IJBPAS, April, 2022, 11(4): 2063-2079

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ANALYSIS OF NANOPARTICLES SYNTHESIZED USING *Terminalia chebula* (HARAD)

KHATAK S*, WADHWA N AND PANDEY AK

Department of Biotechnology, University Institute of Engineering & Technology, Kurukshetra University, Kurukshetra-136119, Haryana, India

***Corresponding Authors: Dr. Sunita Khatak: E Mail: sunitakhatak2019@gmail.com**

Received 25th April 2021; Revised 26th May 2021; Accepted 30th July 2021; Available online 1st April 2022

https://doi.org/10.31032/IJBPAS/2022/11.4.6045

ABSTRACT

Terminalia chebula is a medicinal tree having immense importance in the Ayurvedic System of Medicine. The decoction of fruit is given to infants for digestive disorders even before the plant was discovered by the scientific community. In the current experiment, monometallic silver and zinc nanoparticles were synthesized by using different plant parts which include seed, fruit pulp, bark and leaf extracts. The nanoparticles synthesized were further evaluated for changed antimicrobial activity against *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus Subtilis*. All four plant parts resulted in silver nanoparticle synthesis and showed absorption peaks at 434 nm for leaf, 453 and 492 nm for bark and 419nm for fruit pulp while a peak at 400nm, 450nm for seeds, which confirmed the synthesis of the silver nanoparticles. The research confirmed the plant *Terminalia* to be efficient for silver nanoparticle synthesis. In addition zinc oxide nanoparticles were also synthesized using only leaf from the plant which resulted in dual peak at a wavelength of 326 nm and 357nm which confirmed the synthesis of nanoparticles from Zinc in addition to color change observed in the solution from green solution to cream colored paste which on further heating in muffle furnance converted to fine powder. XRD analysis further confirmed the zinc nanoparticle of size in the range of 22-83nm.

Antimicrobial analysis revealed significant zones of inhibition using *Terminalia* authenticating the plant to be a great bio-resource to result in nanoparticle synthesis of all types.

Keywords*:* Antimicrobial activity, *Terminalia chebula, nanoparticles, XRD Analysis*

INTRODUCTION

Man has exploited the biological resources since Vedic civilization. The plants have been the constant source of medicines and pharmaceuticals products. Nanotechnology in present scenario has merged the electronic and biological sciences to come up with novel materials and have mushroomed in recent past years as a new field of science and technology. Processing on micro scale has its own setbacks which have forced the researchers to incline to nano scale materials. The decrease in scale from micro to nano has altered biological, optical and physiochemical properties which can be manipulated according to the application area. Large scale stable synthesis of nanoparticles is feasible only using plants. Although chemical and physical methods are persistent but use of plant parts to synthesize all sort of nanoparticles like gold, platinum, palladium, copper and zinc offers clean, eco-friendly, economical, and reliable procedure paving path to greener chemistry **[1, 2]**. In comparison to the microbes' plant-based synthesis needs no maintenance of microbial cultures which is major issue in synthesis of the nanoparticles. Moreover, being single step and time saving the procedure is cost effective also. The literature showing nanoparticle synthesis using plant extracts is well documented but using all plant parts of same plant is rarely reported. Being stable at high temperature and pH nanoparticles have shown potent antimicrobial activity specifically silver nanoparticles. They can prove to be the best alternative to existing antimicrobial agents being par in their magnetic and optical properties **[3]**. Chemical methods used are capital intensive and employ hazardous chemicals and further use of synthetic capping agents precludes them to be used in biomedical applications. Silver nanoparticles have vast application in optical cables and in piezoelectric crystals. Being highly catalytic in activity they are used frequently in ceramics, sunscreens, wastewater treatment, and as fungicidal and bactericidal agents and contribute to a new generation alternative to antimicrobial agents **[4]**. Nanoparticles from noble metals viz-zinc, gold, platinum, silver, palladium, have demand in consumer goods for shampoo, detergents, cosmetics, products, soaps and shoes along with toothpaste, medical and

pharmaceutical products. Where gold is used in medicines, drug delivery and disease diagnosis, silver has its demand in sensor technology, biological leveling and biomedical applications **[5]**. Plant derived nanoparticles have different shapes, sizes stability and other parameters being variable have gain considerable attention due to growing need of society for eco-friendly products/ materials. Their novel characteristics add white feather in standing separated from bulk materials. The prime cause of concern is effluents from industries like food, plastic, pulp, paper, rubber, cosmetics, varnish, ink, textiles and dye stuffs. These release toxic products in water bodies which have acute toxic effects on marine life which has resulted in reduced sunlight penetration, reduced BOD and decreased photosynthetic activities. Dyes released are mutagenic and carcinogenic. Treating effluents is challenging problem as dyes are stable to the light, oxidizing agents and exhibit poor biodegradability. Treatment procedures have their own limitations. Use of activated carbon is restricted due to high cost involved and difficult disposal **[6]**. Silver nanoparticles have been documented in dye degradation application as major effective agents. *Terminalia chebula* commonly known as "Harad" belongs to family *Combretaceae* and is said to be "Sarv Avgun Nivaran" as the herb which cures all the ailments, and this clearly depicts the applicability of different plant parts in different ailments. It is found in the forests of Uttar Pradesh, Northern India and Bengal and is quite common in existence in Southern part of India. The plant is a medium to large sized tree distributed throughout subtropical and tropical Asia including mainland China. Ailments like cough, gastroenteritis, diarrhea, fever, skin disease, urinary tract infection are cured using "Harad" by Tribal people of Karnataka and Tamil Nadu **[7]**. The plant parts are boon to nano-researchers as all type of nanoparticles can be synthesized using this plant as sole source confirmed based on visual observation of initial color change experiments using different salts.

