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Studies On Antioxidant, Antimicrobial Properties And Factors Affecting The Extraction Of Nanoparticles From Withania Somnifera

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Keywords:

Silver nanoparticle, Withania somnifera, phytochemical screening, antioxidant assay, antimicrobial assay, UV, FTIR,

ABSTRACT

Withania somnifera commonly known as Ashwaganda belongs to the Solanaceae family, also popularly known as the nightshade family. It is an important herb in Ayurvedic and indigenous medical systems. To evaluate the antioxidant and antimicrobial properties of the aqueous bark extract of Withania somnifera against gram positive and gram negative bacteria. Biosynthesis of silver nitrate nanoparticles using an aqueous extract of Withania somnifera barks were observed by color change in the reaction mixture. These silver nitrate nanoparticles were characterized by Ultraviolet-visible spectroscopy, Fourier-transform infrared spectroscopy techniques. The phytochemical screening of Withania somnifera revealed the presence of alkaloids, steroids, terpenoids and other cardiac glycosides. Since the plant extract contains various secondary metabolites, it acts as a reducing and stabilizing agent for bioreduction reaction to synthesized novel metallic nanoparticles. The phytochemical activity of the extract varied with the time, temperature and pH..

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1. Introduction

Withania somnifera commonly known as Ashwaganda belongs to Solanaceae family and as also popularly known as the nightshade family. Withania somnifera is also known by other names such as Indian ginseng, poison gooseberry, or winter cherry. It belongs to the Solanaceae family, order-Tubiflorae, class-Dicotyledons and its species-somnifera Dunal. Withanaia somnifera was grown mostly in dry area and it is also abundant in Nepal, China and Yemen. The plant crude extract contains novel secondary metabolites such as phenolic acid, flavonoids, alkaloids and terpenoids and these compounds are mainly responsible for the reduction of ionic into bulk metallic nanoparticles from Withania somnifera**.** Nanotechnology is an attractive area of research related to production of nanoparticles of variable sizes and shapes as well as their possible benefits in clinical medicine. Biosynthesis of nanoparticles is the intersection of nanotechnology and biotechnology that increase the attention because of the growing need to develop eco-friendly technologies for materials synthesis. Silver nanoparticles exhibiting antimicrobial activity have been synthesized and these biological synthesized nanomaterials have potential applications in different areas such as treatment, diagnosis. In the recent years silver nanoparticles (AgNps) have received enormous attention of researchers due to their extraordinary defense against a wide range of microorganisms and also due to the appearance of drug resistance against commonly used antibiotics.

2. Materials And Methods

2.1 Collection Of Samples

The barks of Withania somnifera collected from Tirisulam hills, Chennai, Tamil Nadu. The sample was washed with deionized water.

2.2 Drying Of Samples

Withania somnifera barks were kept for drying in the oven at a temperature of 40-50°C for 3-7 days till the weight became constant. The plant material was regularly examined to check for any fungal growth or rotting. These were finely powdered using an electric grinder.

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2.3 Extraction

The extraction process was carried out using a Soxhlet apparatus. 20 g of Withania somnifera powdered sample material was uniformly packed into a thimble and extracted with 250 ml of distilled water. The process of extraction was continued for 24 hours or till the solvent in siphon tube of extractor became colorless. After that the extract was taken in a beaker and kept on a hot plate and heated at 30-40º C till all the solvent got evaporated. For further use the dried extract of Withania somnifera was refrigerated at 4º C.

2.4 Synthesis of Silver Nanoparticles

The silver nitrate at a concentration of 1 mM solution was prepared in a 100 ml flask. 1 ml of plant extract was mixed with 9 ml of 0.9 % of silver nitrate. The aqueous bark extracts of Withania somnifera and silver nitrate solution was used as a control throughout the experiment (Smetana et al., 2005). The final solution of 200 ml was centrifuged at 18,000 rpm for 25 min. The collected pellets were stored at -40 C. The supernatant was heated at 500 C to 950 C and colour changes observed during the heating process.

