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"Study on Green synthesis of ZnO nanoparticles using *Withania somnifera* leaf extract and its pharmacological evaluation"

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Abstract: *Withania somnifera* is being extensively used from ancient times in India especially for rejuvenation of the body as well as longevity enhancement. This herb is considered as Indian ginseng in Ayurveda. As Ayurvedic preparations, various parts of the *Withania somnifera* are being used to treat variety of ailments that affect the human health, especially dried roots are widely used for the treatment of nervous and sexual disorders. Presently green synthesis of nanoparticles has emerged as extensive research area to develop new drugs using noble metals, zinc. In this study, Methanolic extract of *Withania somnifera* were evaluated for phytochemical screening as well as antimicrobial activity against gram negative bacteria (*E.coli and Salmonella*), gram positive bacteria (*S. aureus*) and fungi (*Candida albicans*) before the green synthesis ZnO nanoparticles. ZnO Nanoparticles were synthesized using Zinc acetate. ZnO nanoparticals were characterized by using various techniques UV- Visible, XRD and FTIR. Later these ZnO green nanoparticles were evaluated for antioxidant activity & antimicrobial activity.

***Index Terms* - *Withania sonifera*,** Green synthesis, Phyrtochemicals, Antimicrobial activity and Antioxidant activity.

I. Introduction

Introduction:

Plant based medicines are attracting the interest of researches to combat new developing diseases worldwide. The development of new herbal formulations having a significant role in prevention and treatment of severe. Herbal medicines have distinctive therapeutic effect and fewer side effects compared to traditional pharmaceuticals (Kesarwani ***et al.,*** 2013).

Nature is a repository of a no of plants comprising active bio-molecules which are precursor for drugs against health and diseases. Nature is being an integral part of all civilization from centuries and humans were depends on it for everything including medicine to treat various diseases by evolving a strong medicinal system. Plants are utilized for treatment of various ailments have been employed in all over the world namely from countries like China, Japan, Egypt, Brazil and India (**Jamshidi-Kia *et al.,* 2018**). From this point of view plants are still employed as medicinal alternative by 80 percent population from poor and underdeveloped countries due to their easy availability, cost-effectiveness, biocompatibility, natural origin, and acceptability.Moreover the plant based medicines , Aspirin, artimesinin, colchicine, serpentine, digoxin, ephedrine, morphine, physostigmine, Z guggulsterone, pilocarpine, reserpine, taxol, tubocurarine, vinblastine and paclitaxel, are some of the Plant derived medications (**Dar *et al.,* 2017**). Hence, the future medicine therefore will be the basis of future investigation of safe and effective drugs by holding the hands of medicinal herbs. As a result research and development in this arena requires the advanced and latest upcoming technologies.

The quantity of phytochemicals is varied parts to parts in the same plant and in different plants. The presence of phytochecals viz. alkaloids, flavonoids, saponins, terpenoids, steroids, phlobatannins, glycosides, tannins, etc characterizes the plants for therapeutic efficacy. All these secondary bio metabolites are recognized for curing one or other diseases. As an instance Alkaloids are identified for antispasmodic, antimalarial, analgesic, diuretic activity. Terpenoids are known for antiviral, anthelmintic, antibacterial, anticancer, antimalarial, anti-inflammatory properties. These are also control cholesterol synthesis and possess insecticidal properties hence useful for storing agricultural products. Biometabolites saponins are contained with anti-inflammatory, antiviral, plant defence and for cholesterol reducing property. Phlobatanins have astringent properties. Glycosides are account for antifungal and antibacterial properties. Phenols and flavonoids are identified for their antioxidant, anti-allergic, antibacterial, etc (**Moteriya *et al*, 2015; Padalia *et al*, 2015**)

Findings over the phytochemical profile of different parts in Withania somnifera plant states that one can decide the concerned part to be investigated for any specific activity and that can be helpful to decide for synergistic evaluation. phytochemical profile is the key starting base for investigation in than random selection of the plants. taking into account the above, the present study was carried out on Withania somnifera plant which are traditionally used in curing or treating many diseases and disorders were screened for their preliminary qualitative phytochemical constituents.

Withania somnifera is a perennial plant with remarkable medicinal properties including anti-bacterial and anti-fungal properties. This medicinal plant belongs to the division: Magnoliophyta, class: Magnoliopsida, and family: Solanaceae. Genus: *Withania* and species: *somnifera*. *Withania somnifera* consist of anti-inflammatory and antioxidant characteristics **(Bandhan *et al.*, 2014).** This plant's extract contains active components such as tannin, alkaloids, lactones, and flavonoids. Extracts of Root, fruit, and leaf part of *W. somnifera* are significant to the pharma industry (**Raut, *et al.*, 2014; Marslin, *et al.*, 2015**).Thus the medicinal importance of *Withania somnifera* makes its plantation on large scale mainly in East Asia, India, and Africa. It also have anti-fungal property.

Withania somnifera plant advantageous in tumor healing and soothing mind and reduces anxiety and depression **(Chauhan, *et al.*, 2016)**.These plants also show immune modulation properties and strengthen the weakness of bone, muscle tension, impotency and bronchitis **(John, 2014).** *Withania somnifera* is a rich source of bioactive compounds, about 62 has been isolated from leaves and 48 from roots **(Chatterjee, *et al.*, 2010)**.

2.0 Material & methods

II. RESEARCH METHODOLOGY

2.1. Collection and processing of plant material

The plant *Withania somnifera*was collected from Gwalior district (M.P.), India. The Plants leaves were washed under running tap water, air dried in the shade and ground to a fine powder and stored in the airtight bottle.

2.2 Preparation of Leaf extract

Leaf powder (10 g) of the *Withania somnifera*were extracted in 100 methanol and double distilled water separately water using the soxhalet apparatus soaked. The filtrates were concentrated on a rotary evaporator and then stored at 4°C till further use (**Swaminathan, *et al.*, 2017**).

2.3. Green Synthesis of ZnO particles

Zinc oxide nanoparticles was formed by using Zinc acetate dehydrate. In this experiments 100 ml of Zinc acetate dihydrate 0.2 M solution was prepared and filtered through 0.45 µ filter. In Subsequent steps 90 ml of 0.02 m Zinc acetate dehydrate solution kept on magnetic stirrer at 60 °C for 30 min. by drop wise mixing of 10 ml of aqueous extract of plant leaves. This solution further heated on oven at 150°C until dried. Now this nano materials were washed two times with double distilled water and Ethanol to remove impurities and again dried the ZnO NP at 60 °C and transferred to muffle furnace for annealing at 450°C for 4 hrs. Now cool the disc and collect the ZnO NP powder for further study.

