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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING EUCALYPTUS LEAVES EXTRACT AND EVALUATION OF THEIR ANTIBACTERIAL ACTIVITY

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Abstract:
Green chemistry principles were employed to manufacture green NPs of Ag from aqueous leaves extract of Eucalyptus. Secondary metabolites present in leaves extract to reduce the Ag^{+} into Ag NPs. Secondary metabolites are important in the production of NPs. Secondary metabolites play a significant part in the production of NPs, due to which biosynthesized NPs are gaining attention and being used in the biological area. Gram(+ve) and Gram (-ve) bacteria were employed for the evaluation of the antibiotic activity of Ag NPs. FT-IR, UV-Visible spectroscopy, SEM-EDAX, and XRD were used to analyse the fabricated NPs. The identification of functional groups on the surface of NPs was done using FT-IR spectroscopy. The peak was found at 430 nm in the UV-Visible spectrum. Obtained results confirm that the green method is a rapid, single-step and environmentally benign process and an alternative chemical process without using any hazardous chemicals.
Keywords: Green Synthesis, Ag NPs, FT-IR, UV-Visible Spectroscopy, XRD, SEM-EDAX.

I. Introduction

Green nanotechnology is the world's fastest sector for the production of new nanomaterials using green chemistry principles. [1] It has been well established that plant cells are the green, reducing agent. In addition, plant extracts are non-toxic, hence creating a more favourable environment for NPs synthesis. Green nanotechnology can improve the interaction of living organisms with NPs. Plant cells have long been recognised as the greatest example of a green, reducing agent. In addition, plant extracts are free from harmful compounds; consequently they provide a superior foundation for the NPs production. Additionally, microorganism isolation and culture medium costs can be reduced by using plant extracts, boosting the cost-effectiveness of microorganisms synthesising nanoparticles [2]. The chemical, physical, and biological approaches can all be used to create nanoparticles. But the chemical process requires the use of toxic agents for the stabilization of NPs and leads to toxic byproducts. Hence, there is a requirement for the green synthesis of NPs. In modern material science, nanotechnology based on green chemistry is the most active area of research. Some characteristics of NPs, such as size, shape and morphology, exhibits improved properties [3]. Nanoparticles display entirely new or enhanced properties as a result of their size, dispersion, and shape.

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The biogenic fabrication of NPs is an environment-friendly, low-cost method. Green NPs are free from toxic chemicals. This is the most important fact of green nanotechnology. Also, the coating of bioorganic compounds makes them useful for medical applications. The biogenic synthesis of NPs has specific benefits over conventional approaches as a result of these factors. [4] For example, nanoparticles (NPs) are being employed in cancer therapy as a novel tool, however, NPs that have

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been chemically manufactured are harmful in nature. Biologically produced nanoparticles (NPs) coated with biocompatible compounds have been used to solve this problem. [5]

Green synthesis of NPs was splited into two stages: nucleation and growth. Researchers will synthesize the green NPs by understanding and manipulating these two stages. According to the green chemistry perspective, the application of environmentally benign reducing and capping agents and non-toxic solvent these three factors should be considered. Phytosynthesis of NPs is an accessible alternative for large scale production because it implies plant material as a green, reducing agent. Medicinal plants are a rich source of green reducing agents. However, in some cases, metal ions can be reduced, only part of the plant which can play an essential role in NPs synthesis. Hence plant extract can be directly used for the biogenic synthesis of NPs. Environmentally benign precursor for the NPs synthesis is also critical requisite of Green nanotechnology.

In the present work, we have used Eucalyptus leaves for the synthesis of NPs. Secondary metabolites present in leaves extract reduce the silver ion into Ag NPs and prevent the aggregation of synthesized NPs. Hence they act as capping agents also. Therefore there is no need for any toxic chemical for the fabrication of NPs. Due to presence of capping agents, NPs are used in biological applications. Ag NPs are used in several organic transformations as heterogeneous catalysts and the pharmaceutical industry [6]. In addition, silver nanoparticles have effective antimicrobial properties due to which they are used in biological activity. They have great potential for use in antimicrobial activity. In addition to being a very powerful antibacterial agent, silver is also safe to use. To prevent infection of open wounds, Ag NPs are used in ointments due to their physicochemical properties.[7] Ag NPs also shows antimicrobial, antiviral activities. [8] Interactions between living cells and the molecular level can be better understood using nanotechnology. For infections, vaccinations, and kidney disorders, a number of nanoparticle-based therapies have been approved for clinical trials [9].