2.5 Tests For Pathogenic Bacteria

Pathogenic bacteria, such as Staphylococcus aureus (MTCC 96) and Escherichia coli (MTCC 443) were used for in vitro antimicrobial activity. These selected pathogenic strains were procured from Institute for Microbial Type Technology (IMTECH), Chandigarh, India.

3. Phytochemical screening for Withania Somnifera

3.1 Test For Saponins

5 ml of Withania somnifera aqueous bark extract was shaken with 5 ml of distilled water in a test tube and warmed at room temperature. The formation of stable foam indicates the presence of saponins.

3.2 Tests For Glycoside

Liebermann's test: 2 ml of the Withania somnifera aqueous bark extract was dissolved in 2 ml of chloroform (CHCl3) in addition of 2 ml of 2% acetic acid (CH3COOH). The solution was cooled well with ice gel pack. 500 µl of 1% sulphuric acid (H2SO4) was

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added carefully. A color change from a violet to blue or green indicates the presence of glycosides.

3.3 Tests For Coumarin

2 ml of the Withania somnifera aqueous bark extract and 3 ml of 10 % alcoholic sodium hydroxide (NaOH) were added in a test tube. Appearance of mild yellow color indicates the presence of coumarin.

3.4 Tests For Alkaloids

3 ml Withania somnifera aqueous bark extract was shaken with 3 ml of 1% Hydrochloric acid (HCl) on water bath. 400 µl of Mayer and Wagner's reagent was added to the mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid.

3.5 Tests For Flavonoids

1 ml of Withania somnifera aqueous bark extract and 1 ml of 10% lead acetate solution was added in the test tube. The formation of a yellow precipitate was taken as a positive test for flavonoids.

3.6 Tests For Tannins

2 ml of Withania somnifera aqueous bark extract was shaken with 2 ml of distilled water in addition of 500 µl of 5% Ferric chloride (FeCl3) solution. Formation of a green precipitate shows the presence of tannins.

3.7 Tests For Terpenoids

2 ml of Withania somnifera aqueous bark extract were dissolved in 2 ml of chloroform (CHCl3) and 2 ml of 1% concentrated sulphuric acid (Con.H2SO4) was added and heated for about 2 minutes. The appearance of grayish color indicates the presence of terpenoids.

3.8 Tests For Steroids

2 ml of Withania somnifera aqueous bark extract was dissolved in 2 ml of acetic acid (CH3COOH) and 1% of 2 ml concentrated sulphuric acid (Con.H2SO4) was added and red color indicates the presence of steroids.

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3.9 Test For Anthroquinones

2 ml of the Withania somnifera aqueous bark extract was boiled with 2 ml of 10 % hydrochloric acid (HCl) for 5 minutes in a water bath. It was filtered using (Whatman filter paper 1) and then allowed to cool. 2 ml of chloroform (CHCl3) was added to the filtrate sample and 400 µl of 10 % ammonia (NH3) added to the mixture and heated in water bath for 2 minutes. The formation of pink color indicates the presence of anthroquinones.

3.10 Detection Of Phenols

a) Ferric chloride test: 2 ml of Withania somnifera aqueous bark extract was treated with 400 µl of 5% ferric chloride (FeCl3) solution and the formation of bluish black color indicates the presence of phenols.

b) Lead acetate test: 2 ml of Withania somnifera aqueous extract was treated with 400 µl of the lead acetate solution and the formation of yellow color precipitate indicates the presence of phenols.

4. Conformation Test For Nanoparticles

4.1 In Vitro Antimicrobial Activity

The antibacterial activity was determined by well diffusion methods (Holder and Boyce 1994). About 25 ml of molten Mueller Hinton Agar was poured into a sterile petri plate (Himedia, Mumbai, India). The pathogenic bacteria were spread onto the agar using sterile L-rod spreader. After 5 minutes setting of the pathogenic microbes, a sterile cork borer was used to make 5 mm well on the agar. The test samples were dissolved in sterile saline and loaded in two wells with various concentrations such as 25 □g/well, 50 □g/well, 75 □g/well and 100 □g/well. The solvent saline loaded well served as negative control and streptomycin (20 µg/ml) well served as positive control. The plates were incubated at 37° C in a bacteriological incubator for 24 hours. The antibacterial activity was determined by measuring the diameter of the zone of inhibition around the well using Ruler scale (Himedia, Mumbai, India).