2. MATERIALS AND METHODS

Plant and culture collection

Different plant parts *of Terminalia chebula* exploited in the present study for analysis of the antimicrobial activity and for the synthesis of nanoparticles were taken from Kurukshetra University Campus and verified and identified from the Botany Department, Kurukshetra University (Kurukshetra, Haryana, India). The various human pathogenic microorganisms were brought from Microbial Type Culture Collection

(MTCC): Institute of Microbial Technology (IMTECH), Chandigarh, which included Gram-negative bacteria: *Pseudomonas aeruginosa* (MTCC -2295) *Escherichia coli* (MTCC-5704) and two gram-positive bacteria: *Staphylococcus aureus* (MTCC-3160) and *Bacillus subtilis* (MTCC-121). Muller Hilton broth was made to preserve the cultures. All the test tubes containing broths were kept at 4ºC for further use in studies.

Preparation of plant extracts from different plant parts

The leaves, bark, fruit pulp and seeds were oven dried at 50-65^{o}C for 20-40 min for leaf while bark, fruit pulp and seeds take longer time as compared to leaf. The dried samples were pulverized into fine powder and stored in zip bags for further use. The 10gm of powder from each sample was kept in 150 ml of deionized water and heated for only 5 minutes using hot plate. After cooling to room temperature, the extracts were filtered using the Whatman filter paper No.1. The extract, after filtration were stored at 4°C till further use and were used within a week for analysis **[8]**.

Preparation of silver nitrate solutions

Stock solution of silver nitrate solution was prepared by dissolving 3.397g of silver nitrate to the 100ml of de-ionized water giving a concentration of 0.2M and a final concentration of 1mM was prepared from this stock solution to be used finally to synthesize silver nanoparticles **[9]**.

Synthesis and Characterization of silver nanoparticles

The different plant extracts prepared using double distilled water were mixed with 1mM solution of silver nitrate according to method prescribed. All the procedure was carried out in dark room under laminar air flow. Take 2.5ml ammonia solution, add 5ml of 1mM silver nitrate solution followed by addition of 4ml plant extract separately for each sample. Make up a final volume of 50ml using double distilled water. Keep in incubator shaker at 35^{0}C at 100rpm for overnight. Synthesis of silver nanoparticles was confirmed by color change of original solution to light brown colored solution (visual observation) which turned to dark reddish brown on standing and taking absorption maxima within the wavelength range of 300-600nm **[9]**.

Preparation and Confirmation of zinc oxide nanoparticles solution

Five gram of powdered zinc nitrate was dissolved in 50ml of double distilled water in water bath at 70^{0}C till the solution became homogeneous. The 50ml of this dissolved zinc nitrate solution was added drop wise into 12.5ml of plant extract and the solution

was incubated in water bath at 70^0C for one hour. The prepared solution was analyzed for antimicrobial analysis. Synthesized ZnO nanoparticles were confirmed by visual observation via color change of original solution to cream colored solution and taking absorption maxima at the wavelength range of 300-600nm **[3]**.

3.4. Antimicrobial activity of plant extracts by Agar Well Diffusion Assay

The antimicrobial activities of the plant extracts were evaluated by agar well diffusion assay **[10]**. The microbial inoculums were inoculated aseptically spread uniformly on surface of pre-solidified Mueller Hinton Agar (MHA) plates with the help of sterile cotton swabs. A well of about 6.0 mm diameter was aseptically punctured using a cork borer (sterile). The cut agar was removed with the use of sterile forceps. Plant extract was used as control in one of the wells. The petriplates were kept in the laminar for 30 minutes for pre-diffusion to occur and afterwards, petriplates were incubated at 37^0C for 24 hours. The antimicrobial spectrum of the extract was determined in terms of zone sizes (inhibition zone diameters) around each well. Zones were measured by using Zone Scale.

4. RESULT AND DISCUSSION

The current investigation reports an eco-friendly, economical procedure with prime focus on silver nanoparticle synthesis using silver nitrate solution using different plant parts of an ethno-medicinal plant-*Terminalia chebula* commonly known as "Harad" in India. The study revealed the importance of using different plant parts of *Terminalia* as an exceptional plant material and a boon to nano researchers to act as a better reducing and capping agent as reported by most of the researchers till date.