2.3. **Phytochemicals screening**

The qualitative phytochemicals screening of crude leaf extract was carried out using standard phytochemical methods with slight modifications (Harborne, 1998; Evans, 1989**)**.

2.4. **Bacterial strains**

Klebsiella pneumoniae, *Salmonella sps*, *Fusaium oxusporum*, *Candida albicans* and *E.coli* used in the present study were procured from the MTCC, Chandigarh, India.

2.5. **Determination of antimicrobial activity**

The antimicrobial activities of *Withania somnifera*leaves extracts were determined using the agar-well diffusion method of Akpata and Akinrimisi (1977) with slight modification. Crude leaves extracts in different concentrations 25 µg/µl, 50 µg/µl, 75µg/µl and 100µg/µl (10 mg/ml).

2.7. Characterization of nanoparticles

Synthesized CuO were characterized by using Systronic 2702 UV-vis spectrophotometer at wavelengths ranging from 200 to 700 nm for UV-vis spectroscopy. Shimadzu Fourier-transform infrared in the range of 4500–400 cm^{-1} was applied for the IR-spectroscopy.

3.0 Result & Discussion

The present study was carried out on the methanolic and aqueous extract of leaves of *Withania somnifera plant*. In present study phytochemicals were qualitatively analysed. From the methanolic and aqueous extract of leaves, ZnO nanoparticles were synthesized and characterized. Moreover the present study, antimicrobial study was assessed against gram negative, gram positive bacteria and fungi using plant leaves extract and ZnO particles.

3.1 Extractive Value

The extractive values obtained for *Withania somnifera* ,the aqueous extract yielded the highest extractive value (16.65% w/w), while the methanolic extract showed a comparatively lower yield (13.5% w/w). This suggests that the leaves contain a greater proportion of highly polar constituents that are more soluble in water than in methanol.The higher aqueous extractive value observed in this study indicates that *W. somnifera* leaves are rich in water-soluble phytochemicals. Thus, the present findings contribute useful baseline data for the standardization of *W. somnifera* leaf material.

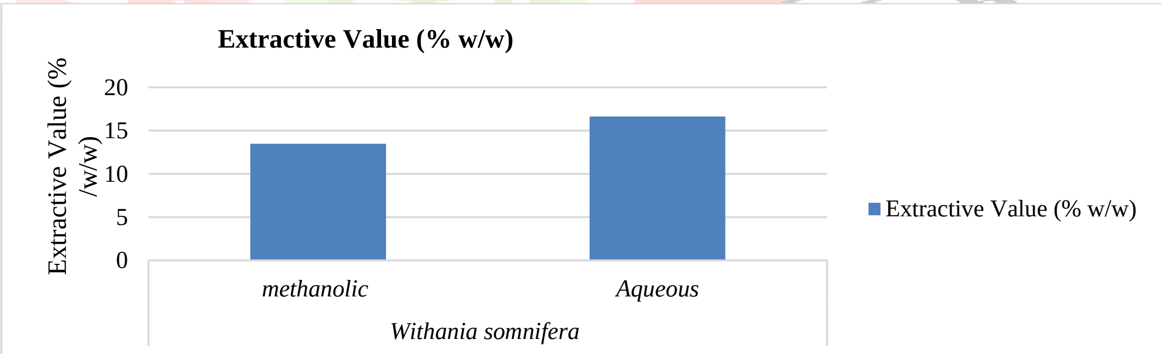


Fig. 3.1: Extractive Value of Plant leaves extract

Overall, the results confirm that solvent selection plays a crucial role in phytochemical extraction, and aqueous extraction appears more suitable for recovering the bulk of soluble constituents from *Withania somnifera* leaves.

3.2 Qualitative Screening of present Phytochemical

The qualitative phytochemical screening of *Withania somnifera* leaves revealed the presence of several important classes of secondary metabolites in both methanolic and aqueous extracts (Table 3.2). The variation in phytochemical composition between the two extracts reflects the role of solvent polarity in determining extraction efficiency.

Table: 3.1 Qualitative Screening of present Phytochemical

Plant Name	Plant Part	Extracts	Type of Phytochemicals						
			A	F	Ph	Tn	SG	T	St
Withania somnifera	Leaves	ME	++	+++	+++	++	-	++	-
		AqE	+	++	++	++	+ ++	-	-

A: Alkaloids;F: Flavonoids ;Ph :Phenols;Tn: Tannin;St: steroids;T : Terpenoids;G: Glycosides and S: saponins; M : Methanolic Extract and Aqueous Extract.

The methanolic extract showed a strong presence of flavonoids and phenolic compounds (+++), along with moderate levels of alkaloids, tannins, and terpenoids. Methanol is widely recognized as an effective solvent for extracting moderately polar compounds, including phenolics and flavonoids, due to its ability to penetrate plant tissues and solubilize a broad spectrum of phytochemicals. The aqueous extract, on the other hand, contained alkaloids, flavonoids, phenols, tannins, saponins, and glycosides, though generally at lower to moderate levels compared with the methanolic extract. The detection of saponins and glycosides specifically in the aqueous extract is consistent with their hydrophilic nature, as these compounds dissolve readily in water.

Terpenoids were detected only in the methanolic extract and Steroids were not detected in either extract, possibly due to low concentrations in the leaves or limitations of qualitative detection methods. The presence of alkaloids, tannins, flavonoids, phenols, saponins, and glycosides is pharmacologically significant compounds and also associated with diverse biological activities such as anti-inflammatory, immunomodulatory, antimicrobial, and adaptogenic effects.

Overall, the results indicate that both extracts contain therapeutically relevant secondary metabolites, with the methanolic extract being richer in phenolic and flavonoid compounds, while the aqueous extract contains more water-soluble constituents such as saponins and glycosides.

3.3 Antioxidant Activity

3.3.1 Total phenolic content of *Withania somnifera* and ZnO-WS nano particles

The total phenolic content (TPC) of *Withania somnifera* leaf extracts and the corresponding ZnO-WS nanoparticles showed considerable variation depending on both the solvent used and nanoparticle synthesis (Fig. 3.2). Among the plant extracts, the aqueous extract exhibited the highest phenolic content (28.1 mg GAE/g), followed by the methanolic extract (22.1 mg GAE/g). This indicates that a substantial fraction of phenolic compounds present in the leaves are highly polar and readily soluble in water.