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4.2 Antioxidant Test

Free radical scavenging ability by the use of a stable (1, 1-diphenyl-2-picrilhydrazyl)

The radical scavenging activity of the extract of Withania somnifera was prepared. Different concentrations of the extract ranging between (25, 50, 75,100 and 125) µg/ml were taken in a test tube. 1 ml of 0.1% DPPH solution in methanol was mixed with 1 ml of various concentrations of the Withania somnifera mixture was allowed to stand for 30 minutes incubation in the dark. 1 ml of methanol and 1 ml of 0.1% DPPH solution was used as a control. The absorbance was calculated using UV-Vis spectrophotometer at 517 nm. The percentage of inhibition was calculated using the following equation.

$$\% \text{ of Inhibition} = \left(\frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100 \right) \quad (1.1)$$

5. Analysis Of Nanoparticles

5.1 UV-Vis Spectroscopy

The aqueous extract of Withania somnifera was diluted with 2 ml of deionized water and it is measured using UV-Vis spectrum at different time intervals regularly. The UV-Vis spectrometric readings were recorded wavelength of (350 to 500 nm). The bioreduced silver solution was centrifuged (Woodley clinispin CT20) at 10,000 rpm for 15 minutes and the pellets were washed 3 times with 20 ml of deionized water [UV-Vis spectrophotometer Perkin-Elmer, Lamda 35, Germany].

5.2 FTIR Spectroscopy

The chemical composition of the synthesized silver nanoparticles were studied by using FTIR spectrometer (Perkin-Elmer LS-55-Luminescence spectrometer). The solutions were dried at 75° C and the dried powders were characterized in the range 4000–400 cm^{-1} using KBr (Potassium Bromide) pellet method.

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6. Factors Affecting The Yield Of Silver Nanoparticle Extracted From Withania Somnifera

6.1 Effect Of Temperature

The crude, synthesized and phytochemical test of Withania somnifera were repeated for optimization of temperature, where the reaction of temperature was maintained at 4°C,50°C,37°C for 10 minutes. The absorbance of the resulting solution was measured by using UV-Visible spectrophotometer (Shimadzu-700).

6.2 Effect Of Time

The crude, synthesized and phytochemical test of Withania somnifera was repeated for optimization of time where the reaction was monitored from 60, 90 and 120 minutes at respective time intervals. The absorbance of the resulting solutions was measured by using UV-Visible spectrophotometer (Shimadzu-700).

6.3 Effect Of pH

The crude, synthesized and phytochemical test of Withania somnifera was repeated for different pH (4, 7 and 8) for the synthesis of silver nanoparticles. The absorbance of the resulting solution was measured by using UV-Visible spectrophotometer (Shimadzu-700).

7. Results

7.1 Phytochemical Screening

Table 1: Phytochemical assay on Withania somnifera

Phytochemicals	Observation	Result
Phenols	Deep blue to black color	+
Flavonoids	Yellow color appearance	+
Coumarin	Formation of yellow color	+
Alkaloids	Reddish brown color precipitate	+
Tannins	Formation of green color	+
Terpenoids	Greyish color	+
Steriods	Formation of red color	-
Anthroquinone	Formation of pink color	+
Saponins	Stable foam /Persistant	-
Glycosides	Violet to blue or Green color	-