II. Experimental

A. Preparation of leaves extract

A collection of leaves was taken from the local area of Indore and cleaned multiple times with distilled water before being sliced into little pieces. Pour 100 mL of distilled water and 25 gm of leaves into a beaker and bring to a boil at 80 degrees Celsius for 30 minutes. The extract was then separated.

B. Biogenesis of silver NPs

One hundred millilitres of 0.1 M $AgNO_3$ solution were treated with ten millilitres of aqueous leaves extract under continuous stirring on a magnetic stirrer at 80 degrees Celsius for three hours. After that, precipitates will be formed, which were separated by centrifugation and rinsed with distilled water. Precipitates were dried for 1 hour at 80^{o}C in a hot air oven and calcined for 3 hours at 300^{o}C in a muffle furnace.

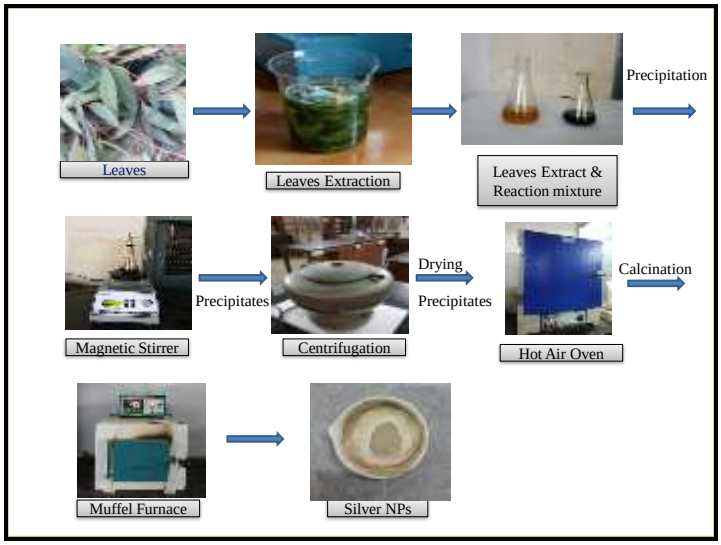

Fig:1Biogenic synthesis of Ag NPs

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C. Characterization of Ag NPs

UV-Visible spectroscopy was utilised to detect optical characteristics of produced NPs. FT-IR spectroscopy was also utilised to identify functional groups on NPs' surfaces. Furthermore, crystalline nature was characterized by XRD analysis. Finally, SEM-EDAX was used for the morphology and elemental composition of NPs'.

X-Ray Diffraction: X-ray diffractometer (XRD) [Proto AXRD] Cu radiation was used for the characterization of NPs. The Debye-Scherrer equation was used to compute the average particle size. Debye-Scherrer equation is as follows:

$$D = K\lambda / \beta\cos\theta$$

D = crystallite size, θ= Bragg's angle, λ = wavelength of X-rays, β = width at half maxima .

SEM-EDAX determined the morphology and elemental composition of synthesized NPs. The images viewed using SEM are created by detecting secondary electrons in the specimen that are ejected by the high energy beam of electrons. SEM-EDAX images of sample was recorded on a JEOL 6390 6390LA/ OXFORD XMX N instrument. The advantages of EDAX are a user can acquire a full elemental spectrum in only a few seconds.

D. General procedure of Knoevenagel condensation by Ag NPs (Green Catalyst)

In a 50 ml round bottom flask 2mmol benzaldehyde, 2mmol malanonitrile and solvent Ag NPs catalyst were mixed. After some time distilled water was used as a solvent. After the reaction was completed, the catalyst was separated using filtration. FT-IR analysis was used to characterise the synthesised product.

E. Antibacterial activity of biosynthesized Ag NPs

Agar well diffusion method

The antimicrobial activity of Ag NPs was tested using Agar well diffusion method[**11**]. In the petri dish, bacterial strains were spread on the surface of the agar medium. A 6-8mm hole was bored with a cork borer, and a 20 µL amount of NPs was put into the well with a pipette. Incubation at 37°C for 24 hours followed. Bacteria growth is inhibited by the diffusion of NPs into the agar media. The nanoparticles (NPs) infiltrate into the agar medium and limit bacterial growth.

III. Result and discussion

A. UV-Visible Spectroscopy

The optical characteristics of nanoparticles (NPs) were investigated using UV-Visible spectroscopy. Some amount of the suspension (NPs dispersed in distilled water) (5mL) was taken into the cuvette and analyzed at room temperature. A broad band was obtained at 435 nm. Mie theory states that when synthesized NPs are spherical, a single band is obtained in the graph [**12**]. Hence, from the UV-Graph, it has been confirmed that the Ag NPs are spherical shaped.