In contrast, both methanolic and aqueous ZnO-WS nanoparticle samples showed lower phenolic content compared with the crude extracts. The methanolic ZnO-WS nanoparticles contained 18.4 mg GAE/g, while the aqueous nanoparticles showed the lowest value (15.8 mg GAE/g). The reduction in phenolic content after nanoparticle synthesis may be attributed to the involvement of phenolic compounds in the reduction and stabilization of zinc ions during nanoparticle formation.

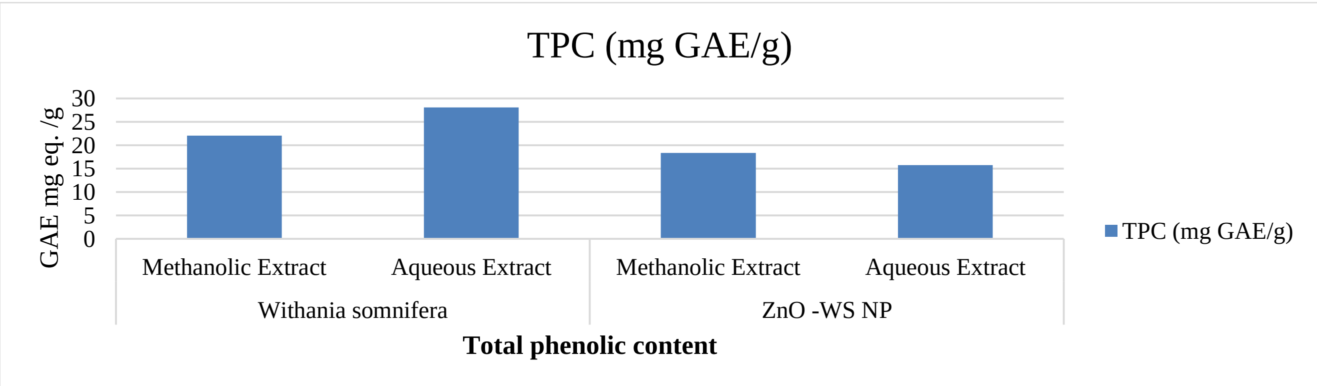


Fig. 3.2. Total phenolic content of *Withania somnifera* and ZnO-WS nano particles

The relatively higher phenolic content in methanolic ZnO-WS nanoparticles compared to aqueous nanoparticles suggests that organic solvent–derived phytochemicals may remain more strongly associated with the nanoparticle surface. This surface-bound phenolic layer can influence the biological activity, stability, and antioxidant potential of the nanoparticles.

Variations in TPC between extracts and nanoparticle formulations are consistent with previous reports indicating that phytochemical-mediated synthesis of metal nanoparticles alters the chemical composition of plant extracts. Such changes are important when evaluating the pharmacological potential of plant-based nanomaterials.

3.3.2 Total Flavonoid Content of *Withania somnifera* and ZnO-WS nano particles

The quantitative estimation of total flavonoids showed that *Withania somnifera* leaf extracts contained higher flavonoid levels compared to the corresponding ZnO-WS nanoparticles (Fig. 4). Among the plant extracts, the aqueous extract exhibited the maximum flavonoid content (43.1 mg QE/g), followed by the methanolic extract (35.1 mg QE/g). In contrast, the flavonoid content decreased in nanoparticle samples, with values of 28.4 mg QE/g for methanolic ZnO-WS NP and 25.8 mg QE/g for aqueous ZnO-WS NP.

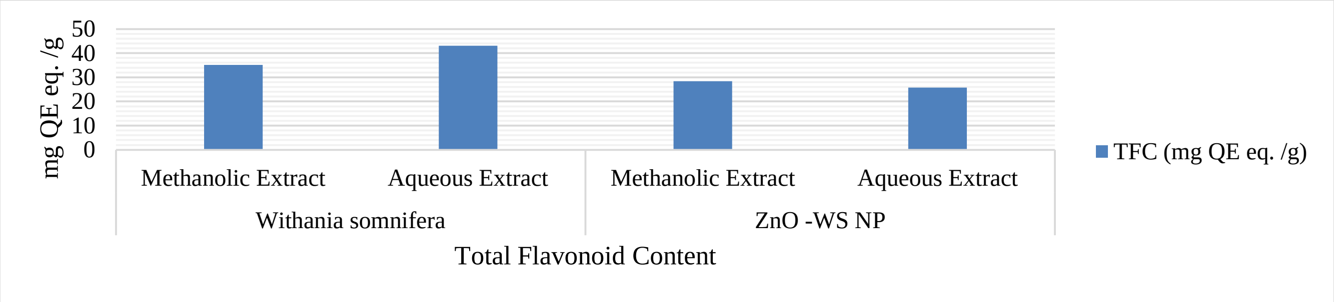
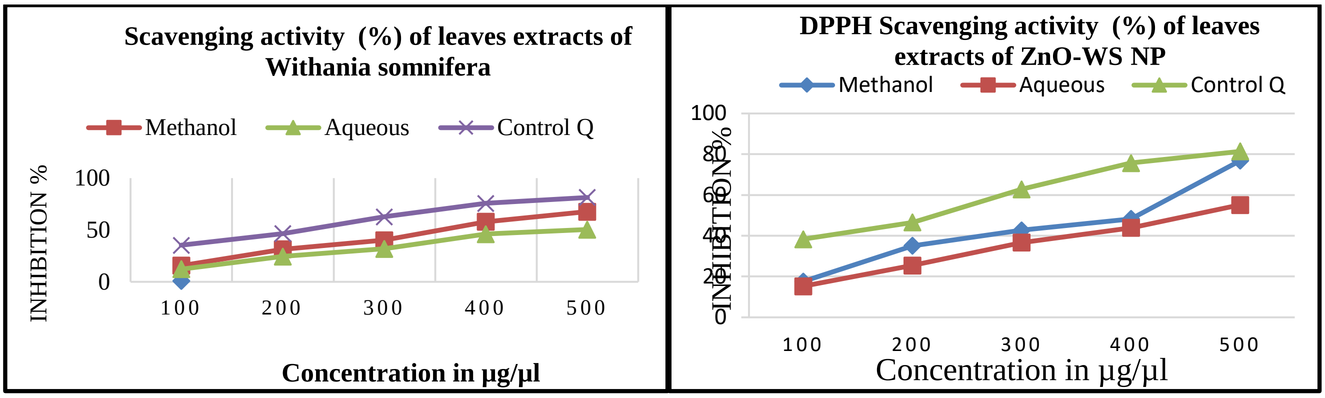


Fig.:3.3 Total Flavonoid Content of *Withania somnifera* and ZnO-WS nano particles

Flavonoids are major bioactive polyphenols responsible for antioxidant, anti-inflammatory, and antimicrobial properties of medicinal plants, including *Withania somnifera*. The higher flavonoid content observed in the aqueous extract (Fig. 4) suggests that most flavonoids present in the leaves are polar and water-soluble, possibly occurring as glycosidic forms. Ashwagandha leaves have been reported to contain appreciable quantities of flavonoids and other phenolic compounds contributing to their therapeutic potential.

