RESEARCH ARTICLE

DOI: 10.54392/irjmt2341

Asian Research Association

INTERNATIONAL RESEARCH JOURNAL OF
MULTIDISCIPLINARY TECHNOVATION

Empowerment the antibacterial activity of Silver Oxide nanoparticles using Woodfordia Fruticosa flower extract

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DOI: https://doi.org/10.54392/irjmt2341

Received: 01-04-2023; Revised: 12-05-2023; Accepted: 18-05-2023; Published: 23-05-2023

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Abstract: The undeniable importance of human well-being microorganisms to strengthen the antimicrobial resistance behavior of inorganic metals has created an avenue towards the development of green nanotechnology. However, numerous physiological issues and challanges need to be addressed from the aspects of microbiological and nanotechnological. In this present work, an endeavour has been made to synthesis silver oxide nanoparticles using Woodfordia Fruticosa flower extracts as reducing agent and also examined their antibacterial activity against S. aureus and E. Coli. The utmost antibacterial activity was obtained for the Ag_2O nanoparticles prepared with extract against S. Aureus than E. Coli.

Keywords: Silver oxide, Ag_2O, Dual phase, Cubic, Monoclinic and Antibacterial activity

1. Introduction

In recent years, the human society faced tremendous severe health related challenges due to inevitable antimicrobial resistance. Around seven lakh peoples are died owing to antimicrobial resistant bacterial infection worldwide every year [1]. Meanwhile, pervasive endeavours have been made by the versatile researchers and scientists to safeguard the human society from the aforesaid serious issues. Hence the recent researchers focussed their attention on innovation in medication to overcome the hazardous microorganisms with antimicrobial resistance properties for the human community [2]. The revolutionized growth in green nanotechnology with low dimensional particles is the most suitable solution for the above problems. Microorganisms like yeast, fungi, algae, bacteria, virus and plant extract played a vital role in the formation of nanoparticles with multifaceted structures for specific issues over the few decades [3]. Plant extract is the most apposite method than among other microorganisms, because they act as eminent shrinking and capping agents due to the omnipresence of prominent phytochemicals like ketones, aldehydes, amides, carboxylic acids, flavonoids, alkaloids, phenolic compounds, terpenoids, etc [4]. Plant-mediated synthesis is a prominent green synthetic route, and it is pronounced as suitable approach among the different biological entities as they provide a clean & safe with eco-friendly and cost-effective, optimally utilizable, and favourable way to the synthesize silver oxide nanoparticles [5]. The first investigation on the plant-mediated synthesis of Ag NPs using Medicago sativa has been published in 2003 [6]. Noble metal nanoparticles specifically gold and silver have substantial properties in photovoltaic, photo-catalytic and bio-medicinal activities against cancer & tumour cells. They also show multi-diversity morphology structures with high destructive property towards bacteria and microorganisms thus they are considered as the most prominent compared with other specimens [7]. Compared to Gold nanoparticles (Au NP's), the silver nanoparticles (Ag NP's) are economically more feasible and they expose atypical properties. Silver has less density and lighter element with greater ability to conduct electric currents compared to gold. It is also reactive to nature and has the supreme ability to be able to transfer heat through their surface than other noble metals [8]. Moreover, silver nanoparticles have gained a considerable popularity in recent years due to their fabulous properties such as good stoichiometric, high chemical stability, high electrical & thermal conductivity, large specific surface area with formidable fraction of surface atoms, admirable catalytic, electro-chromic and optoelectronic properties, most importantly anti-microbial, anti-inflammatory and diagnostic properties [9-12]. These versatile properties of silver oxide nanoparticles enunciated implausible significance in all evolving fields particularly in medicine by enhancing restorative applications of humankind due to its peculiar cytotoxic activity. However, research appraisal should be carried out to validate the formulation, precise function and biocompatibility of silver oxide nanoparticles against various diseases [13].

Numerous physical and chemical methods have been exploited to prepare silver oxide nanoparticles, like sol-gel, solvothermal, micro emulsion, hydrothermal, egg white method, chemical precipitation, biosurfactant-mediated emulsions, and microwave irradiation [14-17]. Among these methods, chemical precipitation is one of the simplest and cost effective methods owing to the varied preparation conditions parameters, extensive production, ease control of size, divergent morphology, homogeneity and composition of nanoparticles, without any mechanical, vacuum conditions or microwave treatments [18].

