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Effect of Crude Extract and Silver Nanoparticles Obtained from *Withania somnifera* as a Source of Antibacterial Compound

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Abstract : *Withania somnifera is the best rejuvenating agent commonly known as "Ashwagandha", belongs to the family Solanaceae. The present study deals with the effect of crude extract and synthesized nanomolecule (AgNP) from Withania somnifera on antibacterial activity. This knowledge can help in better management of swelling, tumour, scrofula, rheumatism, anxiety and neurosis. The crude extract of Withania somnifera were used for synthesis of silver nanoparticles and comparison of antibacterial activity of crude extract with AgNPs and commercial antibiotic against bacteria like Bacillus, Klebsiella, Streptococcus, Stapylococcus and E.coli. From the result, Withania somnifera silver nanoparticle has attained the antibacterial activity against pathogen.*

Keywords: *Nanoparticles, phytochemical, antibacterial, antibiotics, UV-Vis, XRD, FT-IR, HPLC.*

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Introduction:

Characterisctics Of *Withania somnifera*: *Withania somnifera*, commonly known as Ashwagandha, Indian Ginseng, Poison Gooseberry or Winter Cherry is a plant which is classified under the family Solanaceae. It has been extensively used in Indian, Unani and African traditional medicines (Chopra., 1994).

This plant possesses immense therapeutic potential and is known for its immuno-medulatory, anti-stress (Bhattacharya *et al.*, 200l), cardio-protective (Andallu *et al.*, 2000), anti-ageing (Bone., 1996), anti-oxidant (Dhuley., 2007), anti-inflammatory (Begum., 1988), anti-tumour and anti-cancer activities.

Antibacterial Property: Medicinal plants contain secondary metabolites like alkaloids, steroids, tannins and phenolic compounds which are capable of antibacterial activity (Kapoor *et al.*, 2015). *Escherichia coli*, *Staphylococcus*, *Klebsiella*, *Streptococcus* and *Bacillus* are some of the very common plant pathogenic bacteria.

Antibiotics: Antibiotic, also called as antibacterial, is a type of antimicrobial drug used in the treatment and prevention of bacterial infections. These may either kill or inhibit the growth of bacteria.

As medicinal plants are rich source of antibacterial compounds, hence, we used *Withania somnifera* for showing antibacterial activity.

According to our literature review, silver nanoparticles show antibacterial property. Hence, we prepared silver nanoparticles from the extract of leaf, stem and root of *Withania somnifera.*

Concept of Nanotechnology: Nanotechnology is the manipulation of matter on an atomic, molecular and supra-molecular scale. Nanotechnology in size is naturally very broad including fields of Science as diverse as Surface Science, Organic Chemistry, Molecular Biology, Semi Conductor, etc (Saini *et al.*, 2010). The term "Nanotechnology" was first used by Norio Taniguchi in 1974 though it was not widely known.

Materials and Methods :

The plant of *Withania somnifera* was collected from nursery. The leaves, stem and root were separated and were thoroughly cleaned with clean cloth and were dried at room temperature. The leaves were dried and crushed using mortar and pestle, stems were dried and cut into small pieces and roots were dried at room temperature.

Preparation Of Plant Extracts: Soxhlet extractor was used for preparation of plant extract. 250 ml of ethanol was added to round bottom flask of Soxhlet extractor. The dried plant material was loaded into the thimble (made of thick filter paper) which was placed inside the Soxhlet extractor. The nearly transparent liquid obtained was the plant extract. (Redfern *et al.,* 2014)

Phytochemical Analysis: Phytochemicals are chemical compounds produced by plants, generally to help them thrive or thwart competitors, predators or pathogens. The name comes from 'Phyton' meaning 'Plant'. Phytochemicals are chemicals produced by plants through primary and secondary metabolism (Molyneux *et al.*, 2007).

1. **Test for Alkaloids :-**

   Ÿ **Wagner's test :-**

   A few drops of Wagner's reagent was added to 2 ml of plant extract along the sides of test tube. (Wagner., 1993)

2. **Test for Amino Acids :-**

   The extract was dissolved in 10 ml of distilled water and filtered through filter paper. The filtrate was subjected to test for amino acids.

