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U. V. Spectrometric Analysis of Green Synthesized Silver Nanoparticles (AgNPs) Using Aqueous Leaf Extract Of Neem (Azadirachta indica) And Assessment of its Antibacterial Activity.

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ABSTRACT

Neem (Azadirachta indica) plant leaves possess medicinal properties. Green synthesized silver nanoparticles (AgNPs) demonstrated good stabilizing agent due to its reducing and capping nature. After six hours' incubation of silver nanoparticle synthesis showed peak and stabilization. Green synthesis of silver nanoparticles with aqueous leaf extract of Neem leaves exhibited remarkable antibacterial activity against Escherichia coli, Bacillus subtilis and Salmonella typhi. Diameter of zone of inhibition (mm) was more with S. typhi than E. coli and B. subtilis.

Keywords: -Silver nanoparticles (AgNPs), aqueous leaf extract of neem leaves U.V spectrophotometer, E. coli, B. subtilis and S. typhi

Introduction:

Synthesis of nanoparticles by using plant material extract got importance because of its rapidity does not have environment nonpolluting nature. Neem plant leaves contain biomolecules. Nanoparticles synthesis can be done by physical, chemical and biological methods. From ancient times silver metal is used for different purposes because of its antimicrobial activity. Silvernanoparticles (AgNPs) greatly used due to its antibacterial,antifungal and antiviral activities. Green term is indicating technology used in prepararion is clean technology, does not involve any harmful chemical and it is ecofriendly. Biological synthesis of silvernanoparticles consist of simple method,cost effective and harmless to nature i.e environmentally friendly and large production is possible by easy steps (Veerasamy et al., 2011). Silver nanoparticles (AgNPs) have

electrical, thermal and optimal properties.It plays an important role in drug deliverying imaging, sensoring gene delivery, artificial implants and anticancer therapy. Silvernanoparticles can be synthesized by chemical method but it has toxic residues or by products are produced.

The green synthesis of nanoparticles is convenient method over physical and chemical method. The use of plant material for synthesis of nanoparticles is cost effective and ecofriendly method and it provides natural capping and reducing agent .Silvernanoparticles synthesis by using leaf extract of neem (Azardicta indica) has antibacterial activity against E.coli,B.subtilis and Salmonella spp.

Material and method:

Collection of plant material:

Neem (Azadirachta indica) leaves were collected from Vasantrao Naik, Agricultural University, Parbhani . The plant leaves were brought to Department of Microbiology, Dnyanopasak college, Parbhani.

Preparation of fine powder of neem leaves:

Neem leaves were thoroughly washed with distilled water then dried in shed for 7 days at room temperature. Fine powder was prepared with electric blender. It was stained through double layered musclin cloth to get uniform particle size of leaves. Neem powder was stored in glass container for practical use.

Plant leaf extract preparation:

Fine powder of neem leaves 1g was weighted and added in 100ml deionized water. This mixture was heated at 60 c for 30 minutes in waterbath. The leaf extract was filtered through whatmann filter paper no. 1. Aqueous extract was stored in refrigerator, for practical use.

Silver nitrate ($AgNo_3$) preparation:

100 ml of 1 mM silver nitrate solution was prepared by adding 0.169 g of silver nitrate weighed accurately and added in deionized water mixed well, it gives 1 MM silver nitrate solution.

Synthesis of silver nanoparticles. (AgNPs):

To the 250 ml conical flask 5 ml of aqueous extract of neem leaf extract was added drop by drop with continuous stirring to Agno3 (50ml) ,flask was kept for incubation at room temperature for 24 hours in dark to avoid photo activation of silver nanoparticles after 24 hrs. incubation the flask was observed for color change that is pale yellow to dark brown. The colored solution was further used for U.V. spectrophotometric analysis and antibacterial study.

Characterization of silver nanoparticles

U.V spectrophotometric analysis was done by using (shimadzu -1800). The solution (3 ml) was taken in quartz cuvette and subjected to scanning in the range 200-800 nm. (Das et al.,20 16)

Detection of stability of synthesized silver nanoparticles

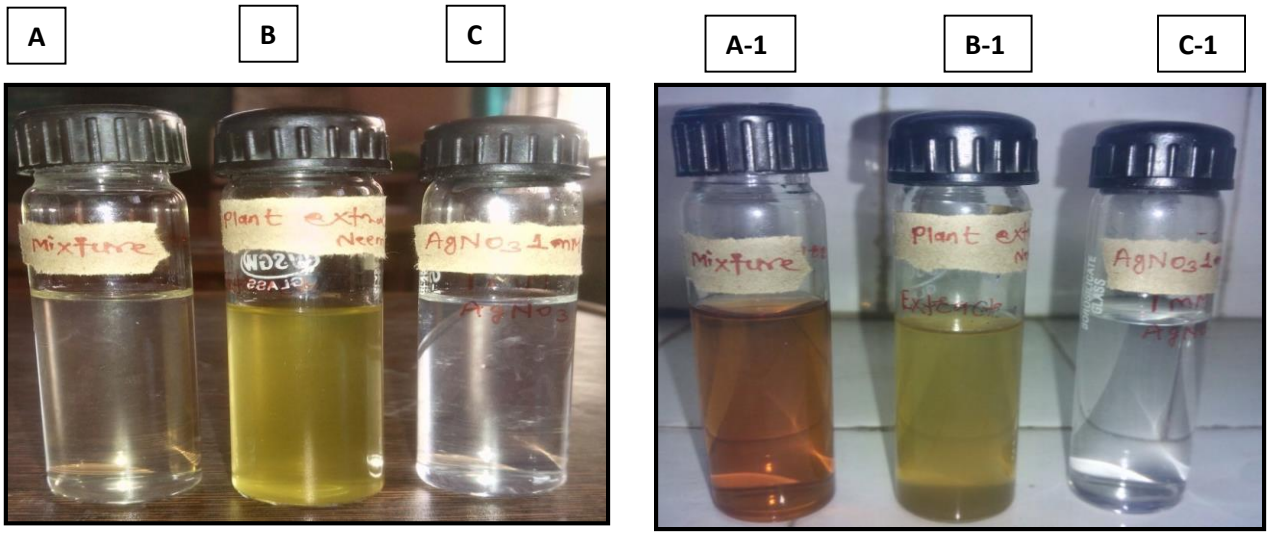
Stability of silver nanoparticles was confirmed by observing any change in the colour of the colloidal solution after every 24 hrs visually as well as spectrophotometrically for 72 hrs.