1. Silver Nanoparticles Synthesis and Antimicrobial Propensity

The study involves seed, fruit pulp and bark in addition to leaf extracts for nanoparticle synthesis and was further tested at three different volumes against four standard bacterial pathogens which included two gram negative and two gram positive bacteria **(Table 1)**. Synthesized silver nanoparticles were further characterized by using UV visible spectroscopy which confirmed the peaks at 434nm for leaf, 453 nm and 492 nm for bark and 419nm for fruit pulp while a peak at 400nm and another at 450nm for seeds. In silver nanoparticles the zone size ranged from a minimum of 10mm to maximum of 24mm. The leaves synthesized nanoparticles resulted in moderate activity against gram positive *Bacillus subtilis*

inhibiting the bacteria by producing a zone of 11mm, 17mm and 19mm at three different concentrations taken (50µl, 75µl and 100µl) while against *Staphylococcus aureus* a comparable zone of 15mm was reported. In comparison to gram positive, gram negative bacteria were more susceptible to nanoparticles synthesized using leaf resulting in zones of 16, 18 and 20mm at three different concentrations in increasing order against *Escherichia coli* while against *Pseudomonas aeruginosa* significant activity was not reported as zone of inhibition ranges from 10-13mm only at different volume of extracts. The reason behind the comparable zones of inhibition might be because of minor difference between the volumes of extracts used for antimicrobial analysis **(Table2) (Figure 1)**. In comparison to leaf extracts the bark extract synthesized nanoparticles showed linear relationship with the increasing concentration with the zones. The zone size increased in size from 17 to 20mm against *Bacillus subtilis* while a comparable zone of 14mm was observed against another gram-positive bacteria *Staphylococcus aureus*. The nanoparticles were effective against *Escherichia coli* giving a zone of 21mm which increased in size to 24mm on doubling the concentration from 50µl to 100µl. While least activity was reported against *Pseudomonas aeruginosa* giving a zone of 14mm at all three volumes of extracts. Earlier reports have been focused on fruit part of plant to be exploited for antimicrobial assay using methanolic solvent extracts and leaves as most preferred part for synthesis of nanoparticles being best reducing agents. The present investigation has exploited almost all plant parts for nanoparticle synthesis. So, in this regard the nanoparticles synthesized using fruit pulps were tested against four bacterial pathogens. Fruit pulp were also effective in inhibiting gram-negative bacteria *Escherichia coli* giving a zone of 15mm at 50 µl, and 75µl while the zone size increased to 20mm at 100µl volume of extract, while against *Pseudomonas aeruginosa* it gave a zone of 12mm at all three volumes of extracts. In comparison to gram negative, gram positive bacteria resulted in a zone of 14mm against *Bacillus subtilis* at all three volumes of extracts and zones of 12, 14, and 16 at three different concentrations of 50,75and 100µl each respectively. Pratibha *et al*., 2015 reported green synthesis of the silver nanoparticles by the using *Terminalia* synthesizing silver nanoparticles similar to the present investigation **[11]**. The researcher also reported the presence of terpenoids, resins and saponins in aqueous and

methanolic extracts while tannins and alkaloids were altogether absent in both extracts. Color change from yellowish to brown and UV visible spectroscopy showed absorption peak at 358nm and 450nm, while SEM analysis revealed a particle size of 90-120nm. Similar bacterial pathogens were tested against the nanoparticle synthesized using fruit extracts. The aqueous extracts having nanoparticles have been found to be more resistant to all microorganisms in comparison to aqueous and methanolic extracts. Present results however observed moderate activity using nanoparticles which in turn is further dependent upon solubility of different plant parts in different solvent system. Another group of researchers observed absorption peak at 358nm in Mesua fruit plant extract **[12]**. Present results well corroborate with Lakshmi and her coworkers as two peaks were observed for leaf compounds at 400nm and 450nm, while the zones were less than 24mm using silver nanoparticles solution. One single observation was that highest zone was observed against *Staphylococcus aureus* at 150µl. Dwivedi *et al*., 2013 synthesized nanoparticles using *Terminalia* and assessed antimicrobial activity against similar pathogens as used in present study but using leaf as plant part instead of fruit as stabilizing and reducing agent. UV visible spectroscopy and visual color change gave a peak at 199nm **[13]**. The highest zone of 20mm was observed against *Klebsiella* which was followed by a zone of 18mm against *Salmonella typhii*, 16mm with *Staphylococcus aureus* and least of 15mm with *Escherichia coli*. The activity was reported to be significant in comparison to original plant extract and silver nitrate taken as control. They further suggested that the antimicrobial activity is due to electrostatic interaction between the negative charged cell membrane and positively charged nanoparticles. Kesarlal *et al*., 2012 synthesized silver nanoparticles using same plant and devised a rapid method of silver nitrate synthesis within 15min using *Terminalia chebula* leaf tissues at room temperature and observed peak at 452nm. The researcher revealed higher sensitivity of silver nanoparticles for *Staphylococcus aureus* as compared to *Escherichia coli* and further explained the sensitivity is due to different strain type and is due to morphology of nanoparticles incorporated into the membrane of the cell **[14]**.