   - **Ninhydrin test :-**

     2 drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was added to 2 ml of aqueous filtrate. (Yasuma *et al.*, 1953)

3. **Test for Carbohydrates :-**

   - **Benedict's test :-**

     To 0.5 ml of filtrate, 0.5 ml of Benedict's solution was added. The mixture was heated on a boiling water bath for 2 minutes.

4. **Test for Glycosides :-**

   5 ml of plant extract was hydrolysed with concentrated HCl for 2 hours on a water bath, filtered and the hydrolysate was subjected to following test :-

   - **Borntrager's test :-**

     To 2 ml of filtered hydrolysate, 3 ml of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia was added to it. (Evans., 1997)

5. **Test for Phenolic Compounds and Tannins:-**

   Ÿ **Lead acetate test :-**

   2 ml plant extract was dissolved in 5 ml distilled water and 1 ml of 10% lead acetate solution was added to it.

6. **Test for Proteins :-**

   Plant extract was dissolved in 10 ml of distilled water and filtered through filter paper and the filtrate was subjected to test for proteins.

   - **Millon's test :-**

     To 2 ml of filtrate, few drops of Millon's reagent was added. (Rasch *et al.*, 1960)

7. **Test for Saponins :-**

   Plant extract was diluted with distilled water and made upto 20 ml. The suspension was shaken in graduated cylinder for 15 minutes. (Kokate., 1999)

8. **Test for Gum and Mucilages :-**

   Plant extract was dissolved in 10 ml distilled water. To this, 2 ml of absolute alcohol was

added with constant stirring. (Whistler *et al.*, 1993)

9. **Test for Volatile Oil :-**

For volatile oil estimation, 50 mg powdered material (crude drug) was taken and subjected to hydro-distillation. (James *et al.*, 1996)

Estimation of Withanolides Present in *Withania somnifera:* Estimation of phytochemicals was done by HPLC technique.

Synthesis Of Silver Nanoparticles: For synthesis of silver nanoparticles, 10 ml of ethanolic extract of plant part was added to 90 ml of $AgNO_3$ solution so as to make its final concentration to 10^{-3} M (0.5 g $AgNO_3$ in 200 ml of distilled water). The solution was allowed to react at room temperature. Periodic sampling after 30 minutes was carried out to monitor the formation of silver nanoparticles. (Ramteke *et al.*, 2012)

Characterization Of Nanoparticles: The synthesied nanoparticles were characterized using UV-Vis spectrophotometer, XRD and FT-IR.

Antibacterial activity: The five bacterial strains used in our study were *Bacillus, Klebsiella, Streptococcus, Staphyloccocus,* and *E.coli*. They were obtained from IGIMS, Patna. The effect of silver nanoparticles were confirmed by disc diffusion method. (Aneja., 2003)

Result and Discussion:

Phytochemical Analysis: The results obtained from phytochemical analysis has been mentioned in the table : Table 1

Estimation of withanolides present in *Withania somnifera*: In sample, percentage of withanolide-A (0.146 %) was quantified at retention time 11 minutes and wavelength 200 nm. This has been depicted in Figure 1.

Synthesis of silver nanoparticles: In the present study, extracts of *Withania somnifera* (leaf, stem, root) were used as reducing agents for the synthesis of silver. Synthesis of silver nanoparticles in leaf extract could be observed by the change in colour from dark green to light green. Synthesis of silver nanoparticles in stem extract could be observed by colour change from light green to orange. Synthesis of silver nanoparticles could be observed in root extract by colour changes from colourless to pale yellow.

Characterisation of silver nanoparticles:

UV-Vis spectrophotometer: The UV-Vis spectra of silver nanopaticles of synthesised using extract of *Withania somnifera* (leaf, stem, root). Maximum absorbance peak of silver nanoparticles of root was seen to be at 450 nm, stem was seen to be at 350 nm and leaf was seen to be at 400 nm wavelength. Absorbance peak of stem increased at 450 nm and further started decreasing. The reading was taken after 24hrs. This has been mentioned in Figure : 3-5

XRD: From XRD Analysis, we observed that intense peaks were observed at 38.1º, 44.1º and 64.1º, corresponding to the index of planes at 111, 200 and 220, respectively and structure was FCC (Face Centred Cubic) structure (according to Bragg's Law). Analysis of XRD further confirmed and synthesised nanoparticles with sharp bands of Bragg's peaks and this might be due to the stabilization of silver nanoparticles of *Withania somnifera* and the provided the crystalline nature of the silver nanoparticles. This has been shown in Figure 2.