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7.2 Antimicrobial Assay

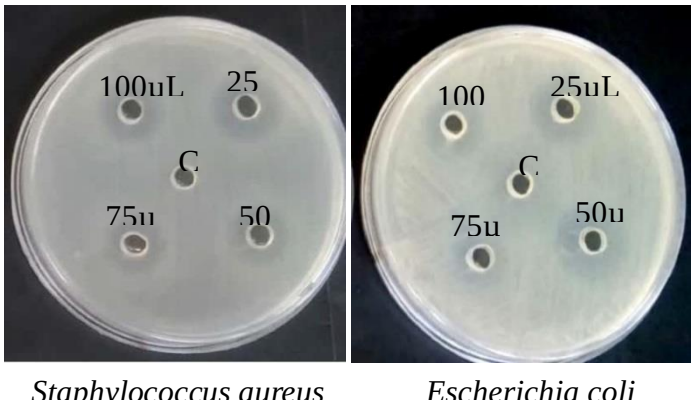

Fig 1: Antimicrobial assay for Withania somnifera with Silver nitrate

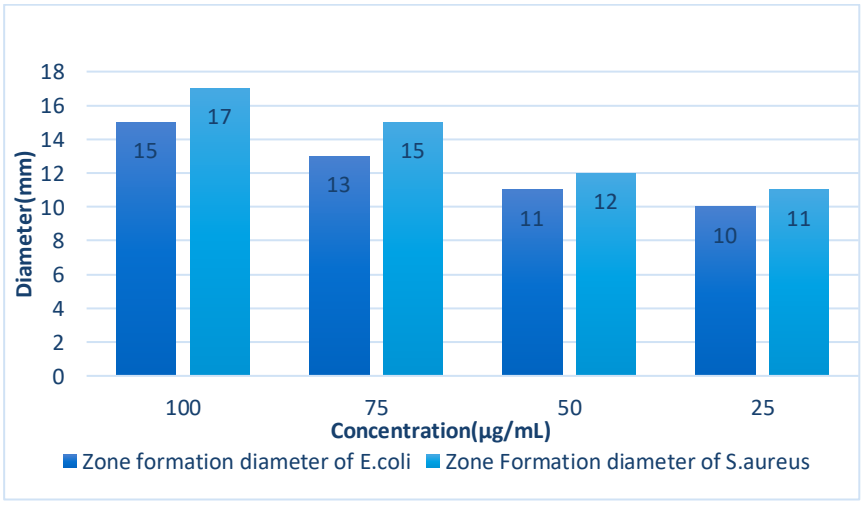

Fig 2: Antimicrobial assay of Escherichia coli and Staphylococcus aureus

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7.3 DPPH radical scavenging assay of Withania somnifera

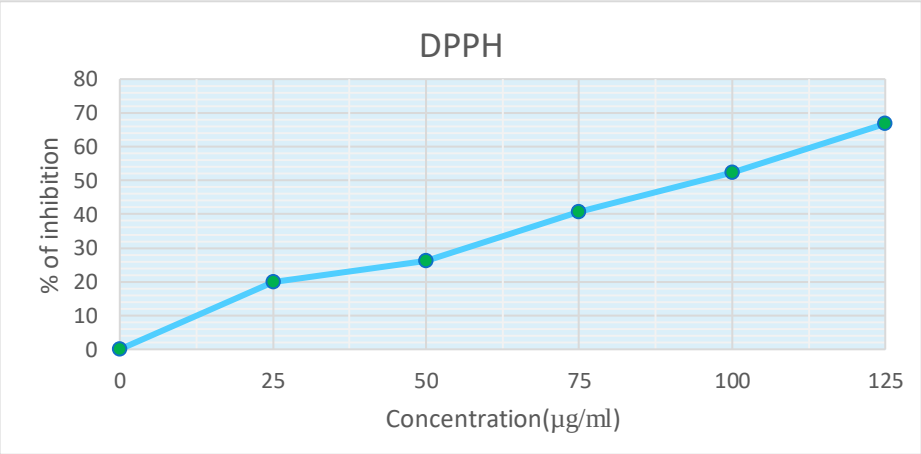

Fig 3: DPPH radical assay of percentage inhibition of Withania somnifera aqueous extract