3.3.4 DPPH Scavenging activity (%) of of *Withania somnifera* and ZnO-WP

The antioxidant activity assessed by the DPPH assay showed both methanolic and aqueous extracts of *Withania somnifera* (Fig. 5) at the lowest concentration (100 mg/ml), the methanolic extract exhibited 15.75% inhibition, which increased progressively to 67.75% at 500 mg/ml. Similarly, the aqueous extract showed lower activity overall, ranging from 12.29% to 50.44% across the tested concentrations. The standard antioxidant, quercetin, demonstrated substantially higher activity, reaching 81.42% inhibition at 500 mg/ml.



A B

Fig. 3.4 DPPH Scavenging activity (%) of of *Withania somnifera (A)*& ZnO-WS Nano materials (B).

Fig. 5 indicate that *Withania somnifera* leaf extracts possess significant antioxidant activity, which increases with concentration. The methanolic extract consistently showed higher scavenging activity than the aqueous extract, suggesting that methanol extracted greater amounts of antioxidant compounds such as phenolics and flavonoids.

The antioxidant potential of ZnO-WS nanoparticles evaluated by the DPPH assay showed a clear concentration-dependent increase in radical scavenging activity (Fig. 6). At 100 µg/µl, the methanolic nanoparticle sample exhibited 17.56% inhibition, which increased markedly to 76.81% at 500 µg/µl. The aqueous nanoparticle sample also showed increasing activity from 15.22% to 55.08% over the same concentration range. The reference standard, quercetin, demonstrated the highest activity across all.

The results presented in Table 6 and Fig. 6 indicate that ZnO-WS nanoparticles possess significant antioxidant activity, which increases with concentration.

Green-synthesized ZnO nanoparticles typically contain a coating of plant-derived biomolecules such as phenolics, flavonoids, and proteins, which contribute to their antioxidant properties. These surface functional groups can donate electrons or hydrogen atoms to neutralize DPPH radicals. In addition, ZnO nanoparticles themselves may participate in redox reactions and enhance radical scavenging through electron transfer mechanisms.

The lower activity of the aqueous nanoparticle sample may reflect differences in phytochemical composition or weaker adsorption of antioxidant compounds on the nanoparticle surface. Organic solvents often facilitate extraction of moderately polar phenolics and flavonoids that exhibit strong radical scavenging activity.

3.3.5 Comparative IC 50 values of *Withania somnifera* Leaf Extract and ZnO-WS Nanoparticles

both methanolic and aqueous leaf extracts of *Withania somnifera* exhibited higher values than the corresponding ZnO-WS nanoparticles. The aqueous leaf extract showed the highest value (551.87), followed by the methanolic extract (356.32). In contrast, the ZnO-WS nanoparticles displayed lower values, with 460 for the aqueous sample and 345 for the methanolic sample. This trend suggests that the crude plant extracts contain a greater quantity of bioactive constituents compared with the nanoparticle formulations.

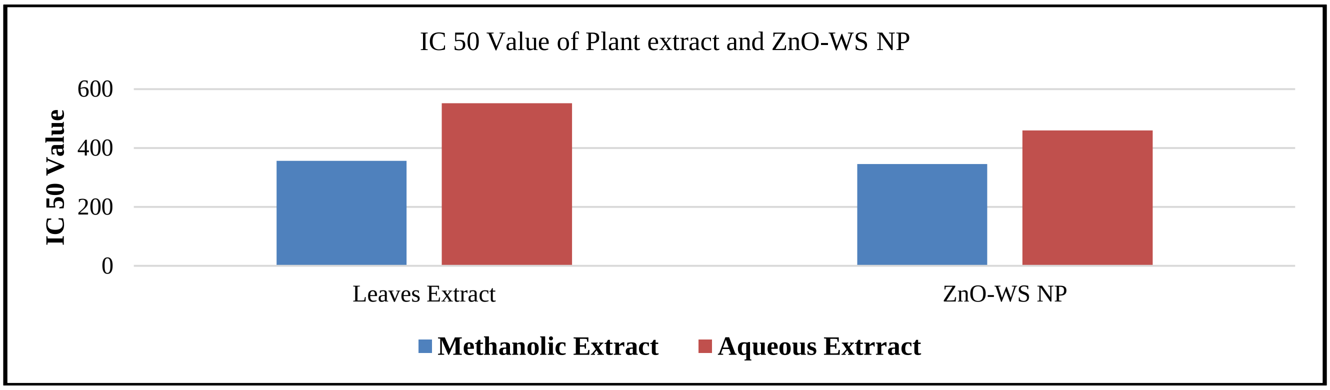


Fig.3.5 Comparative IC 50 values of *Withania somnifera* Leaf Extract and ZnO-WS Nanoparticles

the findings presented in Fig. 3.5 suggest that while nanoparticle synthesis modifies the chemical composition of the plant extract, ZnO-WS nanoparticles still retain considerable bioactivity. The results support the potential of *Withania somnifera* as a suitable biological source for the eco-friendly synthesis of functional ZnO nanomaterials.

3.4 Antimicrobial activity of *Withania somnifera* Leaf Extract and ZnO-WS Nanoparticles

3.4.1 Antibacterial activity of different extracts

3.4.1.1 Antibacterial activity of Levaes extract of *Withania somnifera and ZnO-WS NP* against *S. aureus*

In present study plant extracts and nanoparticles exhibited measurable zones of inhibition against *Staphylococcus aureus*, which increased with concentration. The aqueous extract of *W. somnifera* showed activity even at lower concentrations, whereas the methanolic extract displayed activity only at higher concentrations (≥75 µg/µl). ZnO-WS nanoparticles demonstrated antibacterial activity across all tested concentrations, with the methanolic nanoparticle preparation showing slightly greater inhibition than the aqueous one (Fig. 3.6).