FT-IR: It is an effective analytical technique for detecting functional groups and for characterization of nanoparticles. The results obtained from FT-IR were mentioned in Table 2

Antibacterial activity: In present investigation, we have studied the comparison of antibacterial activity of crude extract with AgNPs and commercial antibiotic against bacteria. AgNP extract shows maximum zone of inhibition in comparison to that of antibiotic against the pathogen *Klebsiella*. Crude extract shows more zone of inhibition in comparison to that of antibiotic against the pathogen *Escherichia coli*. Crude extract shows more zone of inhibition in comparison to that of AgNP extract against the pathogen *Staphylococcus.* Table : 3-5

Conclusion:

From the present work we conclude that extracts of *Withania somnifera* are rich source of nutritional component like carbohydrates and non-nutritional components like alkaloids, volatile oils, tannins and phenolic compounds. Silver nanoparicles can be synthesised from *Withania somnifera*. The crude extract and synthesised nanoparticles both show antibacterial activity. Thus, these particles can be used to treat certain diseases.

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Table 1. Showing Phytochemical Analysis of Plant Extract

S. No.	Phytochemical Test	Leaf	Stem	Root
1.	Wagner's Test	+	+	+
2.	Ninhydrin Test	–	–	–
3.	Benedict's Test	+	+	+
4.	Borntrager's Test	–	–	–
5.	Lead Acetate Test	+	+	+
6.	Millon's Test	–	–	–
7.	Test for Saponins	–	–	–
8.	Test for Gums and Mucilages	–	–	–
9.	Test for Volatile Oil	+	+	+

Table 2. Showing FT-IR Peak Values and Functional Groups of Plant Extracts of *Withania somnifera*

S. No.	Plant Extract	Peak Value	Functional Group	Functional Group Name
1.	Leaf	730	C-Cl	Haloalkane
		816.80	C-H	Alkane
		2951.04	C-H	Alkyl Group
2.	Stem	2900.55	C-H	Alkyl Group
		1274.64	O-H	Hydroxyl
3.	Root	290.11	C-H	Alkyl Group
		1274.92	O-H	Hydroxyl
		875.50	C-H	Alkane

Table 3. Showing Comparison of Zone of Inhibition of AgNP Extract and Zone of Inhibition of Antibiotic

S. No.	Pathogenic Strain	Plant Part	Zone of Inhibition of AgNP Extract	Zone of Inhibition of Antibiotic
1.	***Escherichia coli***	Leaf	0.4 cm	0.6 cm
		Stem	0.3 cm	0.2 cm
		Root	0.3 cm	0.05 cm
2.	***Bacillus***	Leaf	0.6 cm	1 cm
		Stem	0.2 cm	1.2 cm
		Root	0.5 cm	1.1 cm
3.	***Klebsiella***	Leaf	0.2 cm	0.1 cm
		Stem	0.4 cm	0.2 cm
		Root	0.2 cm	0.1 cm
4.	***Streptococcus***	Leaf	0.3 cm	0.6 cm
		Stem	0.5 cm	0.8 cm
		Root	0.2 cm	1.2 cm
5.	***Staphylococcus***	Leaf	1 cm	0.8 cm
		Stem	0.5 cm	0.8 cm
		Root	0.3 cm	1 cm

Table 4. Showing Comparison of Zone of Inhibition of Crude Extract and Zone of Inhibition of Antibiotic

S. No.	Pathogenic Strain	Plant Part	Zone of Inhibition of Crude Extract	Zone of Inhibition of Antibiotic
1.	***Escherichia coli***	Leaf	0.3 cm	0.1 cm
		Stem	0.4 cm	0.04 cm
		Root	0.2 cm	0.1 cm
2.	***Bacillus***	Leaf	0.5 cm	1.3 cm
		Stem	0.5 cm	0.7 cm
		Root	0.8 cm	1 cm
3.	***Klebsiella***	Leaf	0.4 cm	0.6 cm
		Stem	0.4 cm	0.8 cm
		Root	0.2 cm	0.6 cm
4.	***Streptococcus***	Leaf	0.2 cm	0.1 cm
		Stem	0.2 cm	0.04 cm
		Root	0.9 cm	1 cm
5.	***Staphylococcus***	Leaf	0.1 cm	1 cm
		Stem	0.2 cm	1 cm
		Root	0.2 cm	1.2 cm