Woodfordia Fruticosa (Figure 1a & 1b), also commonly called as Fire Flame Bush, is an acrid, astringent, and they have the properties to be depurative, uterine sedative and febrifuge. They have high antibacterial properties and are useful during the treatment towards leprosy, skin diseases, diarrhoea, liver disorders, ulcers, internal haemorrhage, and so on. It can also be used over wounds to reduce the discharge and promote granulation [19]. Finally it has been concluded that to find out a solution for the bacterial infection in wounds by using silver oxide nanoparticles. At this juncture, an endeavour has been made to synthesis silver oxide nanoparticles using Woodfordia Fruticosa flower extracts as reducing agent and also examined their antibacterial activity against S. aureus and E. coli.

2. Experimental details

2.1 Preparation of extract

This method involves heating the solvent to reflux and then letting the solvent vapor travel up a distillation arm to be bleached into a chamber. The condenser in the apparatus makes sure that any solvent vapor that enters the chamber drips down into the chamber. Some of the pertinent elements are dissolved in the warm solvent, while the undesired compounds stay down in the thimble. When the Soxhlet chamber is completely filled, then the chamber is removed and clears into distillation flash by the siphon. The thimble ensures that the undisclosed solid doesn't mix with the solid when moving the solvent out of the chamber. This cycle is repeated to many times over for a few days to get the required extract mixed in the desired solvent.

100g finely-powdered flower woodfordia fruticosa was tightly packed in the Soxhlet extractor. The extraction process is carried out using 250 ml of methanol solvent being heated to reflux. The pale brown colored solution was formed after completing the extraction process multiple times and then collected in a 500 ml beaker. Carrying out a set of distillation process to concentrate the solution, the small amount of solvent is kept for a slow evaporation process. Finally, after the completion of extraction process a brown oily precipitate is obtained for synthesis.

2.2 Synthesis of Ag_2O nanoparticles

In this process, 0.2 M of silver nitrate was dissolved in 50 ml of deionized water in a beaker and the resulting solution was continuously stirred in the magnetic stirrer for 30 minutes to acquire homogeneous solution. The white colored slurry was formed after the drop-wise addition of ammonia into the above solution with continuous stirring. The obtained slurry washed several times with water and ethanol to eradicate the redundant impurities. Then the slurry was heated in hot air oven for 3 hours to attain fine and smooth particles. The same procedure was followed for the synthesis of Ag_2O nanoparticles with flower extracts of woodfordia fruticosa. The color of the slurry was turned into pale brown.

The prepared powders are named as Ag_2O without extract (Figure 2a) & Ag_2O with extract (Figure 2b) and the same characterized using varied techniques to assess the various properties particularly antibacterial activity.

3. Result and Discussion

Impact of Woodfordia Fruticosa flower extract on various properties

Figure 1. Illustration of Woodfordia Frutiocosa flower (a) fresh flower from plant, (b) dried flowers

Figure 2. Photograph of the prepared samples (a) Ag_2O without extract, (b) Ag_2O with extract

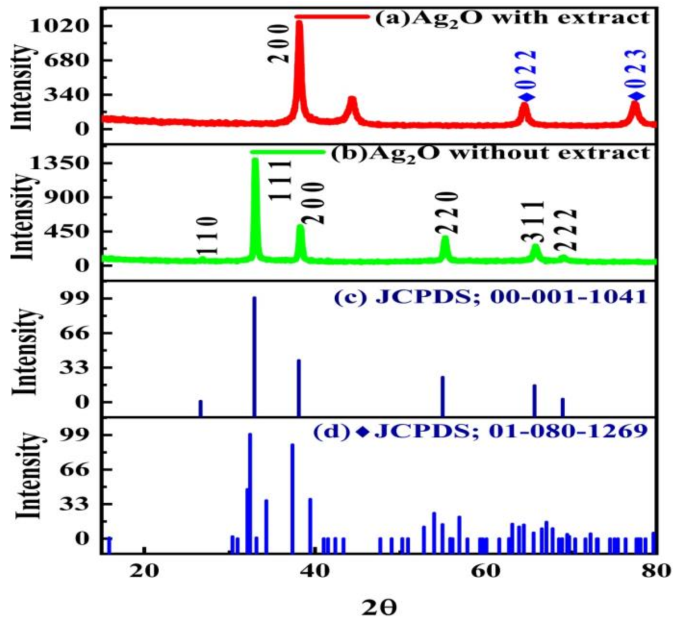

Figure 3. XRD patterns of the prepared samples **(a)** Ag_2O with extract, **(b)** Ag_2O without extract, **(c & d)** JCPDS reference card for the samples

