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Research Article

SCREENING OF ANTIBACTERIAL POTENTIAL OF BIOSYNTHESIZED SILVER NANOPARTICLES BY USING SOME MEDICINAL PLANTS

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ABSTRACT

The Silver nanoparticles (AgNPs) were biosynthesized from the leaf extracts of *Prunus Dulcis* & *Syzygium Cumini* by ecofriendly means. In this study, rapid, simple approach was applied for synthesis of silver nanoparticles using *Prunus Dulcis* & *Syzygium Cumini* aqueous solution and leaf extract. The plant extract acts both as reducing agent as well as capping agent. To identify the compounds responsible for reduction of silver ions, the techniques used to characterize synthesized nanoparticles are UV-Visible spectrophotometer. UV-Visible spectrophotometer showed absorbance peak in range of 400nm & 422nm and 400nm. The silver nanoparticles showed antibacterial activities against *Escherichia coli (MTCC1679)* microorganisms. Results confirmed this protocol as simple, rapid, one step, and eco-friendly, nontoxic and alternative conventional physical/chemical methods. only 24hrs. were required for the conversion of silver ions into silver nanoparticles at room temperature, without the involvement of any hazardous chemical.

Keywords: Silver nanoparticles, Biosynthesis, Medicinal plants, Green synthesis, Nanotechnology.

INTRODUCTION

A nanoparticle or ultrafine particle is a particle of matter 1 to 100 nanometres (nm) in diameter. The term is sometimes used for larger particles, up to 500 nm, or fibers and tubes that are less than 100 nm in only two directions (Vert *et. al*., 2012). At the lowest range, metal particles smaller than 1 nm are usually called atom clusters instead. Nanoparticles are distinguished from microparticles (1-1000 µm), "fine particles" (sized between 100 and 2500 nm), and "coarse particles" (ranging from 2500 to 10,000 nm), because their smaller size drives very different physical or chemical properties, like colloidal properties and ultrafast optical effects (A. López-Suárez *et. al.*2015) or electric properties. Nanoparticles occur widely in nature and are objects of study in many sciences such as chemistry, physics, geology, and biology. Being at the transition between bulk materials and atomic or molecular structures, they often exhibit phenomena that are not observed at either scale. They are an important component of atmospheric pollution, and key ingredients in many industrialized products such as paints, plastics, metals, ceramics, and magnetic products. The production of nanoparticles with specific properties is a branch of nanotechnology.

Nanotechnology refers to the branch of science and engineering devoted to designing, producing, and using structures, devices, and systems by manipulating atoms and molecules at nanoscale, i.e. having one or more dimensions of the order of 100 nanometers (100 millionth of a millimeter) or less. In the natural world, there are many examples of structures with one or more nanometer dimensions, and many technologies have incidentally involved such nanostructures for many years, but only recently has it been possible to do it intentionally. Many of the applications of nanotechnology involve new materials that have very different properties and new effects compared to the same materials made at larger sizes. This is due to the very high surface to volume ratio of nanoparticles compared to larger particles, and to effects that appear at that small scale but are not observed at larger scales.

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Medicinal plant has been identified as a part of the evolution of human healthcare for thousands of years. Medicinal components from plants play an important role in traditional as well as modern medicine. Antibacterial resistance is an increasingly serious threat to global public health. According to World Health Organization (WHO) report on antimicrobial resistance in 2014, overcoming the antibiotic resistance is the major issue to the WHO for the next millennium. Screening of plants for antimicrobial agents has gained much importance because WHO is encouraging and promoting in the development and utilization of medicinal plant resources in the traditional system of medicine. Accordingly, the last decade witnessed an increase in the investigation of plants as a source of human infectious disease management.

The aim of this study was to screening the antibacterial activity of some medicinal plant used in Ayurveda and traditional medicinal system for treatment of manifestations caused by microorganisms. Therefore, extracts of the two plants *Prunus Dulcis* & *Syzygium Cumini,* commonly *Prunus Dulcis* referred to as the Almond tree is a deciduous tree renowned for its edible seeds, which are almond, belonging to family of Rosaceae and *Syzygium cumini*, commonly known as Malabar plum, Java plum, black plum, jamun, jambul, or jambolan, is an evergreen tropical tree in the flowering plant family Myrtaceae were tested for their potential activity against bacterial pathogens.