Table- 3.2: Antibacterial activity of leaves extract of *Withania somnifera and ZnO-WS NP* against *S. aureus*

Bacteria used	Conc. of extract in µg/µl	Zone of inhibition (mm)					
		Withania somnifera			*ZnO-WS NP*		
		Extract M	Extract Aq	Streptomycin	Extract M	Extract Aq	Streptomycin
S. aureus (MTCC 3160)	25	00	14.4±1.20	17.5±0.17	13.6±1.5	12.9±0.9	25±1.00
	50	00	14.7±0.2		14.4±0.6	13.1±0.9	
	75	12±1.0	16.4±0.6		16.3±0.7	15.7±0.3	
	100	15.1±0.9	17.1±0.9		16.9±0.9	16.3±0.7	

Extract used: M= Methanolic; Aq=Aqueous; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

A B

Fig.3.6: Antibacterial activity of Methanol extract of *Withania somnifera (A)* & *ZnO-WS nano material (B)* against *S. aureus*

.ZnO-WS nanoparticles exhibited enhanced antibacterial activity compared with crude extracts (Table 4.9; Fig. X). This observation is consistent with the known antimicrobial properties of ZnO nanoparticles. When synthesized using *Withania somnifera* extract, ZnO nanoparticles acquire a phytochemical coating that further enhances antibacterial efficacy.

the findings presented in 3.2 and Fig. 3.6 demonstrate that green-synthesized ZnO nanoparticles using *Withania somnifera* extracts possess significant antibacterial activity against *Staphylococcus aureus*, surpassing crude extracts and supporting their potential biomedical applications.

3.4.1.2 Antibacterial activity of Levaes extract of *Withania somnifera and ZnO-WS NP* against *Salmonella.*

Table- 3.3: Antibacterial activity of leaves extract of *Withania somnifera* & *ZnO-WS nano material* aga Salmonella

Bacteria used	Conc. of extract in µg/µl	Zone of inhibition (mm)					
		Withania somnifera		*Standard*	*ZnO-WS NP*		
		Extract M	Extract Aq	Streptomycin	Extract M	Extract Aq	Methanol
Salmonella (MTCC 425)	25	11.8±0.60	10.0±0.50	20.0±0.60	0.0±0.00	0.0±0.00	18.9±0.20
	50	12.2±0.30	11.4±0.30		11.5±1.00	0.0±0.00	
	75	12.9±0.60	11.9±1.10		12.0±0.50	9.8±1.20	
	100	14.0±0.80	12.3±0.60		12.8±0.70	10.2±0.60	

Extract used: M= Methanolic; Aq=Aqueous; NA= No activity
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

A B

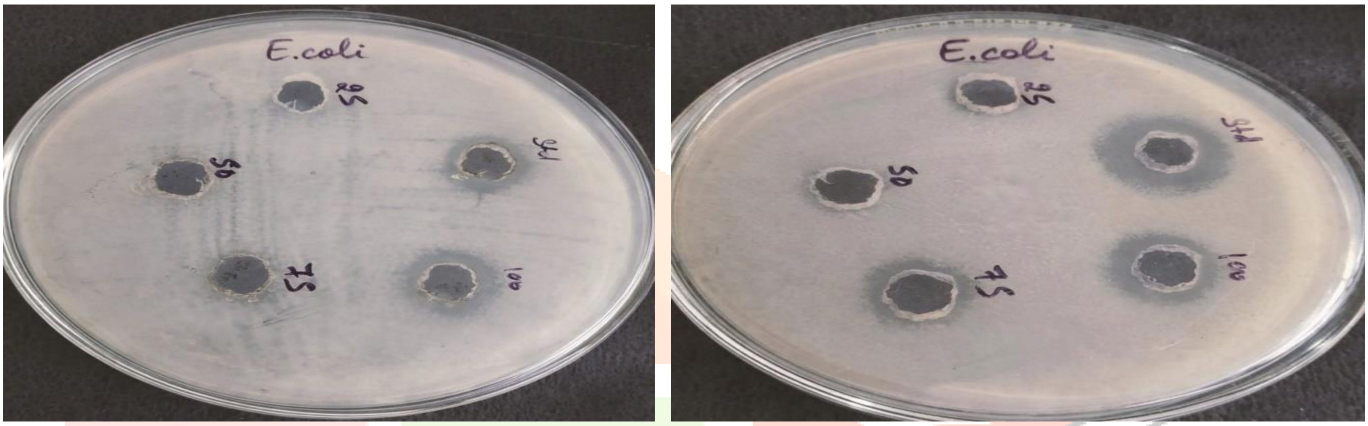
Fig. 3.7. Antibacterial activity of leaves extract of *Withania somnifera (A)* & *ZnO-WS nano material (B)* against *Salmonella*

3.4.1.3 Antibacterial activity of Levaes extract of *Withania somnifera and ZnO-WS NP* against *E.oli*

Table- 3.4 : Antibacterial activity of leaves extract of *Withania somnifera* & *ZnO-WS nano material* against *E.coli*

Bacteria used	Conc. of extract in µg/µl	Zone of inhibition (mm)					
		Withania somnifera			***ZnO-WS***		
		Extract M	**Extract Aq**	**Streptomycin**	**Extract M**	**Extract Aq**	**Streptomycin**
E.coli (MTCC 1610)	25	11.5±0.40	11.0±0.10	21.0±0.40	13.4±0.30	12.8±0.50	21.0±0.40
	50	12.9±0.10	12.1±0.25		15.1±0.10	15.0±0.20	
	75	13.8±0.1	12.9±0.4		16.0±0.2	15.9±0.2	
	100	14.7±0.4	14.7±0.2		18.0±0.3	17.5±0.3	

Extract used: M= Methanolic; Aq=Aqueous; NA= No activity
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)



A B

Fig.3.8 Antibacterial activity of leaves extract of *Withania somnifera (A)* & *ZnO-WS nano material (B)* against *E.coli*

The ZnO-WS nanomaterial exhibited markedly enhanced antibacterial activity compared to the crude extracts. At 25 µg/µl, inhibition zones of 13.4 ± 0.30 mm (methanolic) and 12.8 ± 0.50 mm (aqueous) were observed, which increased to 18.0 ± 0.3 mm and 17.5 ± 0.3 mm, respectively, at 100 µg/µl. These values approached those of streptomycin, indicating substantially improved antibacterial efficacy following nanoparticle formulation.