Table 1. Structural parameters of dual phase silver oxide nanoparticles

Sample	Peak Composition	2 theta	FWHM	hkl	Cry. Structure	Cry. Size (nm)	Dis. density	Strain	Lattice constants		
									a	b	c
Ag_2O without extract	Ag_2O	27.09	0.4015	110	Cubic	21.2	2.82	1.876	4.7010	-	-
		32.98	0.1506	111		57.4	0.31	0.567	4.7036	-	-
		37.14	0.1506	200		58.3	0.26	0.484	4.7103	-	-
		56.07	0.3346	220		28.1	0.81	0.712	4.7002	-	-
		65.42	0.4684	311		20.2	1.32	0.841	4.7055	-	-
		69.32	0.4015	222		25.1	0.82	0.649	4.7046	-	-
Ag_2O with extract	Ag_2O	38.16	0.4015	200	Cubic	21.9	2.40	1.655	4.7171	-	-
	Ag_2O_2	64.53	0.6022	022	Monoclinic	18.7	2.86	1.063	5.8165	3.4302	5.4532
	Ag_2O_2	77.54	0.5353	023	Monoclinic	22.9	1.92	0.743	5.8314	3.4567	5.4709

3.1 Structural properties

Figure 3a-d illustrates the XRD pattern of silver oxide nanoparticles prepared by co-precipitation method. Pure silver oxide (Figure 3b) nanoparticles clearly portrayed the appearance of distinct intense peaks observed at 2θ values 27.09, 32.98, 37.14, 56.07, 65.42 and 69.32 that corresponds to the lattice planes of (110), (111), (200), (220), (311) and (222) respectively. The strongest and faintest intensity peaks are observed at 32.98 and 27.09 respectively. The substitution of atoms within the unit cell causes the different peak intensity anticipated as a result of constructive and destructive interference of waves in the lattice sites. The obtained peaks are matched with ICDD reference no. 00-001-1041 (Figure 3c) and it confirms the single phase cubic structure (space group: Pm3m) with dominant orientation along (111) direction by comparing with Rashmi et al. [4]. The dominant orientation promotes more number of Ag_2O crystallites oriented in that particular reflection compared with other orientations. The silver oxide nanoparticles prepared with extract explored versatile intense peaks obtained at 2θ values 38.16, 64.53 and 77.54 that ascribed to the lattice planes of (200), (022) and (023) respectively as depicted in Figure 3a. Flower extract ensured that the appearance of distinct dual phase (Ag_2O and Ag_2O_2) and dual crystal structure (Cubic and Monoclinic) occurred during the insertion of specific phytochemicals in the lattice sites of Ag_2O. Due to the dual phase with dual crystal structure existence, the prepared samples exhibit most constructive route for antimicrobial applications. The woodfordia fruticosa flower extract modified the preferred orientation form (111) to (200) with the inclusion of new phase (Ag_2O_2) which denotes the higher crystalline structure. The impact of flower extract introduced three new peaks at 45, 64 and 77° which expressed the annihilation of three major peaks and also retain and augments the peak intensity of (200). The obtained peaks are matched with ICDD reference no. 01-080-1269 (Figure 3d) and it confirms the Monoclinic structure. The obtained results are consistent with the following reports revealed by Belaiche et al. (Artemisia Herba-Alba Leaf extract) [20], Dhoondia et al. (Lactobacillus mindensis) [21], Baruah et al., (Ocimum sanctum leaf extract) [22], Mani et al. (Cleome gynandra leaf extract) [23], Maheshwaran et al. (Zephyranthes Rosea flower extract) [24] and Manik et al. (Artocarpus heterophylus leaf extract) [25].

The crystallite size is found using the Debye-Scherrer formula $D=k\lambda/\beta\cos\theta$ where D is the crystallite size, k is shape factor which is 0.9 (for assumption of spherical particle), λ corresponds to the wavelength of X-ray radiation (0.1541 nm), β is the value of full-width half maxima and θ is the half of corresponding peak angle. The average crystallite size was calculated and found to be 35.1 nm for a pure silver oxide nanoparticle and 21.2 nm for the samples prepared with flower extract. The embodiment of flower extract relentlessly influences the structural parameters (Table 1) owing to the confiscation of some peaks, and creation of new peaks with dual phase and dual crystal structure format [26].

3.2 Morphological properties

FESEM images of silver oxide nanoparticles prepared without extract reveal the existence of agglomerated clusters of distorted spherical shaped particles with reduced size as represented in Figure 4. It also clearly authenticates the formation of smoothest surface with uniform distribution of interlaced spherical shaped particles as network with an average size 48 nm. The similar morphological structure was also obtained using cleome gynandra leaf extract by Mani et al. [23].