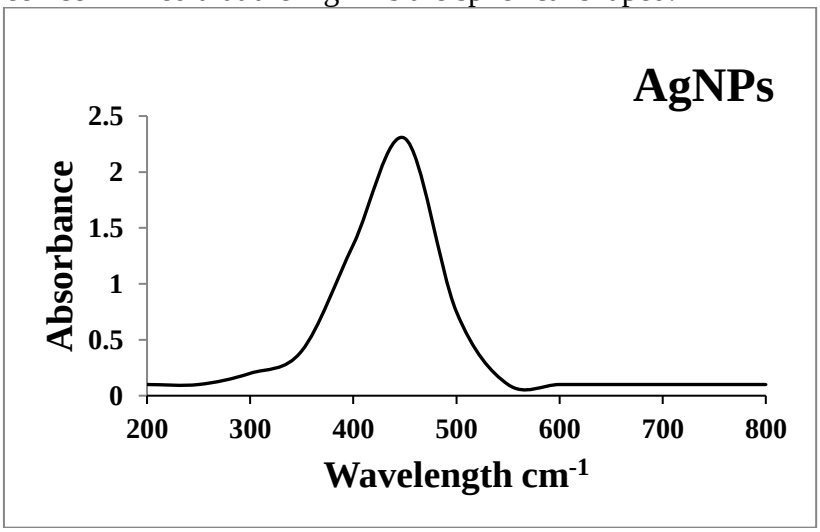

Fig:2 UV-Visible Spectrum of Ag NPs from Eucalyptus leaves extract

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B. FT-IR analysis

FTIR studies were performed to identify potential biomolecules responsible for the capping and stabilisation of Ag NPs produced by Eucalyptus (**Fig:3).** The absorption bands at 3440, 1649.49, 1459.66, 1103.60, and 672 cm^{-1} indicated the presence of capping agents with the nanoparticles.

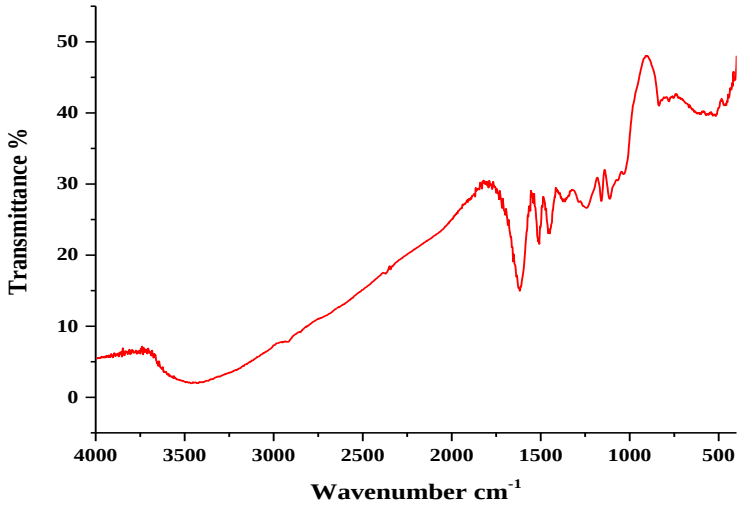

Fig:3 FT-IR Spectrum of Ag NPs Synthesized by Eucalyptus leaves extract

Carboxylate groups make a layer on the surface of Ag NPs and prevent them from aggregation. Thus leaves extract of Eucalyptus have a vital role in producing Ag NPs. Interaction between secondary metabolites presented in plant extract and Ag^{+} causes the reduction of Ag^{+} into Ag. Negatively charged groups such as carboxylate COO^{-}, OH^{-}, attach to the surface of the NPs and act as a capping agent. So, the secondary metabolites reduce and stabilise Ag ions **[13].**