8. Factors affecting the yield of silver nanoparticles

8.1 Effect of Temperature

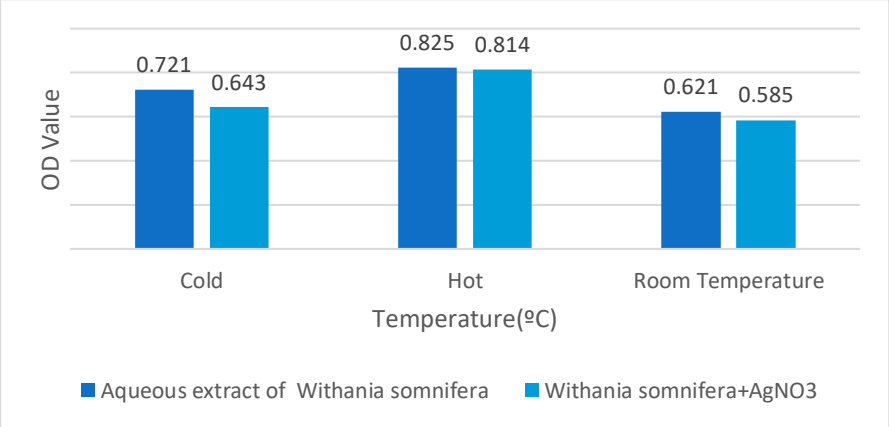

Fig 4: Effect of temperature for crude and synthesized sample of Withania Somnifera