FESEM images of silver oxide nanoparticles prepared with extract unveiled the formation of merely spherical and rod shaped particles with rather rough surface as shown in Figure 5. On close observation, it reveals the arrangement of rectangular rod structured particles with curved edge in one side and flat edge in other side without any voids. Ag_2O phase with cubic structured crystallites are responsible for the formation of spherical shaped particles whereas Ag_2O_2 phase with monoclinic structured crystallites responsible for the arrangement of rod shaped particles consonance with XRD results. From Figure 5, it indicated that the large number of spherical shaped particles compared to rod shaped particles which designated the supremacy in the formation of silver oxide nanoparticles with more Ag_2O. The same morphological patterns composed of spherical and rod shaped particles were also reported by Singh et al. using tulsi leaf extract [27]. Generally spherical and rod shaped nanostructures are the most favorable for all technological applications importantly photo-catalytic, optoelectronic and antimicrobial domains. This result motivated us to explore the antimicrobial properties of the same. The Woodfordia Fruticosa flower extract strongly interact with the silver ion on the surface and changed into interlinked spherical networks with lesser size. The extract intensively impact on the morphological aspects, bespoke the shape of the particles from interlinked clusters of distorted spherical into merely spherical and rod shaped particles with varied edges. Moreover, the size of particles also reduced by the influence of flower extracts which makes them favorable for antimicrobial and antibacterial applications.

The elemental analysis of silver oxide nanoparticles were done by EDAX spectrum as shown in Figure 6. The presence of prime elements like silver atom (92.67%) and O atom (7.33%) were strongly evidenced form the tenacious absorption peaks occurred at 3 keV. No additional peaks except Ag and O were obtained from Figure 6 corroborate the formation of pure silver oxide nanoparticles in accordance with XRD and SEM results.