C. XRD analysis

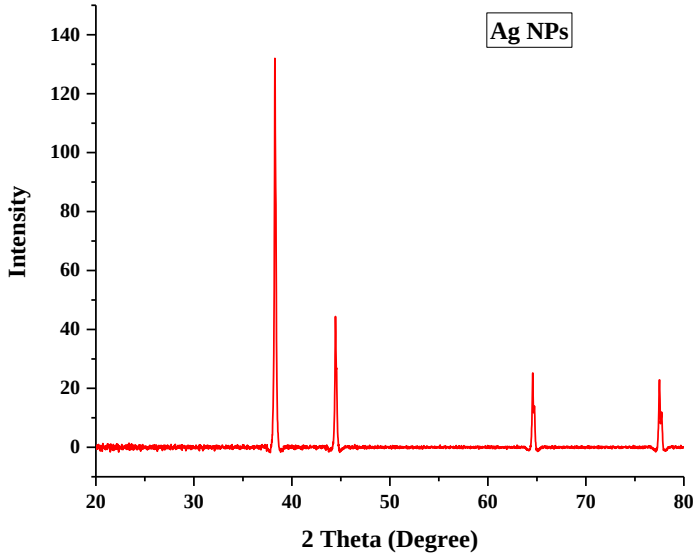

Fig:4 XRD graph of Ag NPs synthesized by Eucalyptus leaves extract

Table:1 XRD analysis

2 Theta	Height	FWHM	d-spacing	Rel. int %	Particle size
38.30	111.89	0.23	2.34	100	36.56
44.48	36.25	0.23	2.03	32.39	37.31

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64.60	19.15	0.27	1.44	17.11	34.80
77.55	17.06	0.31	1.22	15.24	32.87

Average particle size of the Ag nanoparticles: 35.39 nm.

To measure the size of Ag NPs, XRD analysis was carried out. Fig:3 show the XRD peaks obtained from the XRD analysis of Ag NPs synthesized of Eucalyptus leaves extract. Four peaks were obtained in the XRD graph between the range of 20 to 80 nm. Obtained peaks at **38.30, 44.48, 64.60** and **77.55** confirm the crystalline nature of Ag NPs. Sharp peaks indicate the spherical nature of Ag NPs. Using Debye Scherrer's equation, particle size was calculated. Synthesized Ag NPs are 35.39 nm in size.

D. SEM-EDX Analysis

SEM images indicated that the produced Ag NPs had a spherical form. The elements produced from Eucalyptus leaf extract were discovered using X-ray energy dispersive analysis. **(Table 1)** From EDAX spectrum, it is clear that Eucalyptus have recorded weight per cent **88.26** of silver and **11.74** of Oxygen .

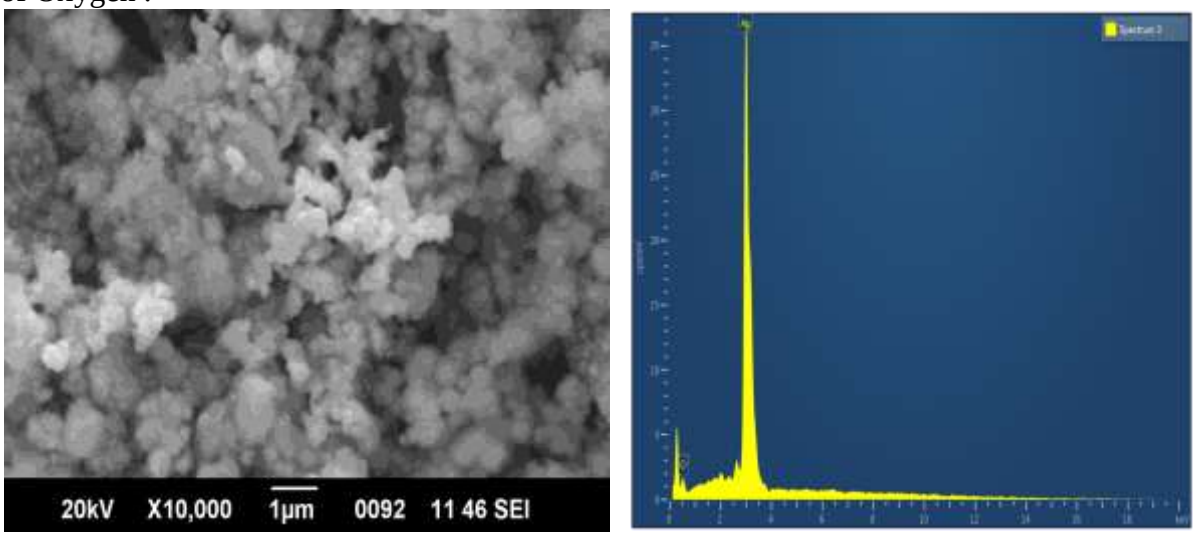

Fig:5 SEM-EDAX analysis of Ag NPs

Table 2: Energy dispersive analysis of X-rays (EDAX) of Ag NPs synthesized by Eucalyptus leaves extract.