Ajmal *et al*., 2016 synthesized silver nanoparticles using *Prunus armeniaca* (apricot) fruit peel extracts and reported 50nm silver nanoparticles synthesis and they

were efficient enough in producing effective zones of inhibitions against selected pathogen similar to the present study. Biggest zone of inhibition was observed with *Staphylococcus aureus* (24mm) followed by *Bacillus subtilis* (21mm) which in turn followed by *Escherichia coli* (19mm) and a minimum zone of 16mm against *Pseudomonas aeruginosa* **[15]**. Awwad *et al*., 2013 synthesized silver nanoparticles using "Loquat leaves" (*Eribotrya japonica*) showing a band at 425nm using UV spectrophotometer and nanoparticles of size 18nm in diameter using FTIR analysis **[16]**. The antimicrobial activity was also confirmed against *Listeria cytogenes* and *Shigella* using 2ug/disc. Kim et al., also supported that *Staphylococcus aureus* is more resistant to silver nanoparticles as compared to *Escherichia. coli*. While blank experiment conducted using aqueous extracts resulted in no zone of inhibition **[17]**. While in contradiction Ruparelia et al.**,** showed silver nanoparticles are more sensitive to *Escherichia coli* than *Staphylococcus aureus* and further explained that this differential observation is depending upon the strain of microorganism and morphology of nanoparticle obtained after synthesis **[18]**. Although a lot of mechanisms have been described for the antimicrobial activity concept but still it is considered that nanoparticles get incorporated into the cell membrane and further cause leakage of the intracellular substances which finally leads to cell death **[14]**. Muthukumaran *et al*., 2015 reported synthesis of silver nanoparticles using plant leaf extract from *Cassia roxburghii* and tested the vulnerability of silver nanoparticles synthesized against most serious human disease –malaria. The disease is caused by *Aedes aegyptii* causing millions of lives every year **[19]**. The silver nanoparticles synthesized come out to be a potent mosquito control agent against *Aedes aegyptii* and *Culex quinquefasciatus* along with *Anopheles stephensi.* The absorption spectrum of *C. roxburghii* leaf extract at different wavelength ranging from 300-800nm giving a peak at 460nm similar to the present study and effective against larvae tested. Awwad *et al*., 2013 used extracts from the leaves of Carob plant (*Ceratonia siliqua)* as capping and reducing agent for reduction of the Ag ions into Ag nanoparticles in a single step process resulting in stable nanoparticle synthesis against gram negative *Escherichia coli* bacteria **[16]**. Zone of inhibition ranged from 8-12mm showing strong activity independent of different concentration of silver nitrate (1 mM to 4 mM). Nanoparticles synthesized

gave a peak at 420nm and particles of size 5-40nm. Yadav *et al*., 2018 also reported silver nanoparticle synthesis from *Ocimum sanctum and Ocimum americana* and tested antimicrobial potential they both observed a peak at 427 and 408nm respectively and tested efficacy of silver nanoparticles against gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* along with *klebsiella pneumonia*. They observed a MIC value of 6.56 at 50µg against *Escherichia coli* and 4.76 at 100µg/ml against *Pseudomonas aeruginosa* using *Ocimum sanctum* while a value of 5.93 at 75µg against *E. coli* and 4.76µg/ml against *Pseudomonas aeruginosa* using *Ocimum americana* **[20]**.

Characterization of nanoparticles

The change in the color of the solution to brown, which was previously colloidal, confirmed the synthesis of nanoparticles from silver. The color change associated with silver and zinc nanoparticles is displayed in **Figure 2 and 3** which confirms the silver nanoparticles synthesis and further confirmed using UV Spectrophotometer analysis within the range of (300-600 nm) which resulted in optical absorption maxima at 434nm for leaf, 453 and 492 nm for bark and 419nm for fruit pulp while a peak at 400nm and another at 450nm for seeds **(Figure 4)**.

2. SYNTHESIS OF ZINC NANOPARTICLES AND ANTIMICROBIAL EFFICIENCY

The Zn nanoparticles synthesized using single-step based process gave a peak at 328 nm and 352nm **(Figure 5)** which confirmed the synthesis in addition to color change from colorless to cream colored solution and was tested against bacteria **(Table 3)** which on heating on hot plate changed to cream colored paste and on further heating in muffle furnace at high temperature of 200^0C resulted in fine particles. The fine particle samples were utilized to deduce the nanoparticle size using XRD analysis and calculating size using Scherer's formula which resulted in nanoparticle of size range of 22 to 83nm size with an average size of 52.5nm sized zinc nanoparticles **(Figure 6), Table 4**.