The antibacterial potential of methanolic and aqueous leaf extracts of Withania somnifera was evaluated against Escherichia coli (MTCC 1610) using the agar well diffusion method. Both extracts demonstrated inhibitory activity, with zones of inhibition increasing progressively with concentration (25–100 µg/µl), indicating a clear dose-dependent response. At the highest tested concentration (100 µg/µl), both methanolic and aqueous extracts produced inhibition zones of 14.7 mm, suggesting moderate antibacterial efficacy against this Gram-negative organism. However, the activity remained lower than that of the standard antibiotic streptomycin (21.0 mm), reflecting the comparatively weaker potency of crude plant extracts relative to conventional antibiotics.

the study indicates that *W. somnifera* leaf extracts possess moderate antibacterial activity against *E. coli*, whereas ZnO-WS nanomaterials exhibit significantly enhanced efficacy, approaching that of a standard antibiotic. These findings highlight the potential of plant-based nanomaterials as promising alternatives to conventional antimicrobial agents, particularly in the context of increasing antibiotic resistance among Gram-negative pathogens.

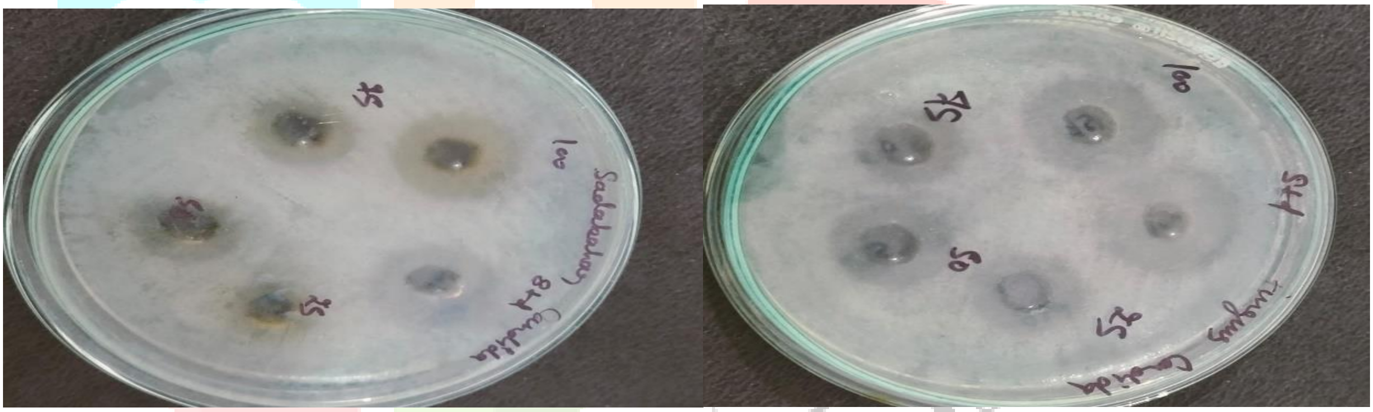
3.4.1.4 Antibacterial activity of Levaes extract of *Withania somnifera and ZnO-WS NP* against *C. albicans*

The leaf extracts of *Withania somnifera* exhibited clear antifungal activity against *C. albicans*, with inhibition zones increasing as concentration increased (Table 4.14; Fig. 14). The methanolic extract showed slightly higher activity than the aqueous extract at all concentrations. At 100 µg/µl, the methanolic extract (20.8 mm) approached the activity of the standard antifungal drug fluconazole (21.4 mm).

Table- 3.5 Antifungal activity of leaves extract of *Withania somnifera* against *C. albicans*

Fungi used	Conc. of extract in µg/µl	Zone of inhibition (mm)					
		Withania somnifera			*ZnO-WS*		
		Extract M	Extract Aq	Fluconazole	Extract M	Extract Aq	Fluconazole
C.albicans (MTCC 3958)	25	11.9±0.70	11.7±0.40	21.4±0.3	12.5±0.30	10.2±0.70	24.0±1.00
	50	13.2±0.40	12.6±1.10		15±0.90	12.5±0.30	
	75	15.5±0.50	14.5±0.90		18.3±0.40	14.8±0.20	
	100	20.8±0.80	18.9±0.60		22.0±0.70	18.5±0.40	

Extract used: M= Methanolic; Aq=Aqueous; NA= No activity
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)



A B

Fig. 3.9 **Antifungal activity of leaves extract of *Withania somnifera (A)* & *ZnO-WS nano material (B)* against *C. albicans***

ZnO-WS nanoparticles exhibited enhanced antifungal activity compared with crude extracts (Table 3.5; Fig. 3.9). This enhancement is consistent with the known antifungal properties of zinc oxide nanoparticles. ZnO nanoparticles can attach to fungal cell walls, generate reactive oxygen species (ROS), and release Zn^{2+} ions that disrupt cellular integrity.

The methanolic ZnO-WS nanoparticles showed the highest activity, suggesting stronger retention of bioactive compounds from methanolic extracts on the nanoparticle surface. Plant-mediated nanoparticles have been reported to be particularly effective against fungal pathogens due to combined physical and biochemical modes of action.

Overall, the findings presented in Tables 4.14 and 4.15 and Figs. 14 and 15 demonstrate that green-synthesized ZnO nanoparticles using *Withania somnifera* extracts possess potent antifungal activity against *Candida albicans*, exceeding that of crude extracts and approaching conventional antifungal drugs.

3.5 Characterization of ZnO-WS nano materials

3.5.1 UV absorbance Spectrum

The UV–Visible absorption spectra of ZnO-WS nanomaterials synthesized using *Withania somnifera* leaf extracts showed characteristic absorption bands in the UV region (Fig. 3.10.A–D). All samples exhibited strong absorption peaks between approximately **350–400 nm**, which is typical for zinc oxide nanoparticles.

Sample A: Sharp peak near ~380–390 nm with rapid decline beyond 450 nm
Sample B: Similar peak position with slightly broader tail toward visible region
Sample C: Broad and intense absorption band extending from ~300 to 500 nm
Sample D: Strong peak near ~370–380 nm with extended absorption tail into visible region

The presence of pronounced absorption in this region confirms the formation of ZnO nanoparticles. Differences in peak intensity and width among samples indicate variation in particle size, distribution, and surface characteristics depending on extraction conditions or synthesis parameters.