Element	Weight	Atomic (%)
O	11.74	43.5
Ag	88.26	55.5
Total	**100**	**100**

E. Catalytic Activity of Ag NPs

The activity of Ag NPs as a catalyst was investigated for Knoevenagel Condensation. In the presence of 25 mg Ag NPs, the reaction of 2mmol benzaldehyde and 2mmol malononitrile was carried out in the water. The reaction was carried out at room temperature. Solid product formation was clearly observed in 20 min.

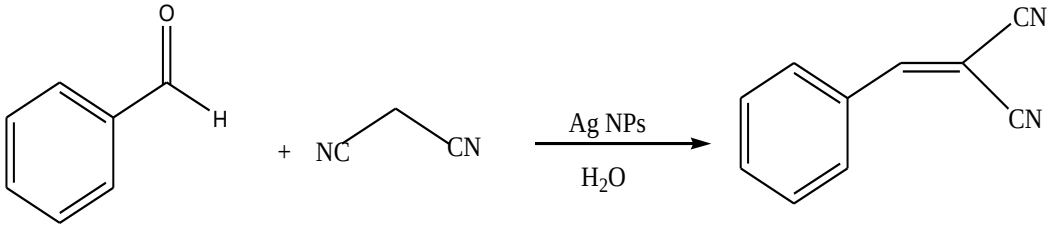

Fig: 6 Knoevenagel Condensation

It has been found that the mole ratio of malononitrile affects the reaction. Reaction activity was increased with an increase in the amount of malononitrile. The reaction mixture was converted into

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the product in just 15 min **[14].** The effect of amount of catalyst affects the reaction activity. 25mg and 50mg catalysts were used in the reaction. On increasing the amount of catalyst, active sites also increased for reactants. We observed that a greater amount of catalyst reaction was completed in just 20 min, and with a minimum amount of catalyst reaction was completed in 30 min.

Table 3- Effect of amount of Malanonitrile and catalyst

No.	Benzaldehyde (mmol)	Malononitrile (mmol)	Time required for completion of reaction (min)
1	2	2.0	30
2	2	2.5	20
3	2	3.0	20

Table 4- Effect of amount of catalyst

No.	Amount of Catalyst (mg)	Time required for completion of reaction (min)
1	50	20
2	25	20
3	10	30

Because of the presence of cationic metal centres and anionic atoms, i.e., Oxygen, metal oxides act as both acidic and basic catalysts. Previously published literature indicated that the bifunctionality of metal oxide catalysts was advantageous for Knoevenagel and other organic transformations **[15].** Compared to all other possible routes for the Knoevenagel condensation, the Ag NPs nanocatalyst exhibits significantly higher catalytic activity. In addition, the use of water helps to make the system more environmentally friendly.

Plausible Reaction Mechanism

It is assumed that the more acidic proton of malononitrile is accepted by O^{-2} of the catalyst. The presence of electron-withdrawing CN^- makes it more acidic, due to which it is easily removed during the reaction. Due to conjugation, conjugate base is more stable and attacks on the carbonyl carbon. When O^{-2} accepts a proton from the catalyst, a β- hydroxy compound is generated. After that water molecule eliminates from the reaction, and α,β- unsaturated dicyano compound is formed.

