotic (Tetracycline) (in mm)	Filtrate without AgNO ₃ (in mm)	Filtrate with AgNO ₃ (50µl) (in mm)	100µl (in mm)	150µl (in mm)	200µl (in mm)
1.	<i>Bacillus subtilis</i>	10	3	5	6	6	3
2.	<i>Salmonella enterica</i>	15	5	6	7	8	10
3.	<i>Pseudomonas aeruginosa</i>	0	2	4	6	6	6

Dye Decolourization:-

Decolourize of the dye Congo red, Orange G and Rhodamine B using silver nanoparticles, synthesized from leaf extract of *C. torulosa* D.Don. After 24 hour samples were withdrawn and analyzed by UV-visible spectrophotometer at 498 for Congo red, 476 for Orange G and 550 for Rhodamine B. Significant decolourization for the dye Congo red, Orange G and Rhodamine B was observed. The decolourization absorbance illustrated in the table. The readings were taken after 24 hours respectively for 8 days. The percentage (%) of dye decolourization was calculated by using the formula:

$$\% \text{ Decolourization} = \left(\frac{\text{O.D}_{\text{control}} - \text{O.D}_{\text{test}}}{\text{O.D}_{\text{control}}} \right) \times 100$$

Table 3:- % Decolourization of dyes by silver nanoparticles

Time duration (in days)	% reduction of dye Congo red	% reduction of dye Orange G	% reduction of dye Rhodamine B
1	17.29	21.57	29.89
2	39.93	31.74	38.79
3	44.02	34.64	46.61
4	60.06	45.43	56.93
5	66.66	55.39	64.05
6	75.15	62.03	68.68
7	81.44	68.25	75.44
8	90.56	80.08	83.27

Silver nanoparticles decolourize Congo red fast as compared with the Orange G and Rhodamine B. The order of decolourization is Congo red > Rhodamine B > Orange G.

Conclusion:-

The rapid biological synthesis of silver nanoparticles from leaves extract of *Cupressus torulosa* provides environmental friendly, straightforward and economical route for synthesis of nanoparticles. Leaf extract of *C. torulosa* contains various phytochemicals which leads to the formation of silver nanoparticles. This biosynthesis methodology is economic, capable of manufacturing silver-nanoparticles in laboratory atmosphere. UV-visible spectroscopy, Scanning Electron Microscopy, Transmission Electron Microscopy and X-Ray Diffraction techniques confirmed the synthesis of silver nanoparticles.

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