CLASSIFICATION OF NANOPARTICLES

Nanoparticles can be broadly grouped into two namely organic nanoparticles which include carbon nanoparticles (fullerenes, graphene, carbon black) while, some of the inorganic nanoparticles include magnetic nanoparticles, semiconductor nanoparticles like zinc oxide and titanium oxide) and noble metal nanoparticles like gold and silver.

1) **Organic nanoparticles:** These nanoparticles are biodegradable, non-toxic and some particles such as micelles and liposomes which has a hollow core, also known as nano-capsules and are sensitive to thermal and electromagnetic radiation such as heat and light.
2) **Carbon nanoparticles:** The nanoparticles made completely of carbon are known as carbon nanoparticles. They can be classified into fullerenes, graphene and carbon black.
3) **Fullerenes:** Fullerenes is a carbon molecule that is spherical in shape and made up of carbon atoms held together sp^2 hybridization. About 28 to 1500 carbon atoms form the spherical structure with diameters up to 8.2 nm for a single layer and 4 to 36 nm for multi-layered fullerenes (Ealias *et al*., 2017).
4) **Graphene:** Graphene is an allotrope of carbon. Graphene is a hexagonal network of honeycomb lattice made up of carbon atoms in a two-dimensional planar surface, generally, the thickness of the graphene sheet is around 1om.
5) **Carbon black:** An amorphous material made up of carton, generally spheric in shape with diameters from 20 to 70 nm.
6) **Inorganic nanoparticles:** Inorganic nanoparticles are particles that are not made up of carbon. Metal and metal oxide-based nanoparticles are generally categorized as inorganic nanoparticles,
7) **Magnetic nanoparticles:** Magnetic nanoparticles are nanoparticles type which can easily be tracked, manipulated and targeted using magnetic field. These are composed of elements iron, cobalt, nickel and their oxides. Magnetic nanoparticles are useful for catalysis, magnetic fluids, data storage, biomedicine, magnetic resonance imaging (MRI) and environmental remediation (Xiangqian Li *et al*., 2011).

SYNTHESIS OF NANOPARTICLES

Various methods can be employed for the synthesis of NPs, but these methods are broadly divided into two main classes i.e. (1) Bottom-up approach and (2) Top-down approach (Wang and Xia *et. al.* , 2004) as shown in Scheme 1 (Iravani *et. al.* 2011). These approaches further divide into various subclasses based on the operation, reaction condition and adopted protocols.

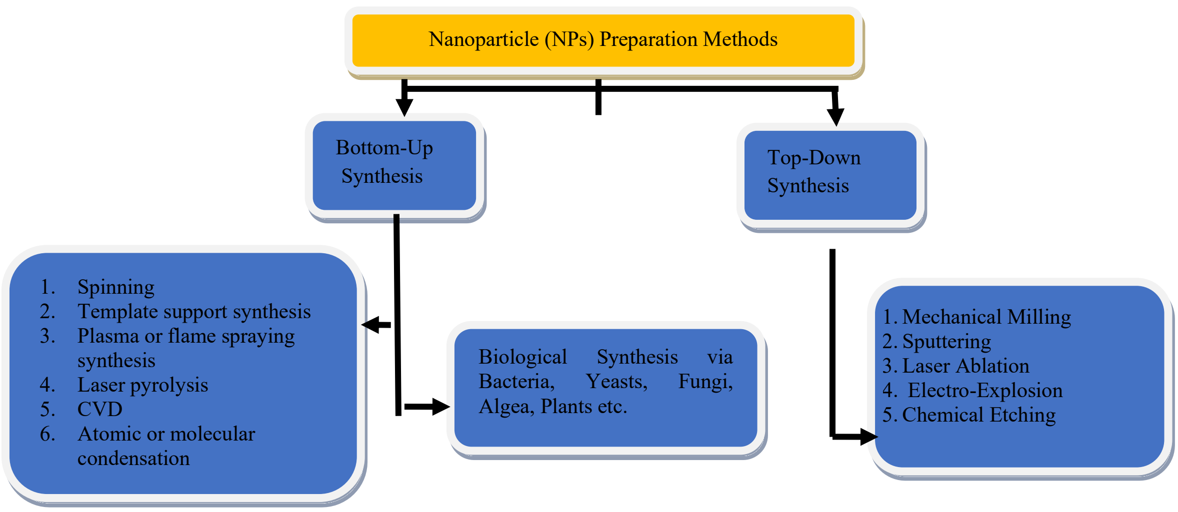


Figure 1. Typical synthetic methods for NPs for the (a) top-down and (b) bottom-up approaches.