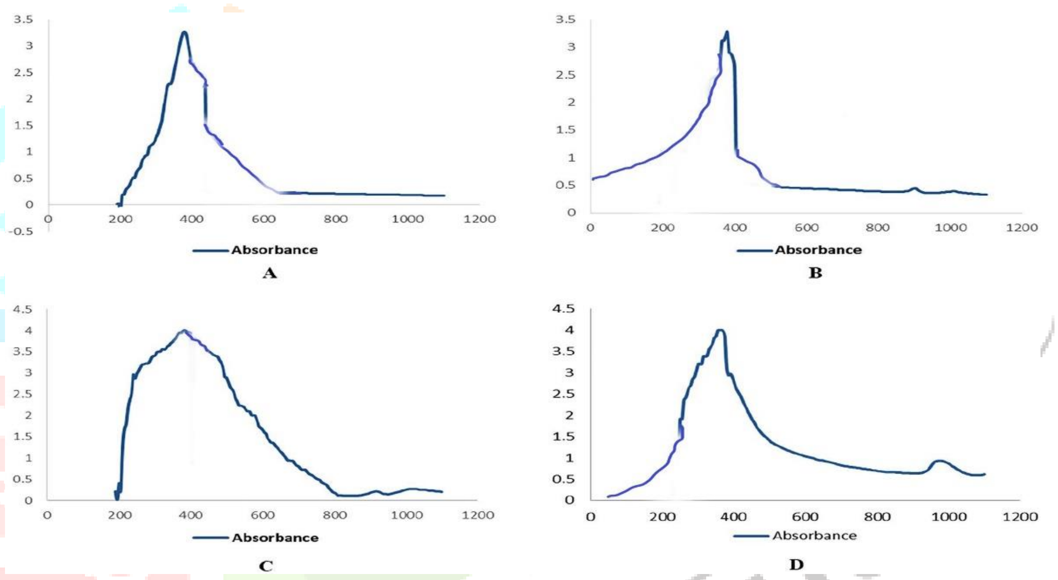


Fig. 3.10. UV-Visible spectrum at different solvents (A. *Withania somnifera* Methanolic extract,B: Aqueous Extarct;C methanoic ZnO-WS, D Aqueous Zno-WS nanomaterials)

Zinc oxide nanoparticles exhibit a characteristic excitonic absorption band in the near-UV region due to electron transitions from the valence band to the conduction band. The peaks observed around 350–400 nm in all spectra (Fig. A–D) correspond to the intrinsic band-gap absorption of ZnO, confirming successful nanoparticle formation. ZnO is a wide band-gap semiconductor (~3.37 eV), and nanoscale particles typically show absorption in this region.

Plant-mediated synthesis using *Withania somnifera* involves phytochemicals such as flavonoids, phenolics, and withanolides that act as reducing and stabilizing agents. The relatively sharp peaks in Samples A and D suggest smaller and more uniform nanoparticles, while the broader absorption observed in Sample C indicates a wider particle size distribution or possible aggregation. Broadening of the absorption band is commonly associated with polydispersity and surface defects. The extended absorption tail into the visible region observed particularly in Samples C and D may be attributed to defect states, oxygen vacancies, or organic capping molecules derived from the plant extract.

Compared with bulk ZnO, nanoscale ZnO typically shows a blue shift or slight variation in peak position due to quantum confinement effects. The peak positions around 370–390 nm observed here are consistent with previously reported values for ZnO nanoparticles synthesized using medicinal plant extracts, including *Withania somnifera*.

the UV–Vis spectra confirm successful green synthesis of ZnO nanoparticles using *W. somnifera* extract. Variations among samples likely reflect differences in particle size, morphology, crystallinity, and surface chemistry, all of which influence optical behavior and potential biological activity.

3.5. 2 FT-IR spectra of synthesized ZnO WS Nanomaterial

The Fourier Transform Infrared (FTIR) spectrum of ZnO-WS nanomaterials exhibited several characteristic absorption bands across the range 4000–500 cm^{-1}, indicating the presence of various functional groups associated with plant biomolecules and zinc oxide formation.

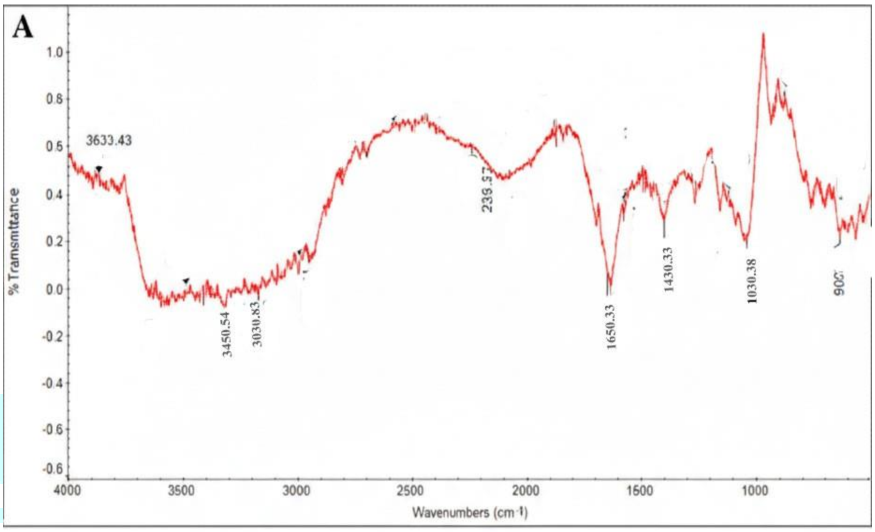


Fig.3.11 FT-IR spectra of synthesized ZnO WS Nanomaterial

FTIR spectroscopy is commonly used to identify functional groups involved in nanoparticle synthesis and stabilization. The broad absorption bands around 3633–3450 cm^{-1} indicate hydroxyl and amine groups, which originate from phenolics, flavonoids, proteins, and other phytochemicals present in *Withania somnifera*.

The peak near 3030 cm^{-1} corresponds to C–H stretching vibrations of organic compounds, suggesting the presence of plant-derived biomolecules attached to the nanoparticle surface. Such organic residues act as natural capping agents that prevent aggregation and enhance stability.

The strong band around 1650 cm^{-1} is characteristic of carbonyl (C=O) stretching or amide groups, indicating proteins or polyphenolic compounds bound to the nanoparticle surface. Plant proteins can adsorb onto nanoparticles and stabilize them through electrostatic interactions.