Figure 1: Zone of inhibition observed using *Terminalia* nanoparticles against *Staphylococcus aureus* (Gram Positive), *Escherichia coli* (Gram Negative) (left to right)

Table1: Inhibition zone diameters (in mm) of different plant extract of *Terminalia chebula* silver nanoparticles at three different volumes of plant extracts

	Leaves (Zones of inhibition) (mm)			Bark (Zones of inhibition) (mm)			Fruit Pulp (Zones of inhibition) (mm)		
Volume (µl)	**50**	**75**	**100**	**50**	**75**	**100**	**50**	**75**	**100**
Bacillus subtilis	**11**	**17**	**19**	**17**	**18**	**20**	**14**	**14**	**14**
Staphylococcus aureus	**14**	**16**	**24**	**14**	**14**	**16**	**12**	**14**	**16**
Escherichia coli	**16**	**18**	**20**	**21**	**21**	**24**	**15**	**15**	**20**
Pseudomonas aeruginosa	**10**	**13**	**13**	**14**	**14**	**14**	**12**	**12**	**12**

Table 2: Inhibition zone diameters (in mm) of different silver nanoparticles of *Terminalia chebula* against different pathogens at (100µl) volume of extract

Plant Part	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Seed	**16**	**18**	**20**	**16**
Leaf	**15**	**24**	**20**	**12**
Bark	**13**	**18**	**18**	**14**
Fruit Pulp	**11**	**18**	**22**	**12**

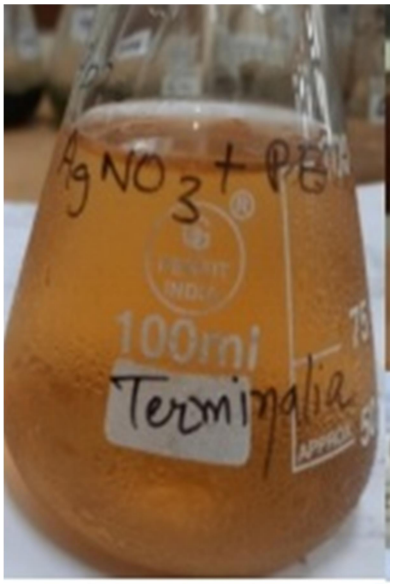

Figure 2: Visual color change confirmation for nanoparticle synthesis from Silver

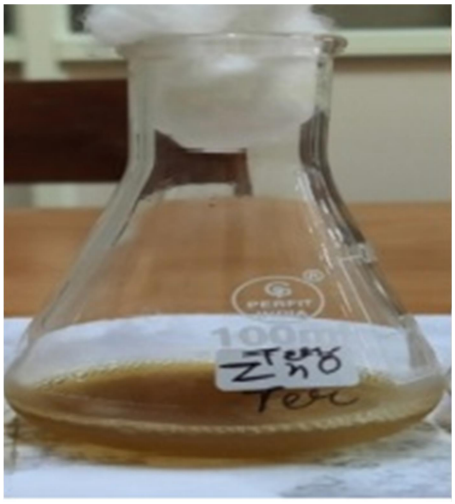

Figure 3: Visual color change confirmation for nanoparticle synthesis from Zinc

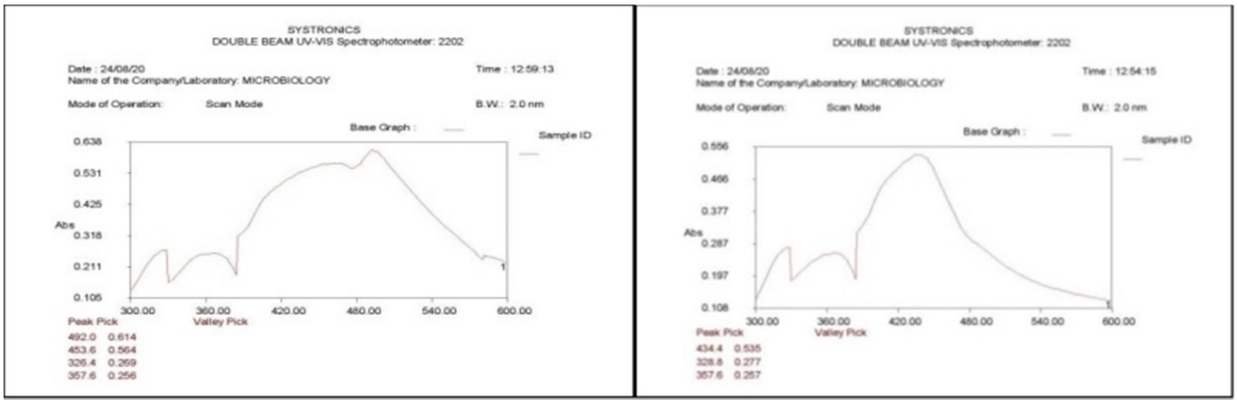

Figure 4: Peaks observed using UV spectrometer of silver nanoparticles synthesized using *Terminalia chebula* Bark (left) and Leaves (right)

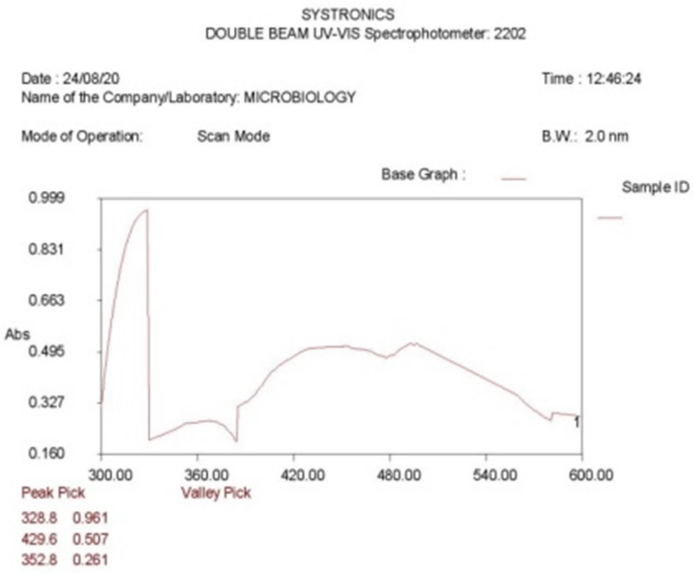

Figure 5: Peaks observed using UV spectrometer of Zinc nanoparticles synthesized using *Terminalia chebula* leaves.