MATERIALS AND METHODS

Survey and collection of plant leaves

In the present research work, the plant samples were collected from the various regions of the Jabalpur regions such as Govt. M.H. College of Home Science campus of Jabalpur region. The collected leaves were used in the preparation of extracts for the green synthesis of AgNPs, and the screening of their biological activities. The list of collected plants and their site are given in the below table number and some selected Indian medicinal plants used to treat various kinds of human.

Preparation of plant metabolite

Fresh leaves of four different plants, that were Jamun (*Syzygium Cumini*) and Badam (*Prunus Dulcis*), were collected from Govt. M.H. College of Home Science campus and some of the samples were collected from rural areas of Jabalpur region. The leaves were rinsed with sterilized distilled water & ethanol and air dried under shade for 10 days. Then the plant leaves were grind into fine powder. Then take 5gm of the leaf powder was mixed with 100ml distilled water then boil for 10 min., after boiling, the mixture was cooled and filtered with Whatman filter paper number 1 used for synthesis of silver nanoparticles.

Table 1. list of collected plant material and their site.

S.No.	Common name	Host Plants (Botanical name)	Part of plant used	Location of collection site
1	Jamun Tree	*Syzygium Cumini*	Leaves	Govt. M.H. College of Home Science campus
2	Badam Tree	*Prunus Dulcis*	Leaves	Sukha Power Grid, Patan Road, Jabalpur

Table 2. Select Indian medicinal plants used to treat various kinds of human.

S.No.	Common name	Medicinal plants (Botanical name)	Part of plant used	Ayurvedic or Traditional Uses
1	Jamun Tree	*Syzygium Cumini*	Leaves	Leaves of plant are used to treat Antidiabetic Effects, Anti-Hyperlipidaemic, Anti-Obesity and Anti-Hypertensive and Antioxidative and Anti-Inflammatory Effects
2	Badam Tree	*Prunus Dulcis*	Leaves	Tannins released by Indian almond leaves kill bacteria, fungus, and viruses. Indian almond leaves contain flavonoids. The flavonoid, Quercetin has been shown to offer anti-inflammatory properties. Indian Almond Leaves can slightly reduce pH in the substrate as the tannic acid is released.

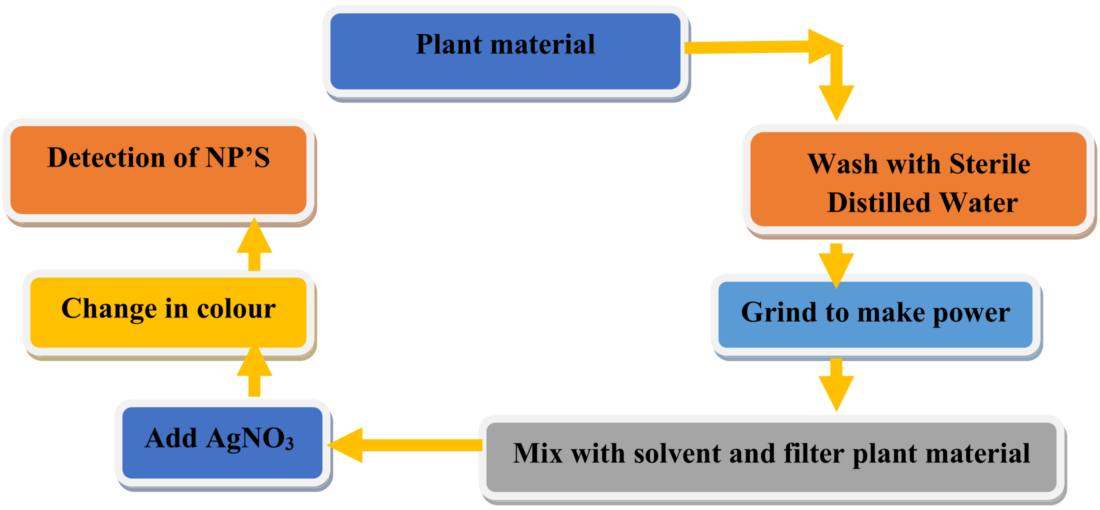


Figure 2. Bio- synthesis of nano-particles by using plant material.

Bio-Synthesis of silver nanoparticles

In the present study, 1mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. For synthesis of silver nanoparticles 50 ml filtrate distilled water & ethanol of leaf extract of *Syzygium Cumini* and *Prunus Dulcis* was added for bio-reduction process at room temperature in dark condition for 24 hours. After the proper incubation period the change of color was observed visually (light brown & dark brown colour).