Table 5. Showing Comparison of Zone of Inhibition of Crude Extract and Zone of Inhibition of Antibiotic

S. No.	Pathogenic Strain	Plant Part	Zone of Inhibition of Crude Extract	Zone of Inhibition of Antibiotic
1.	***Escherichia coli***	Leaf	0.3 cm	0.1 cm
		Stem	0.4 cm	0.04 cm
		Root	0.2 cm	0.1 cm
2.	***Bacillus***	Leaf	0.5 cm	1.3 cm
		Stem	0.5 cm	0.7 cm
		Root	0.8 cm	1 cm
3.	***Klebsiella***	Leaf	0.4 cm	0.6 cm
		Stem	0.4 cm	0.8 cm
		Root	0.2 cm	0.6 cm
4.	***Streptococcus***	Leaf	0.2 cm	0.1 cm
		Stem	0.2 cm	0.04 cm
		Root	0.9 cm	1 cm
5.	***Staphylococcus***	Leaf	0.1 cm	1 cm
		Stem	0.2 cm	1 cm
		Root	0.2 cm	1.2 cm

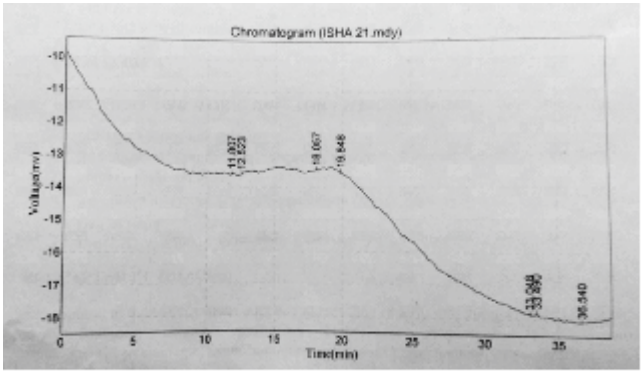


Fig. 1. Estimation of Withanolides Present in *Withania somnifera* by HPLC Chromatogram

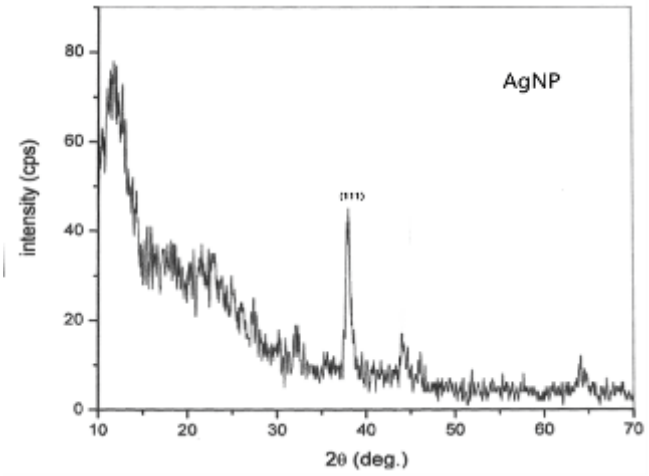


Fig. 2. X-Ray Diffraction Pattern of Biosynthesised Silver Nanoparticles of *Withania somnifera*