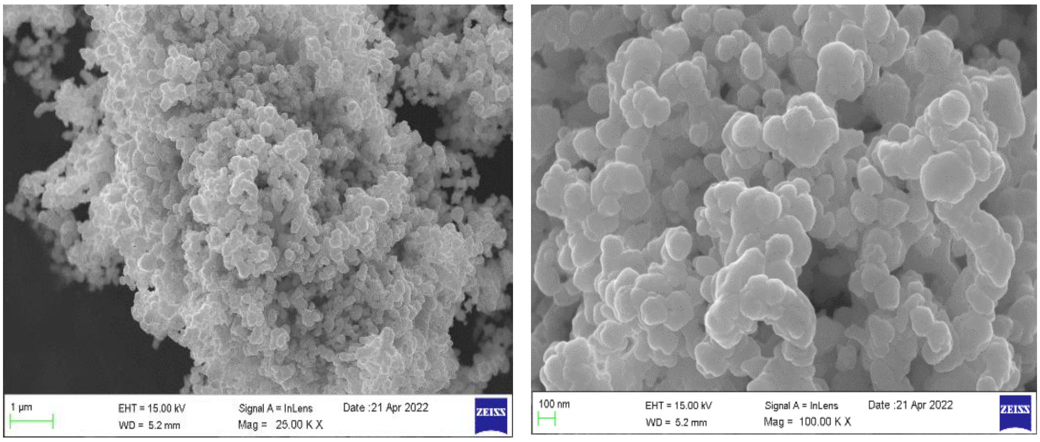

Figure 4. FESEM of Ag2O nanoparticles prepared without extract

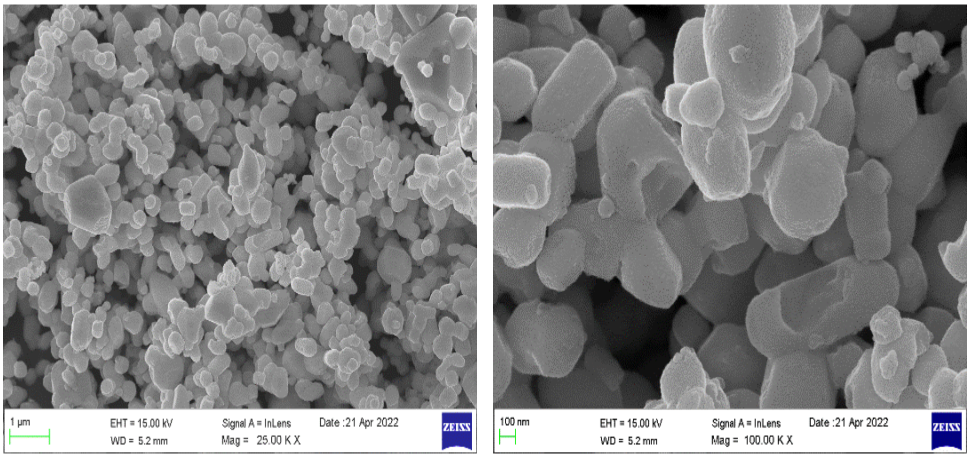

Figure 5. FESEM of Ag_2O nanoparticles prepared with extract

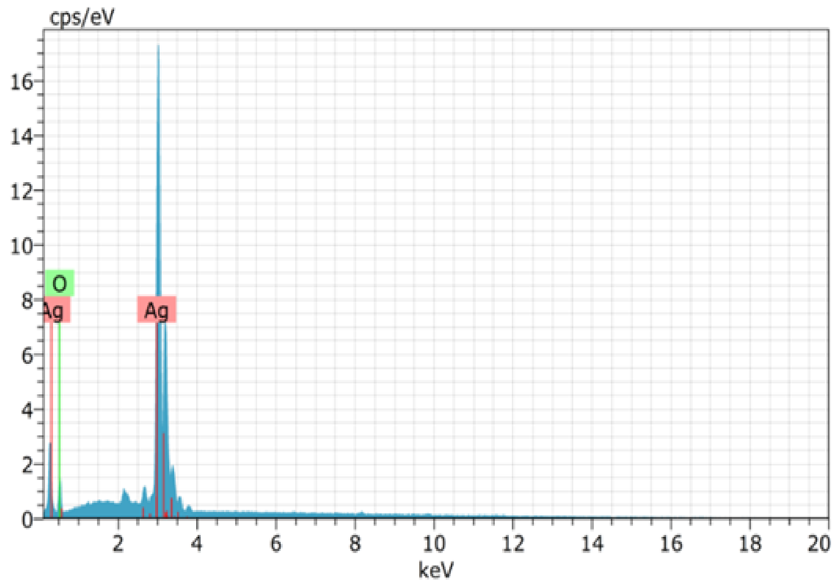

Figure 6. EDS analysis of Ag_2O nanoparticles

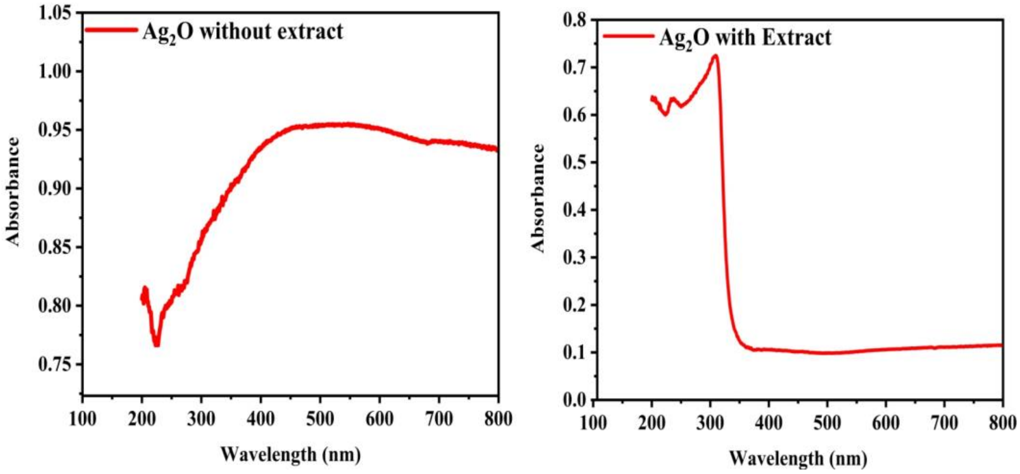

Figure 7. UV-Vis spectra of the prepared samples **(a)** Ag_2O without extract, **(b)** Ag_2O with extract

3.3 Optical properties

The optical properties of the prepared samples were analyzed with the help of UV-Vis spectrophotometer. Absorbance range in the spectra of metal nanoparticles may reveal broad bands in the UV-Vis range due to the excitation of Plasmon resonances or quadrupole and higher order of Plasmon resonances [28]. The absorbance spectra explore the presence of absorption peak at 420 nm (Figure 7a) ascribed to the combined oscillations of outermost electrons in the orbital due to the vibrations of Surface Plasmon Resonance (SPR). It is a phenomenon that transpires where electrons in a thin metal film becomes excited by light that is directed to the film with a particular angle of incidence, and then travel parallel to the film. The existence of merely homogeneous spherical nanoparticles causes the phenomenon of single SPR peak according to Mie's theory. Generally, the isotropic particles of spherical and cylinder shaped should exhibit a single SPR peak whereas the anisotropic particles exhibit two or more SPR peaks depending on their size and shape [29]. The samples prepared with extract authenticate the appearance of strong and sharp absorption peaks at 225 and 300 nm (Figure 7b) attributed to violet-blue shift in the visible region. The heterogeneous anisotropic rod and spherical shaped particles obtained from SEM morphological pattern exhibit the appearance of two sharp SPR bands due to out-of-plane quadrupole resonance reflecting violet-blue shift in the visible region. The shift in the SPR peak towards violet-blue region of light radiation indirectly envisages the decreased size of nanoparticles obtained from FESEM results due to the influence of flower extract.