Screening of plant metabolites

For screening of antibacterial activity of plant extract of the plants were observed by using agar well diffusion method. In this study bacterial strain *Escherichia coli (MTCC1679)* were used.

Agar well diffusion method

The antibacterial bioassay was performed by Agar well diffusion method given by Egrov *et. al*., 1995 against pathogenic bacteria *Escherichia coli (MTCC1679).* The nutrient agars plates were prepared and well of 8mm were made in the plates with the help of a cork borer. Nutrient agar plates were seeded with two sets 20µl (DW) & 40µl (Ethanol) of standardized broth culture of the test bacteria. Each plate was spread evenly on the plate bacterial solution and after 20 min of spreading; the wells were loaded with 100 µl plant metabolites.

The Petri plates were then placed in an incubator at 370C, and the antibacterial activity of AgNPs was evaluated by measuring the diameter of the zone of inhibition (in cm) surrounding and the wells measured with the help of Hi-media antibiotic zone scale, Hi media Laboratories Mata Gujri Mahila Mahavidyalaya (Autonomous) Jabalpur, indicating the antimicrobial effectiveness of the silver nanoparticles. In the present study, potential *Syzygium Cumini* and *Prunus Dulcis* leaf extract was used to synthesize AgNPs. The synthesized AgNPs were characterized using UV-visible spectroscopy and the antibacterial activities of these AgNPs were screened.

RESULT AND DISCUSSION

Plant leaves are used as medicines against gastroenteritis, diarrhoea and toothache for replace antibiotics. The present study screened the antibacterial effects of silver nanoparticles of *Syzygium Cumini* and *Prunus Dulcis*. The *Syzygium Cumini* and *Prunus Dulcis* plant metabolite mixed with in the aqueous solution ion complex, color change from light green to dark brown was observed due to the reduction of silver ions. The color change is reportedly confirmation of formation of nano-particles. The change of color of silver nanoparticles was observed after the 24 hrs of dark incubation of the sample on the rotary shaker.

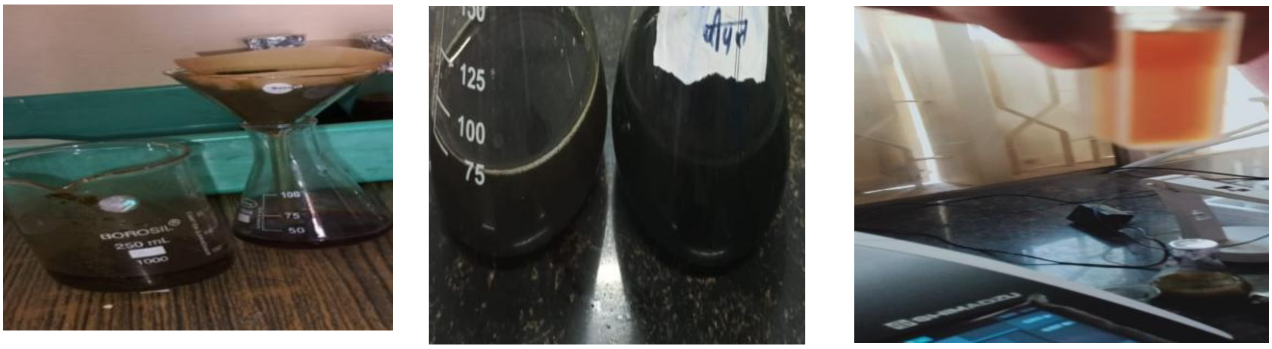


Figure 3. Biosynthesis of silver nanoparticles from leaf extract.

Table 3.Characterization of bio synthesized silver nanoparticles on the basis of colour change.

S.No.	Name of Bacterial strain isolates	Name of Host plant	Colour change
1	*Escherichia coli* (MTCC1679)	*Syzygium Cumini*	Light Brown colour
2	*Escherichia coli* (MTCC1679)	*Prunus Dulcis*	Dark Brown colour