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8.2 Effect Of Time

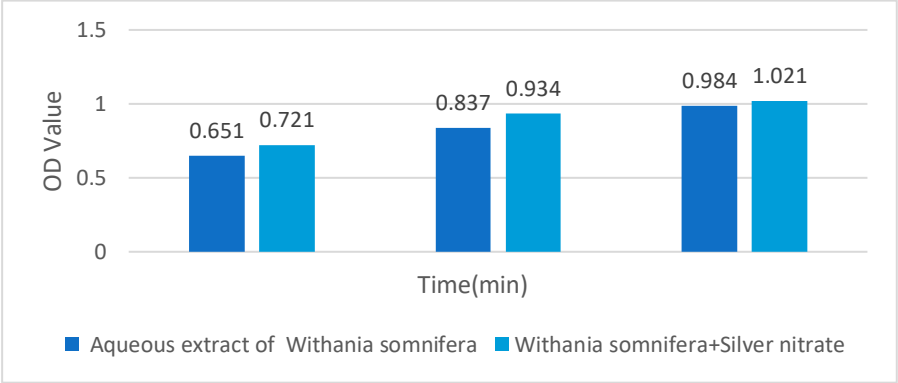

Fig 5: Effect of time for crude and synthesized sample of Withania somnifera

8.3 Effect of pH

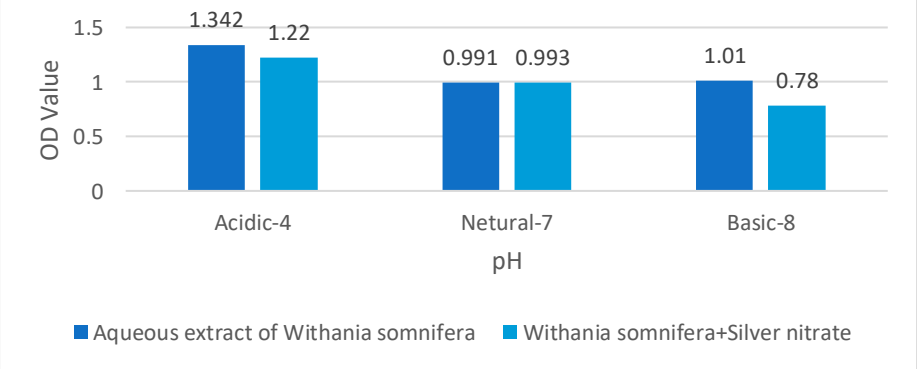

Fig 6: Effect of pH for crude and synthesized sample of Withania somnifera

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9. UV Analysis

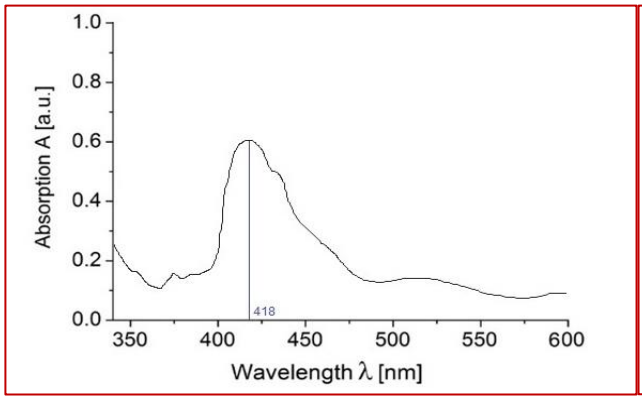

Fig 7: UV analysis of crude sample

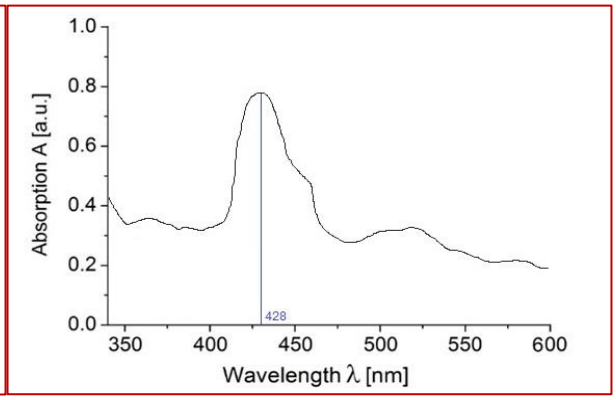

Fig 7.1: UV analysis of synthesized sample

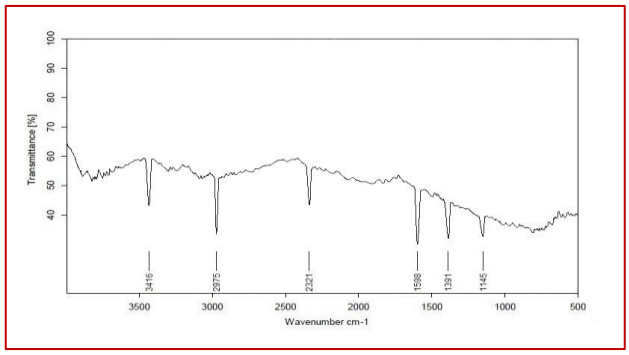

Fig 8: FTIR analysis of crude sample

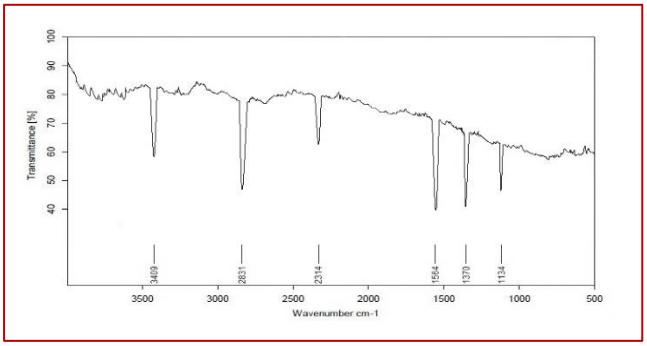

Fig 8.1 FTIR analysis of synthesized sample

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Table 8: Effect of temperature, time and pH

Sl.No	Fixed factors				Factors altered	Quantitative tests									
						Phytochemical Test									
	Solvent	Temp	pH	Time		Glycoside	Saponins	Phenolics	Flavonoids	Carbohydrates	Alkaloids	Tannins	Terpenoids	Triterpenoids	Anthroquinone
1.	Water	4ºC	Neutral	30 minutes	Cold	–	+	–	–	+	–	–	+	–	–
2.	Water	50º C	Neutral	30 minut	Hot	+	+	+	+	+	+	+	+	-	+
3.	Water	37° C	Neutral	30 minutes	Room Temperature	–	–	+	–	–	–	+	–	+	+
4.	Water	50 ºC	4	30 minutes	Acidic	–	–	–	–	+	+	–	–	–	–
5.	Water	50 ºC	7	30 minutes	Neutral	-	+	+	+	+	+	+	+	-	+
6.	Water	50 ºC	8	30 minutes	Basic	+	+	-	-	-	-	-	-	+	+
7.	Water	50 ºC	Neutral	-	0-60 minutes	+	+	+	+	–	–	–	+	+	+
8.	Water	50 ºC	Neutral	-	0-90 minutes	-	-	+	+	+	+	-	+	-	+
9.	Water	50 ºC	Neutral	-	0-120 minutes	+	+	+	+	+	+	+	+	–	–

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11. Discussion

The silver nanoparticles were synthesized from the aqueous bark extract of Withania somnifera within 24 hours of incubation period. The aqueous bark extract of Withania somnifera and silver nitrate solution turned into yellowish brown color within 30 minutes. Intensity of yellowish brown color increases in direct proportion to the incubation period. The color change is due to the excitation of electrons in silver nanoparticles that indicates the formation of silver nanoparticles.