The optical band gap energy of the nanoparticles estimates the energy deserved to excite the electrons from the highest energy level into the possible lowest energy level. The calculated band gap energy of the nanoparticles prepared without extract is 1.76 eV (Figure 8a) whereas the same for the samples prepared with extract is 3.735 eV (Figure 8b). The extracts augment the band gap energy of the spherical and rod shaped particles from 1.76 to 3.735 eV due to the introduction of new phase Ag_2O_2 at the lattice sites of Ag_2O, this result favorable for optoelectronic and photovoltaic applications.

3.4 Photoluminescence properties

The excitation wavelength for the samples was found to be 270 nm. PL spectra enunciate the presence of one sharp peak at 420 nm in the violet region and two strong and sharp emission peaks at 455 and 465 nm in the blue emission as shown Figure 9a&b. The peak observed at 420 nm due to the excitation of silver ion in the surface of oxygen attributed to SPR frequency according to UV results. The prominent emission peak occurred at 465 nm may correspond to a transition between interstitial and oxygen vacancies arise in the lattice sites of Ag_2O [30]. The faint peak encountered at 455 nm may be the irradiative excitation annihilation of unstable phases in the surface [31]. There is no significant change except peak intensity changes occurred due to the influence of flower extract.

From these investigations concluded that the Woodfordia Fruticosa flower extract can synthesize nanoparticles with intrinsic defects, oxygen vacancy and surface defects on the lattice sites which aimed to enhance the antimicrobial and photo-catalytic activities.

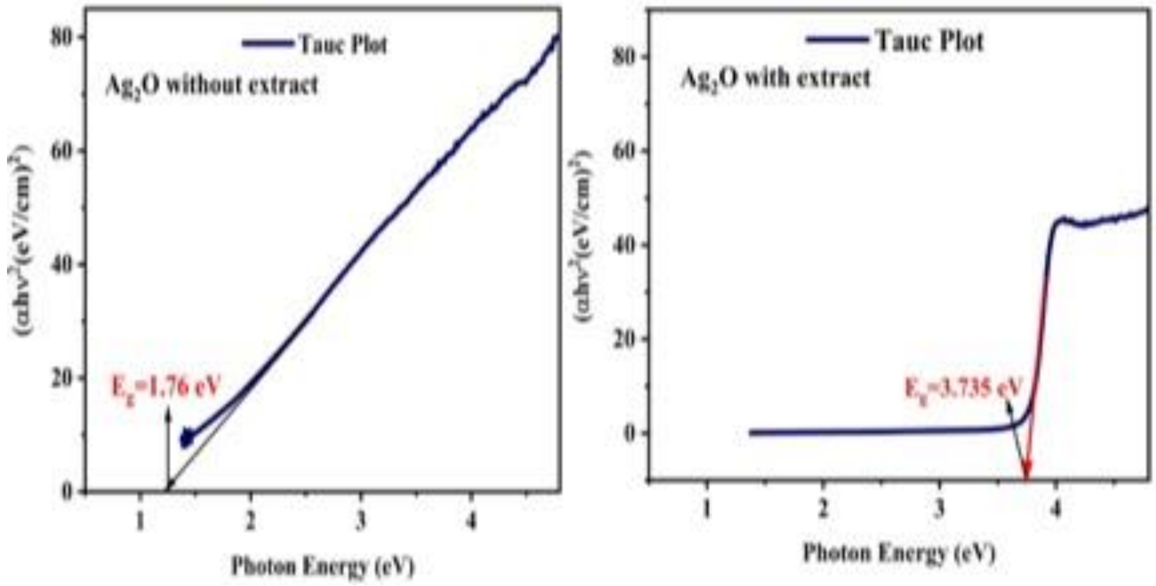

Figure 8. Tauc plot of the prepared samples **(a)** Ag_2O without extract, **(b)** Ag_2O with extract