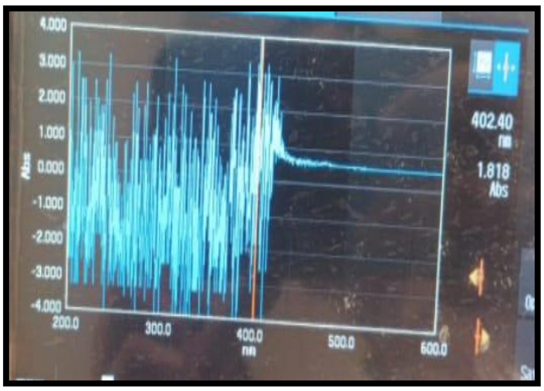
After the biosynthesis of silver nanoparticles by using leaf extract were further characterized by using various approaches such as UV-Visible spectrophotometer to detect the absorbance on the nanoparticles. The findings from the UV-Vis absorption spectrum were considered as a novel technique widely used for structural characterization of nanoparticles. In this case reduction of silver nanoparticles during exposure to fungal metabolite was observed as a result of the color change. The color change is due to the SPR phenomenon. The SPR pattern is dependent on the characteristics of the individual metal particles, such as size and shape, as well as the dielectric properties of the medium used for synthesis and the inter-nanoparticle coupling interactions. The intensity of the SPR band increased with reaction time, indicating the synthesis of the AgNPs.

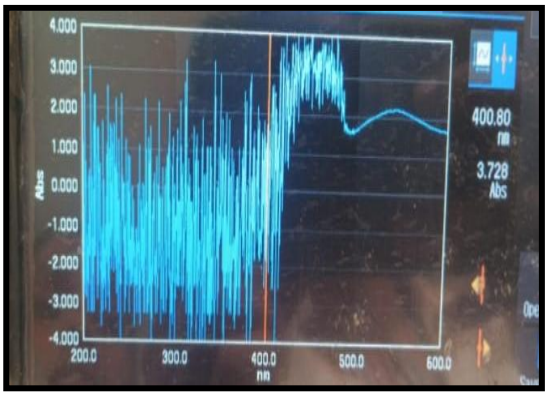
The presence of an absorbance peak at about 400nm (*Syzygium Cumini*), 402nm and 400nm (*Prunus Dulcis*) clearly indicates the formation of AgNPs in the solution due to surface plasmon resonance (SPR) electrons present on the nanoparticle surface as shown in graph 1. Similarly, the formation of silver nanoparticles synthesized from Ocimum tenuiflorum, Solanum tricobatum, Centella asiatica and Citrus sinensis were monitored by UV-vis spectrophotometer analysis. The UV-vis spectra showed maximum absorbance at 420 nm, which increased with time of incubation of

silver nitrate with the plants extract. The curve shows increased absorbance in various time intervals (1 h, 24 h and 48 h) and the peaks were noticed at 420 nm corresponding to the surface plasmon resonance of silver nanoparticles. The observation indicated that the reduction of the Ag+ ions took place extracellularly (Logeswari *et. al*., 2015). The green synthesis of silver nanoparticles (AgNPs) from plant extract of Buddleja globosa was also confirmed by UV-Vis spectroscopy. A wide absorption peak localized between 300 nm and 380 nm of wavelength related to B. globosa extract was observed. After one hour of reaction, the reduction of Ag ions into AgNPs shows a minor absorption peak centered at 445 nm of wavelength related to the localized surface plasmon resonance of AgNPs. Furthermore, as the reaction time rises, the intensity of this absorption peak also increases. For screening the antibacterial activity of the plant metabolites extract were determined by agar well diffusion method against the test *Escherichia coli (MTCC1679)* bacterial strain. The *Prunus Dulcis* plant metabolite was showed the maximum zone of inhibition against *Escherichia coli (MTCC1679)* in aqueous and ehanolic extract 18mm, 26mm, 34mm, & 33mm as shown in bacterial plates, table.4.2, graph 4.1.

Table 4. Spectrophotometric analysis of biosynthesized silver nanoparticles on the basis of absorbance (200nm-600nm).

Molar solution of $AgNO_3$ for different plant leaves extract	Range of wavelength (200-600)	Absorbance (in nm)
1mM $AgNO_3$	400	2.653(ethanol)
(*Syzygium Cumini*)	241	0.273(DW)
1mM $AgNO_3$	400	3.728(ethanol)
(*Prunus Dulcis*)	402	1.818(DW)





Graph 1. UV- Spectroscopy of 1mM $AgNO_3$ *Prunus Amygdalus* biosynthesized silver nanoparticles