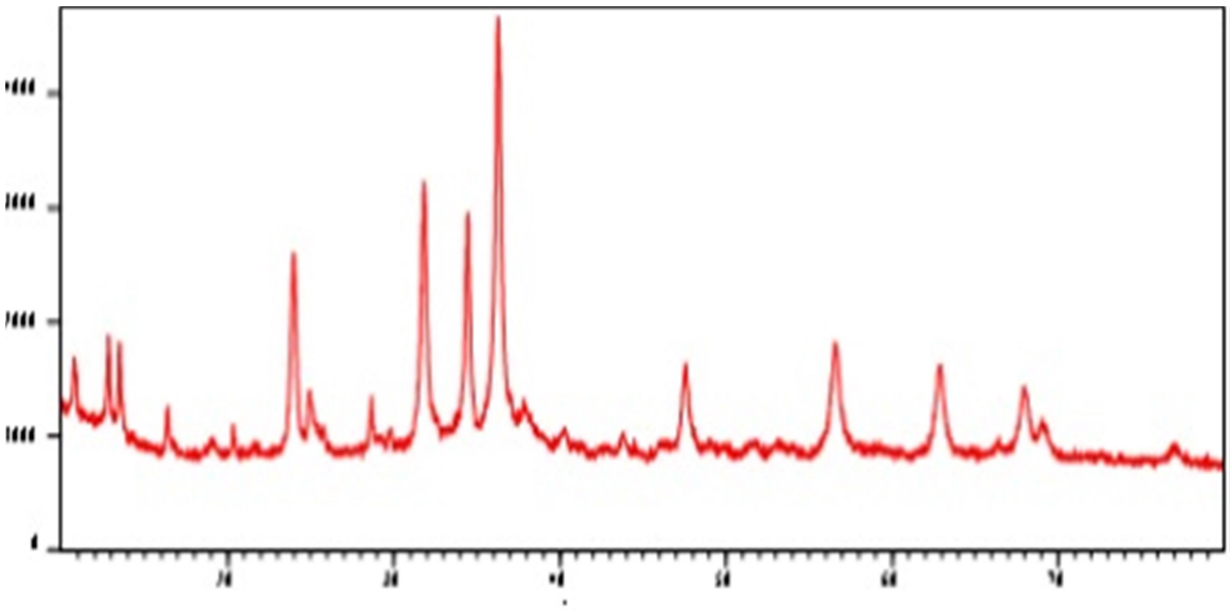

Figure 6: XRD analysis of zinc nanoparticles using leaf extracts of *Terminalia chebula*.

Table 3: Inhibition zone diameters (in mm) of zinc nanoparticles of *Terminalia chebula (leaf)* against different pathogens

Sr. No.	*Concentration of extracts(µl)*	*Staphylococcus Aureus*	*Escherichia coli*
1.	50	17	17
2.	75	18	20
3.	100	20	22
Plant Extract(Aqueous)	100	Nil	nil

Table 4: XRD Analysis of Zinc Nanoparticles using Leaf Extracts of *Terminalia chebula*

S. No.	Pos.[°2θ]	FWHM Total[°2θ]	Diameter (in nm)
1	18.7786	0.1201	70.05
2	24.0740	0.2441	34.77
3	31.8633	0.1095	78.83
4	34.5242	0.1043	83.34
5	36.3479	0.1161	75.25
6	47.6323	0.1370	66.23
7	56.6762	0.1536	61.40
8	62.9488	0.1713	56.82
9	66.4563	0.1963	50.56
10	68.0295	0.1881	53.25
11	69.1586	0.1637	61.60
12	69.1876	0.4593	21.96
13	72.6498	0.1656	62.23
14	77.0419	0.1968	53.92