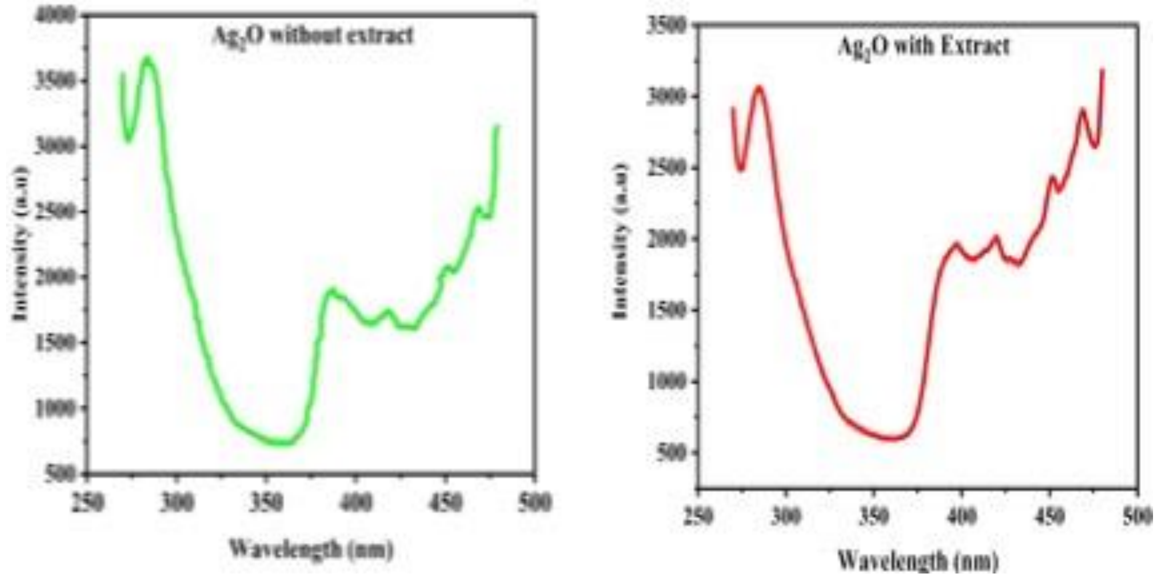

Figure 9. PL spectra of the prepared samples (a) Ag2O without extract, (b) Ag2O with extract

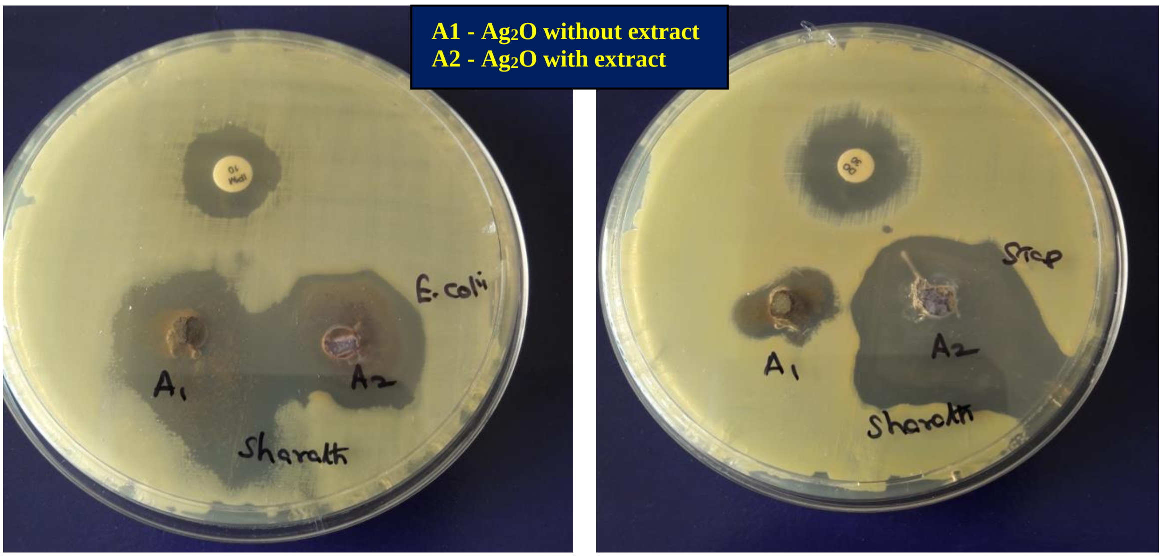

Figure 10 Antibacterial activity of the prepared samples **(A1)** Ag_2O without extract, **(A2)** Ag_2O with extract

3.5 Antibacterial activity

Escherichia coli (E. Coli) is a member of the family called Enterobacteriaceae, which are Gram-negative rod shaped bacteria containing both a fermentative and respiratory metabolism. Certain strains of E. Coli are harmless and commonly found inside the intestine of mammals, while others can cause infections of the digestive and urinary tracts, blood and central nervous system. Considering versatile and idiosyncratic properties with high disinfectant behaviour of silver, it has been found to be proficient disinfecting agent against E. Coli [32, 33]

Staphylococcus aureus (S. Aureus) belongs to the family called Staphylococcaceae. It can cause a variety of infections in skin and soft tissues, endovascular sites and in internal organs, causing high morbidity and mortality. This type of bacteria can enter the bloodstream and into internal organs where it can set up foci of infections causing local damage to the host. Silver nanoparticles are also very effective against S. Aureus, and it also helpful in sterilizing and wound healing treatments [34].