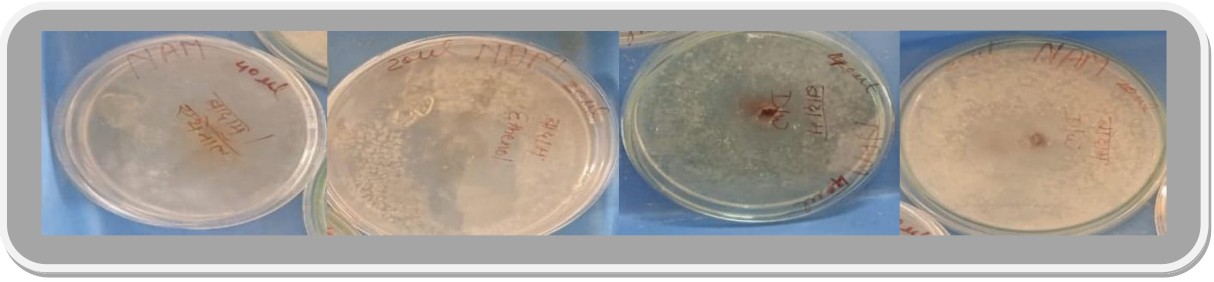
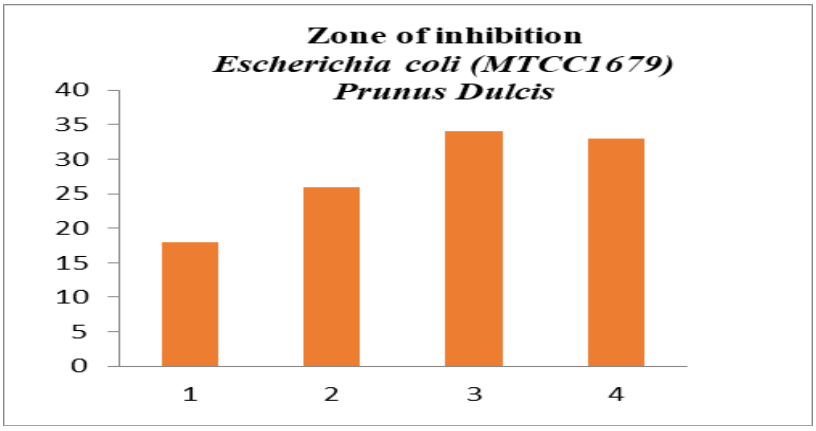


Figure 4. Screening of Silver Nanoparticles for Antibacterial Activity.

Table 5. Zone of inhibition in mm against *Escherichia coli (MTCC1679).*

Plant sample	Zone of inhibition *Escherichia coli* (MTCC1679)			
	20µl (Distilled Water)	40µl (Distilled Water)	20µl (Ethanol)	40µl (Ethanol)
Prunus Dulcis	18mm	26mm	34mm	33mm



Graph 2. Antibacterial Activity of the plant metabolites.

Similarly, leaf extracts of *A. officinalis* were examined for its antibacterial potential using five different solvents against some reference strains of human pathogenic bacteria for the crude extract and showed remarkable antibacterial activity with zones of inhibition of 13mm against *Eschericia coli* (MTCC1679) and 11mm against *Staphylococcus aureus*. Fraction 13(ethyl acetate: methanol 8:2) as the most potent one against with the minimal inhibitory concentration of 30 mm against *E. coli* (MTCC1679) and 25 mm against *S. aureux* (Bhimba *et al.*, 2010).

CONCLUSION

The green synthesis of silver nanoparticles offers a potentially, ecofriendly, non-toxic, and cost-effective approach for the synthesis of nanoparticles. Different plant extracts can be used for the synthesis of silver nanoparticles. In the present research work the plant extract of *Prunus Dulcis* was used for the synthesis of silver nanoparticles. The antibacterial activity was examined against the test bacterial strain *Escherichia coli (MTCC1679)*. It is understood that different types of natural compounds present in plant extracts can act as reducing and stabilizing agents in the synthesis of silver nanoparticles. Plant-mediated silver nanoparticles are also stable due to the presence of natural capping agents such as proteins, which prevent the particles from aggregation. Furthermore, silver nanoparticles generated by green synthesis have potential applications, especially as antibacterial agents of certain microorganisms for which their efficiency has been scientifically proven, in biomedicine as therapeutic agents and wastewater treatment, in agriculture, in food safety and in food packaging. Therefore, the green synthesis of silver nanoparticles from *Prunus Dulcis* leaves extracts has several advantages such as eco-friendly, biocompatibility and cost-effectiveness. It is concluded that due to these unique properties, silver nanoparticles will have a key role in many of the nanotechnology-based processes.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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