The absorption near 1430 cm^{-1} is associated with bending vibrations of CH_2 groups or aromatic ring structures, further confirming the presence of organic constituents from the plant extract. The band at approximately 1030 cm^{-1} corresponds to C–O stretching vibrations of alcohols, ethers, or polysaccharides, which are commonly found in plant extracts and contribute to nanoparticle stabilization.

Most importantly, the band observed in the lower wavenumber region (~900 cm^{-1} and below) corresponds to Zn–O stretching vibrations, confirming the formation of zinc oxide nanoparticles. Metal–oxygen vibrations typically appear below 600–900 cm^{-1} and are considered a fingerprint region for ZnO.

The overall spectrum indicates that phytochemicals from *Withania somnifera* not only reduced zinc ions to ZnO but also remained attached to the nanoparticle surface as stabilizing agents. Plant-mediated synthesis often results in nanoparticles coated with bioorganic molecules, which can influence biological activity and biocompatibility.

Overall, the FTIR analysis confirms successful green synthesis of ZnO nanoparticles using *Withania somnifera* leaf extract and demonstrates that plant biomolecules are involved in both reduction and capping processes.

3.5.3 X Ray Diffraction

The X-ray diffraction (XRD) pattern of ZnO-WS nanomaterials showed multiple sharp diffraction peaks, indicating the crystalline nature of the synthesized nanoparticles. the intense peaks near ~10° and ~22–23° represent major crystalline reflections. The presence of numerous well-defined peaks indicates that the ZnO-WS nanoparticles possess a polycrystalline structure. No broad amorphous halo was observed, confirming successful formation of crystalline ZnO material.

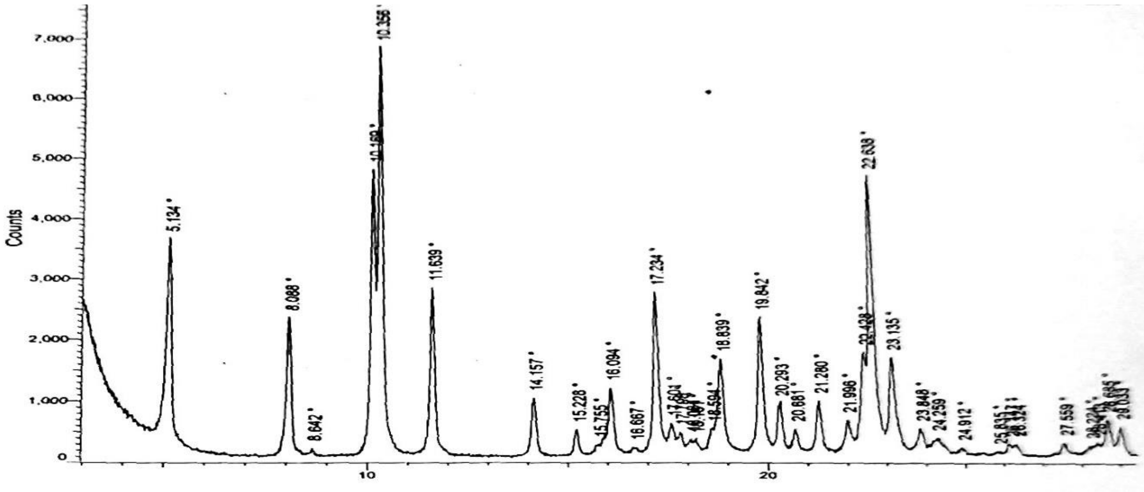


Fig. 3.12. XRD analysis

XRD analysis is a primary technique for determining the crystal structure, phase purity, and crystallinity of nanoparticles. The sharp and distinct diffraction peaks observed in the present spectrum confirm that the ZnO-WS nanomaterials are crystalline in nature. Zinc oxide typically crystallizes in a hexagonal wurtzite structure, which is thermodynamically stable at ambient conditions.

In biosynthesized nanoparticles, variations in peak positions and intensities may occur due to nanoscale size effects, lattice strain, and the presence of organic molecules from plant extracts. Phytochemicals from *Withania somnifera* can adsorb onto particle surfaces and influence crystal growth, resulting in slight shifts or additional reflections in the XRD pattern.

The strong peak intensities suggest good crystallinity, while the presence of multiple reflections indicates a polycrystalline arrangement. Plant-mediated synthesis often produces nanoparticles with smaller crystallite sizes, leading to peak broadening compared with bulk materials. According to Khan et al. (2023), *“ZnO nanoparticles synthesized via plant extracts exhibit characteristic crystalline peaks corresponding to the wurtzite phase, with variations attributed to biomolecule interactions.”*

Crystallinity plays a crucial role in determining the physical, optical, and biological properties of ZnO nanoparticles. Highly crystalline ZnO typically exhibits enhanced stability, photocatalytic efficiency, and antimicrobial activity compared with amorphous forms. The observed diffraction pattern therefore supports the suitability of the synthesized ZnO-WS nanomaterials for potential biomedical and environmental applications.

3.5 Conclusion

Present study on crude, extracts of *Withania somnifera* revealed the wide range of antimicrobial activity against a bacteria and fungi. The aqueous and methanolic extract of *Withania somnifera* leaves showed antimicrobial activities, which may be due the presence of bio metabolites. Subsequently, the bioactive compounds from *Withania somnifera* can be used to produce antimicrobial medicines for the treatment of microbial infections. Identification, Isolation, biochemical characterization, antimicrobial potentiality and cytoxical evaluation of present phytoconstituents is the base of development of natural pharmacological formulations and becoming the new areas of future research.

The present study confirms that *Withania somnifera*leaves are a rich source of bioactive phytochemicals, including flavonoids, phenolics, alkaloids, and terpenes, which contribute significantly to their antioxidant and antimicrobial properties. Methanolic extracts consistently demonstrated higher phenolic and flavonoid content, along with superior DPPH scavenging activity, indicating better extraction efficiency and bioactivity compared to aqueous extracts.

The green synthesis of ZnO-WS nanoparticles further enhanced these properties, showing increased phenolic and flavonoid content and improved antioxidant potential with lower IC_{50} values. Spectroscopic and diffraction analyses (UV–Vis, FT-IR, and XRD) confirmed the successful formation of stable, crystalline ZnO nanoparticles, with plant phytochemicals acting as reducing and stabilizing agents.

Additionally, both crude extracts and synthesized nanoparticles exhibited notable antimicrobial activity against bacterial and fungal strains, supporting their potential as natural therapeutic agents.

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