Zinc nanoparticles were used to test their efficacy against four standard pathogens as given in **Table 3**. Although, all four pathogens were used to test efficacy of zinc nanoparticles but the plates of *Pseudomonas aeruginosa* and *Bacillus subtilis* were not considered because of contamination. The results can't be deduced so here we presented antimicrobial efficacy against two bacteria, *Escherichia coli* (gram negative) while another is *Staphylococcus aureus (*gram positive) so the purpose was solved to test efficacy of synthesized nanoparticles against two different strains. Three different volumes of extracts were used viz 50, 75, 100µl and an aqueous plant extract was taken only at higher concentration of 100µl. The synthesized nanoparticles extract resulted in almost equivalent zones of inhibition of 17mm and 18mm at two different volumes of extracts of 50 and 75 µl. Most probably the minimum difference of 25µl between two is the possible cause for observing almost similar zone of inhibition. While at 100µl the zone size against *Staphylococcus aureus* increased to 20mm which is more significant as the volume of extract get doubled to initial volume of extract. But the aqueous plant extract was unable to produce any zone of inhibition even at a higher concentration of 100µl. Similar results reported by Shah *et al* (2017) **[21]** using zinc nanoparticles resulting in significant zones of inhibition against *Staphylococcus aureus* and *Escherichia coli* by distorting and damaging the cell membrane of bacteria which in turn resulted in leakage of intracellular contents and eventually cell-death. Spherical shaped 23-64nm sized zinc nanoparticles were synthesized using plant extract of *Camellia chinensis* which proved to be potent antibacterial agent resulting in a zone of inhibition against *E. coli* (gram negative bacteria) of 21mm size followed by *Staphylococcus aureus* (15mm). So, *Terminalia chebula* plant parts act as best capping and reducing agent in synthesis of nanoparticles of various types and breaks the myth that nanoparticles are to be more specific and selective in activity against gram positive bacteria in comparison to gram negative bacteria. Research is available on the mechanism-action of ZnO nanoparticles where ZnO nanoparticles in the membrane of bacteria cause membrane integrity changes, interfere in the DNA replication etc., and exert stress on membrane leads to cellular content oozing out and further leading to the cell death **[22, 23]**. Negatively charged ZnO nanoparticles interacted with the gram-positive bacteria by electrostatic forces and hence causing the inhibition **[24]**. Various

methods have been developed for the synthesis of ZnO nanoparticles by using green approach. Among the metal oxide nanoparticles, ZnO nanoparticles are considered significant and used more because of their chemical stability and strong adsorption ability. Literature is studded with plant material used to synthesize zinc nanoparticles including plants like *Camellia chinensis*, *Olea europea, Trifolium pretense, Solanum nigrum*, *Couroupita guianensis*, *Limonia acidissima L., and Vitex trifolia* L. (leaf) etc **[5]**. Various chemical and physical approaches are available to synthesize ZnO nanoparticles, for example solvo-thermal method, micro emulsion method, hydrothermal method, microwave method, thermal decomposition precipitation, laser ablation and sono-chemical method (Mohammed *et al*., 2016) but phytogenic synthesis has overcome the limitation of these methods and offer advantage of being single step combustion method **[6]**. Rong *et al*., 2017 devised an eco-friendly method for the green synthesis of zinc oxide nanoparticles using *Astragalus membranaceus* (AM) leaf extract and revealed wurtzite hexagonal particles with 35-38nm size. On increasing the plant extract concentration from 0.5% to 1.5% the crystallize spherical size of nanoparticles decreased from 38.54nm to11.68nm **[25]**. FTIR analysis confirmed the presence of functional groups of both leaf extracts and zinc nanoparticles. The selective inhibition of different bacterial strains by zinc oxide nanoparticles needs to be explored to design nanoparticles which in turn are applicable in biomedical and antibacterial processes.

CONCLUSION

Silver nanoparticles have been extensively explored due to their therapeutic and antimicrobial potential. Silver has been used in quite a similar way to gold nanoparticles where an antibody photo sensitizer-nanoparticle complex was fused with specific cancer cells to further create free radicals which would then kill the affected cells. Silver nanoparticles can also be used in other biomedical applications as well due to their high applicability in various areas of research. Their potent antimicrobial effects have been extensively studies and offer promising results. Different plant parts after nanoparticle synthesis were selectively active against specific bacteria like bark was giving a zone of 16mm against *Bacillus subtilis* while fruit pulp extracts were active against *Escherichia coli* a gram-negative bacterium while leaf extracts selectively inhibited the *Staphylococcus aureus* strains. So, the need of the hour is to focus on differential activity

of selective plant parts against tested pathogens at a similar concentration and to draw phytoconstituents to design drugs accordingly considering such types of observations. The synthesized zinc nanoparticle has been in demand for acting against minor burns, skin irritations, and diaper rashes. Silver and Zinc coated bandages have recently implemented in wound healing. The zone size against *Staphylococcus aureus* of 20mm showed potential of zinc nanoparticles against infectious bacteria. *S*o, the potential of each type of nanoparticle should be specifically checked for particular strain of bacteria to make effective inhibition and best alternatives to present antimicrobial agents.

ACKNOWLEDGEMENT

We are thankful to Biotechnology Department, University Institute of Engineering and Technology, Kurukshetra University Kurukshetra, Haryana, to provide the lab facilities to carry out this work.

REFERENCE

[1] Dahl, J.A., Maddux, B. L. S, & Hutchison J. E. (2007). Toward greener nanosynthesis. Chemical Reviews.107, 2228-2269.

[2] Hutchison, J.E. (2008). Greener nanoscience: a proactive approach to advancing applications and reducing implications of nanotechnology. ACS Nano, 2, 395-402.