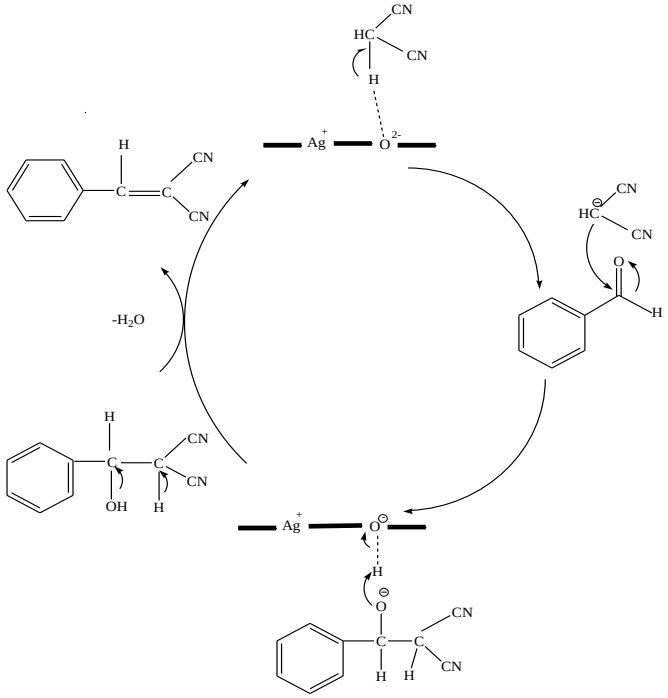

Fig: 7 Plausible mechanism of Knoevenagel condensation in the presence of Ag NPs

Recyclability of Catalyst

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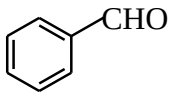
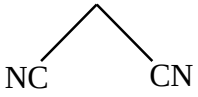
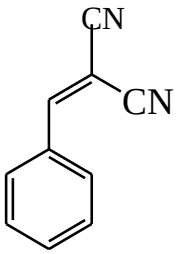
After the reaction was completed, the catalyst was separated using filtration. The solid product was dissolved in ethylacetate and filtered. The catalyst was cleaned with methanol to remove any impurities. Now cleaned catalyst was used for same reaction. Reaction cycle was repeated for five times. Activity of catalyst remains unchanged. Knoevenagel reaction carried out by this method is free from use of organic solvent, reaction completes in short duration with inexpensive catalyst and provides a pure product after isolation. The synthesized product was characterized by FT-IR Spectroscopy.

Table: 5 FT-IR data of Benzylidenemalononitrile

Reactant		Product	Spectroscopic Data of Product
			IR (cm^{-1}): 3034, 2223, 1673, 1585, 1568, 1450, 1375, 1293, 958, 858, 754, 678

F. Antibacterial efficiency of Ag NPs

The green approach may be used to investigate the antibacterial properties of Ag NPs, allowing nanotechnology to be used in medicine. Plant extracts have a new awareness for disease control and eco-friendly benefits, and biogenic production of metal oxide nanoparticles is a conventional way.

Fig:8 Antibacterial activity of Ag NPs Synthesized by Eucalyptus leaves extract (i) E. *coli* (ii) S. *aureus*

Table: 6 Minimum Inhibitory Concentration of Green Ag NPs

Microorganism	Minimum Inhibitory Concentration (Ag NPs)
E. *coli*	22.0 ± 5.8
S. *aureus*	18.0

Using the leaves extract of Eucalyptus, researchers discovered that the green creation of Ag NPs was hazardous to E. *coli* and S. *aureus* bacteria. The size and structure of Ag NPs influence their interaction with bacteria. The antibacterial activity increases with surface area. If greater the surface area then the antibacterial activity will be greater. Ag nanoparticles produced from leaf extracts have tremendous promise for biological applications. Biogenic manufacture of metal oxide nanoparticles is a well-established technique, and plant extracts are gaining fresh attention for their ability to manage illness and have an eco-friendly effect.

Ag NPs show antibacterial activity against Gram (+ve) (S. *aureus*) bacteria and gram (-ve) (E. *coli*) bacteria. The MIC of Ag NPs against E. *coli* and S.*aureus* is shown in **Table 3.** The use of Ag^{+} as preventing agents in cosmetics dispersions with known preservative inhibitors was tested by a challenged list in a set of cosmetics. Ag NPs have more antimicrobial efficacy due to silver binding

proteins in which aminoacid moieties serve nucleation sites. [**16**] The present study revealed an economical and straightforward route to the synthesising Ag NPs and their capability to render antibacterial efficacy.

IV. Conclusion

The biogenic fabrication of silver nanoparticles using leaves extract of Eucalyptus is one pot eco-friendly method. Biogenic synthesis is a more efficient method because it avoids the use of toxic chemicals. It is low cost rapid green approach to the synthesis Ag NPs. Hence, biogenic synthesis follows the Green chemistry principles. Secondary metabolites presented in leaves extract are biocompatible for many biomedical applications. Green Ag NPs show efficient antimicrobial activity against E. *coli* and S. *aureus*. Using plant extracts, the formation of NPs is more beneficial because it avoids the use of toxic chemicals, it is energy efficient, and inhibits the production of byproducts during biogenic synthesis. The present investigation concludes that Ag NPs as Green catalysts are an effective catalyst for organic transformation Knoevenagel condensation. α,β- unsaturated dicyano compound synthesized by the reaction of benzaldehyde and malononitrile, which is an important intermediate for the pharmaceutical industry. This Green method could be an alternative to the conventional physical/chemical processes. It has been concluded that using natural reducing agents, green NPs can be produced by applying green chemistry principles.

Conflict of Interests

The authors have no conflict of interest.

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