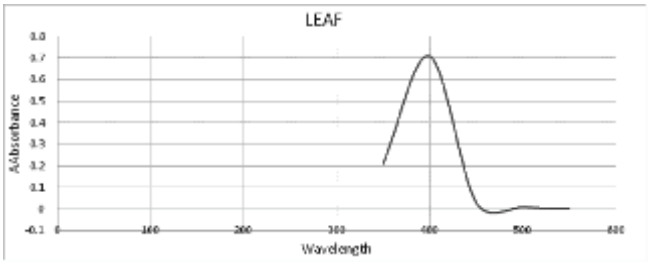


Fig. 3. UV-vis Spectrum of AgNP of Leaf Extract

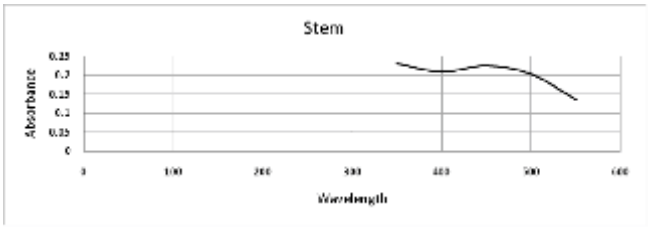
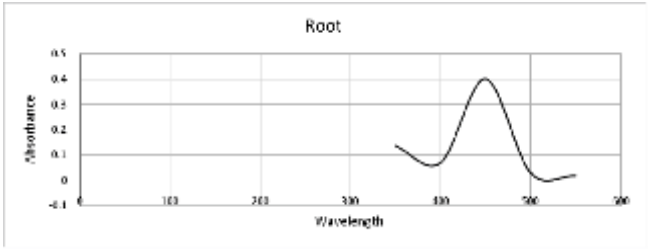


Fig. 4. UV-vis Spectrum of AgNP of Stem Extract



References:

Andallu B. and Radhika B. (2000). Indian Journal Of Experimental Biology, 3,607-609.

Aneja K.R. (2003); Experiments In Microbiology, Plant Pathology And Biotechnology, New Age International Publisher, Delhi, India, 147-156.

Begum V.H. and Sadique J. (1988). Indian Journal Of Experimerntal Biology, 26,877-882.

Bhattacharya A, Bhattacharya S.K. and Ghosal S. (2001). Journal Of Ethnopharmacol, 74,1-6.

Bone K. (1996); Clinical Applications Of Ayurvedic And Chinese Herbs, Queensland, Australia : Phytotherapy Press, 137-41.

Chakrabarti T., Pandey R.A., Ramteke C., Sarangi B.K. (2012). Synthesis Of Silver Nanoparticles From The Aqueous Extract Of Leaves Of Ocimum sanctum For Enhanced Antibacterial Activity.

Chopra (1994). R.N. Glossary Of Indian Medicinal Plants, Academic Press, New Delhi, India.

Dhuley J.N. (2007). Journal Of Ethnopharmacol, pp.57-63.

Evans W.C. (1997). Treaseand Evans Pharmacognosy, Harcourt Brace And Company, Asia Pvt. Ltd. Singapore

Gardner D.R., James L.F., Lee S.T., Molyneux R.J. and Panter K.E. (2000). Phytochemicals : The Good, The Bad And The Ugly?, 68,22-24.

Ichikawa and Yasuma A. (1953). Ninhydrin-Schiff And Alloxan-Schiff Reagent Staining. A New Histochemical Staining Methods For Protiens, Journal Of Clinical And Medical Research, 41,296-299.

James E. and Robbers K. (1996). Pharmacognosy And Pharmaco-Biotechnology, Williams And Willkins, New York, U.S.A.

Kapoor K., Kaur G and Kaur R. (2015). Antimicrobial Activity Of Different Herbal Plant Extracts : A Review.

Kinninmonth M., Redfern J. and Verran J. (2014). Using Soxhlet Extraction To Produce And Test Plant Material (Essential Oils) For Their Antimicrobial Properties.

Kokate C.K. (1999). Practical Pharmacognosy, 4th Edition, Vallabh Prakashan Publication, New Delhi, India.

Miller J.N. and Whistler R.L. (1993). Industrial Gums, Polysaccharides And Their Derivatives, Academic Press, London.

Rajiv, Saini and Santosh (2010). Nanotechnology : The Future Medicine, Journal Of Cutaneous And Aesthetic Surgery, 3 (1) 32-33.

Rasch E. and Swift H. (1960). Micro Photometric Analysis Of The Cytochemical Million Reaction, Journal Of Histochemistry And Cytochemistry, 8,4-17.

Wagner H. (1993). Pharmazeutische Biologic, 5th Edition, AUFT.15 BN 3-437-20 498-X.