11.1 Phytochemical Studies

It was evident the aqueous extract of Withania somnifera was evaluated for the presence of various phytochemical studies that were shown in the Table (1). From this Table, it can be indicated that the aqueous extract of Withania somnifera mainly contains phenols, flavonoids, coumarin, alkaloids, tannins, terpenoids and anthroquinone.

11.2 Antimicrobial Assay

It is well known that silver nanoparticles exhibit a wider bacterial inhibition zone than silver nitrate in all analyzed bacteria (Escherichia coli and Staphylococcus aureus). The silver nanoparticles, which are biosynthesized from plant extract showed enhanced antibacterial activity when compared to chemically synthesized ilver nanoparticles, thus the result substantiates that the highest antimicrobial activity was exhibited by S.auerus than from E.coli

11.3 Antioxidant Activity

The antioxidant activity of Withania somnifera extract was estimated using a free radical scavenging ability by the use of stable (1,1-diphenyl-2-picrilhydrazyl). The antioxidant activity increases, according to the concentration of Withania somnifera extract and maximum antioxidant activity exhibited at 125 µg/ml.

11.4 UV Visible Spectrophotometer

The reduction of silver nanoparticles was observed by measuring in UV spectrophotometer for both the crude sample of aqueous Withania somnifera bark extract and with the addition of silver nanoparticles to aqueous extract of Withania somnifera. The Absorption spectrum of silver nanoparticles formed in the reaction mixture at different wavelength (ie.350nm-600 nm) was recorded. The particles showed maximum absorption

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peak at 418 nm for crude aqueous bark extract of Withania somnifera and 428 nm for biosynthesized sample of Withania somnifera. This is shown in fig (7 and 7.1).

11.5 FTIR

FTIR analysis was used for characterization and to identify the potential biomolecules of the aqueous extract and the resulting silver nanoparticles fig (8 and 8.1). The characterization ranges from 4000–400 cm^{-1} using KBr pellet method. FTIR signal for crude extract of aqueous Withania somnifera bark were observed at 3416 cm^{-1}, 2975 cm^{-1}, 2321 cm^{-1}, 1598 cm^{-1}, 1391 cm^{-1}, 1145 cm^{-1} and for the aqueous biosynthesized of the Withania somnifera bark extract was observed at 3409 cm^{-1}, 2831 cm^{-1}, 2314 cm^{-1}, 1564 cm^{-1}, 1370 cm^{-1}, 1134 cm^{-1}.

11.6 Factors Affecting The Yield

Different parameters were optimized such as temperature, time and pH, which were considered as factors affecting the yields of silver nanoparticles. The reactions were carried at different temperatures, time and pH [temperature (4° C, 37° C and 50° C), time (30 min, 60 min and 120 min) p^H (4.2, 7.0 and 8.5)]. The overall optimized reaction condition was temperature: 75° C, time: 120 minutes, pH: neutral (7).

12. Conclusion

In conclusion, it has been reported that a simple green synthesis of silver nanoparticles from the aqueous bark extract of Withania somnifera can able to synthesis silver nanoparticles from silver nitrate solution effectively which is cost effective and ecofriendly. Withania somnifera found to be a low cost candidate for synthesis of silver nanoparticles. The phytochemical screening was studied to determine the presence of Phenols, Flavonoids, Coumarin, Alkaloids, Tannins, Terpenoid and Anthroquinone. Withania somnifera has been examined well for antimicrobial activity against Escherichia coli and Staphyloccocus aureus and antioxidant activity were determined by DPPH assay. The size and shape of the nanoparticles were also analyzed by UV-Vis and FTIR studies. It is concluded that nanoparticles yield can easily reported effectively with optimized conditions like temperature, time and pH·The above study were proved a simple, economical and ecofriendly way for the synthesis of silver nanoparticles.

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