The microbial activity of flower extract based Ag_2O nanoparticles were analyzed by zone inhibition method using bacterial pathogens, including gram positive (S. Aureus) and gram negative (E. Coli). The inoculums were dried in closed atmosphere at room temperature. Subsequently the discs were placed on the agar plates and Ag_2O nanoparticles. The Ciprofloxacin (100µg) was used as reference sample and were loaded onto each disc in petri dish of respective bacterial pathogens and then incubated for 24 hours and zone of inhibition was measured with a meter ruler.

The antibacterial activities of the prepared samples are shown in Figure 10a&b. The antibacterial activity of spherical and rod shaped silver oxide nanoparticles were estimated against S. Aureus and E. Coli under Agar Well diffusion method. The utmost antibacterial activity was obtained as 16 mm Zone of inhibition (ZOI) by adding 50 µl Ag_2O solution against S. Aureus whereas the same for E. Coli was obtained as 15 mm ZOI. The least ZOI for S. Aureus and E. coli was obtained as 7 mm and 14 mm respectively [35]. The above results indicated that the antibacterial activity of the samples having more accomplished against S. aureus than E. coli. It was explored that the samples prepared with flower extract (Figure 10A1 &A2) having the composition of rod and spherical shaped particles of reduced size with extreme ability to rupture the cell membrane of bacteria and to interact with the essential components such as sulfur and phosphorus of DNA thus extirpate the bacteria [36]. The woorfordia fruticosa flower extract strongly induce the antibacterial behavior of silver oxide nanoparticles due to its dual phase with dual structured crystallites, reduced particle size, higher crystalline nature and composition of dual shaped (rod and sphere) particles.

4. Conclusion

Human society faced tremendous health related challenges and issues in recent years due to inevitable antimicrobial resistant bacterial infections. As a researcher it is a position to safeguard the human society form the aforesaid challenges using versatile fabulous nanobiotechnology. An endeavour has been made to rectify these issues using silver oxide nanoparticles prepared with woodfordia fruticosa flower extract and studied their antimicrobial activities.

The impact of flower extract on silver oxide nanoparticles instigated the various properties in the following aspects,

- ✓ Structural properties
  - ❖ Confiscated some peaks and introduced some new peaks and also changes the preferred orientation form (111) to (200).
  - ❖ Modified the single phase nature into dual phase with dual structured crystallites
  - ❖ Reduced the crystallite size from 35.1 nm to 21.2 nm.
- ✓ Morphological properties
  - ❖ Intensively modified the morphological pattern from clusters of distorted spherical network into merely spherical and rod shaped particles.
  - ❖ Reduced the size of particles which makes them favorable for antimicrobial and antibacterial applications.
- ✓ Optical properties
  - ❖ Strongly authenticates the presence of silver oxide due to the oscillations of SPR peak at 420 nm.
  - ❖ Converted the isotropic particles of single SPR peak into anisotropic particles of dual SPR peak.
  - ❖ Augmented the band gap energy of the spherical and rod shaped particles from 1.76 to 3.735 eV due to the introduction of new phase Ag_2O_2 at the lattice sites of Ag_2O, this result favorable for optoelectronic and photovoltaic applications.
- ✓ Emission properties
  - ❖ Revealed the presence of strong and sharp peaks in violet and blue emission in the visible region with with intrinsic defects, oxygen vacancy and surface defects on the

lattice sites which aimed to enhance the antimicrobial and photo-catalytic activities.

- ✓ Antibacterial properties
    - ❖ Enhanced the antibacterial activity with extreme ability to rupture the cell membrane of bacteria and to interact with the essential components such as sulfur and phosphorus of DNA thus extirpate the bacteria.

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Conflict of interest

The Authors has no conflicts of interest to declare that they are relevant to the content of this article.

Has this article screened for similarity?

Yes

Conflict of Interest

The Author(s) declared no potential conflicts of interest with respect to this research, authorship, and/or publication of this article.

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R. Suresh- Supervision, writing original draft, review and editing; **A. Yogeshwaran & P. Logababu**- Conceptualization, Validation, Review and editing; **P.S. Sharath**- Experimental and Characterization; **G. Aakash**- Experimental and Characterization; **V. Pugazhendhi**- Experimental and Characterization. All the authors read and approved the final version of the manuscript.