[3] Ramesh, P., Rajendran A., & Meenakshisundaram, M. (2014). Green synthesis of zinc oxide nanoparticles using flower extract *Cassia auriculata*. Journal of NanoScience and NanoTechnology, 1, 41-45.

[4] Rai, M., Yadav, A., & Gade A. (2009). Silver Nanoparticles as A New Generation of Antimicrobial. Biotechnology Advances, 27, 76-83.

[5] Kalpana, V. N., Patra, R. P., & Rajeshwari, V. D. (2017). Green synthesis of zinc oxide nanoparticles using fresh stem of *Cissus quadrangularis* extract and its various in vitro studies. Asian J. of Chemistry, 29(6), 1323-1327.

[6] Mohammad S. G., & Ahmed, S. M. (2017). Preparation of environmentally friendly activated carbon for removal of pesticide from aqueous media. International Journal of Industrial Chemistry, 8(2), 121-132.

[7] Dash, B. (1991). Marteria Medica of Ayurveda, 1,170-174.

[8] Ramesh, M., Anbuvannan, M., & Viruthagiri, G. (2015). Green synthesis of ZnO nanoparticles using *Solanum nigrum* leaf extract and their

antibacterial activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 136, 864-870.

[9] Logeswari, P., Silambarasan, S., & Abraham J. (2019). Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. Journal of Saudi Chemical Society, 3, 311-317.

[10] Perez C., Pauli M., & Bazerque P. (1990). An antibiotic assay by agar well diffusion method. Acta Biologiaet Medicine Experimentalia, 15, 113-115.

[11] Pratibha S., Packiyam J. E., Bhatt E. R., Jayadev P., & Shetty S. (2015).Green synthesis of silver nanoparticles from fruit extract of *Terminalia chebula* and their antibacterial activity. Int. J. of Research in Bioscience, 4(2), 29-35.

[12] Lakshmi, N., Packiyam, J. E., Bhat, P. R., & Jayadev, K. (2014). Studies on phytochemical, synthesis of silver nanoparticles from the floral extract of *Mesua ferrea* and their antibacterial activity. International Journal of Green and Herbal Chemistry, 3(4), 1730-1739.

[13] Dwivedi, R. (2013). Green synthesis of silver nanoparticles using *Terminalia chebula* and assessment of its antimicrobial activity. Int. J. Pure App. Biosci., 1(6), 1-6.

[14] Kumar, K.M., Sinha, M., Mandal, B. K., GhoshA. R., Kumar, K. S., & Reddy, P.S. (2012). Green synthesis of silver nanoparticles using *Terminalia chebula* extract at room temperature and their antimicrobial studies. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 91, 228-233.

[15] Ajmal, N., Saraswat, K., Sharma, V., & Zafar, M. E. (2016). Synthesis and Antibacterial activity of Silver nanoparticles from *Prunus armeniaca* (Apricot) fruit peel extract. Bull. Env. Pharmacol, Life Sci. 5(8), 91-94.

[16] Awwad, A. M., Salem, N. M., & Abdeen, A. O. (2013). Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. International journal of Industrial chemistry, 4(1), 1-6.

[17] Kim, J.S., Kuk, E., Yu, K. N., Kim J. H., Park S. J.,& Lee H. J. (2007). Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine. 3(1), 95-101.

[18] Ruparelia, J. P., Chatterjee, A. K., Duttagupta, S. P,& Mukherji, S. (2008). Acta Biomater*,* 4, 707–716.

[19] Muthukumaran, U.,Govindarajan, M., & Rajeswary, M. (2015). Green synthesis of silver nanoparticles from *Cassia roxburghii* –a most potent power for mosquito control. Parasitol Res*,* 114, 4385-4395.

[20] Yadav, A., Kaushik, A., & Joshi A. (2018). Green synthesis of silver nanoparticles using *Ocimum sanctum* and *Ocimum Americana L.* for their antibacterial potential. Int. J. of Life sciences & Pharma Res*,* 8 (1), 42-49.

[21] Shah, R.K., Boruah, F., & Parween, N. (2015). Synthesis, characterization of ZnO nanoparticles using leaf extracts of *Camellia chinensis* and evaluation of their antimicrobial efficacy. Int. J. Curr. Microbiol. Appl. Sci., 4(8), 444-450.

[22] Dobrucka, R. & Długaszewska, J. (2016). Biosynthesis and antibacterial activity of ZnO nanoparticles using *Trifolium pratense* flower extract. Saudi J. Biol. Sci., 23, 517–523.

[23] Visurraga, J. D.,Gutiérrez, C., Plessing, C. V., & García, A. (2011). Metal nanostructures as antibacterial agents. Science and technology against microbial pathogens: research, development and evaluation, 1, 210–218.

[24] Raghupathi, K.R., Koodali, R.T., & Manna, A.C. (2011) Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. Langmuir*,* 27, 4020–4028.

[25] Gao, S., Wang, X., Wang, S., Zhu, S., Rong, R., & Xu, X. (2017) Complex effect of zinc oxide nanoparticles on cadmium chloride-induced hepato-toxicity in mice: protective role of metallothionein. Metallomics, 9(6), 706-714.