Antibacterial activity of silver nanoparticles

The bioassay of silver nanoparticles with neem leaf extract was perform against Escheriachia coli bacillus subtilis and salmonella spp by well (agar) diffusion method. The active culture of test organism was seeded in nutrient agar plate. wells of 2 mm after solidification with sterile corkborrer wells with 0.1ml AgNo3 solution, plant extract distilled water and silver nanoparticles (AgNPs) was added in separate wells. Plates were kept for incubation for 24 hrs. Results were observed and recorded in terms of zone of inhibition surrounding the wells.

Result and discussion

Synthesis of silver nanoparticles by using aqueous leaf extract of neem was confirmed by change in colour from pale yellow to dark brown. Color change was observed after 24 hrs of storage. Brown color development was due to the Plasmon resonance (SPR) which is characteristic property of silver nanoparticle. SPR peak of silver nanoparticle in the range of 420-450 nm surface (Banerjee and et al., 2014). In present study the stable and sharp peak of synthesize AgNPs was recorded at 429 nm.



Photoplate-1: Synthesis of Silver Nanoparticles using leaf extract of Azadirachta indica.

Key: A =Plant extract+ 1mM $AgNo_3$ solution Mixtute. B =Plant extract of neem. C = $AgNo_3$ 1Mm, A-1= =Plant extract+ 1mM $AgNo_3$ solution Mixture (incubated for 24 hours) B- 1 = Plant extract of neem (after 24hours). C-1 =$AgNO_3$ after 24 hour.

UV characterization of silver nanoparticles AgNPs

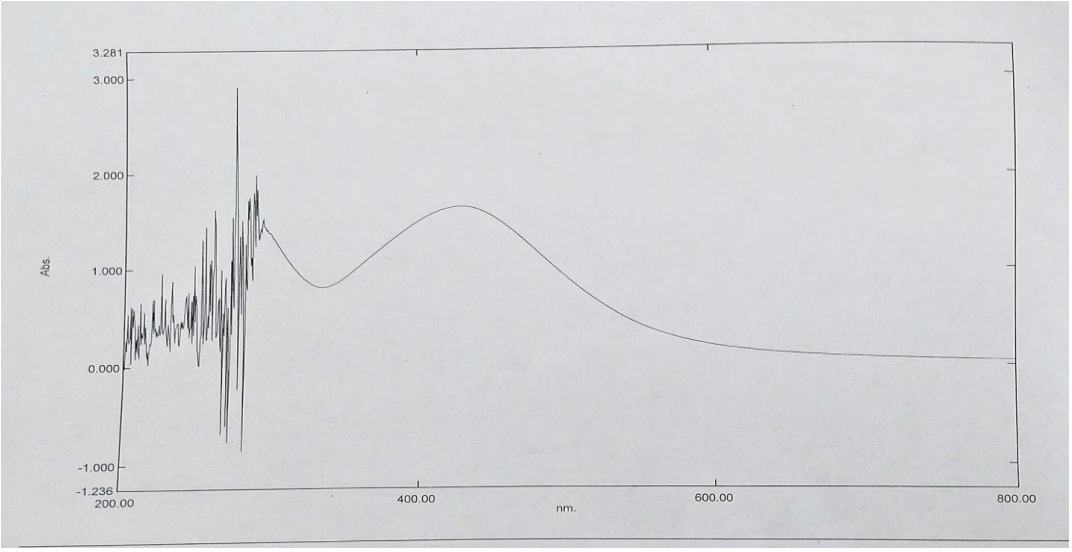


Fig.1: UV- Visible Spectra of AgNPs

After 24 hours of incubation reaction mixture containing silver nanoparticle was subjected to UV spectrum analysis showed peak (Fig.1) spectral band ranges between 400 to 450 nm.

Overlay studies of $AgNO_3$

Overlay studies shows that AgNO3 solution shows only one peak, whereas the brown coloured colloidal solution showed an addition peak at 428 nm confirming the formation of nanoparticles after reacting with plant extract.

Influence of Incubation time on Stability

Results presented in Table-1 shows the stability of synthesized nanoparticles by showing peak and absorbance value of silver nanoparticles using UV- Spectral analysis. Peak values (428nm), (429nm), (429nm) and OD (1.129), (1.651), (1.595) for 24 hrs, 48 hrs and 72 hrs respectively. Peak value and OD values didn't change i.e. remain same which indicates that Nanoparticles are stable indicating that neem extract is acting both as reducing and capping agent.

TABLE – 1: Influence of Incubation period on Stability of AgNPs

Sr. No.	Time of incubation (hrs)	Peak (nm)	OD(429nm)
1	24	428	1.129
2	48	429	1.651
3	72	429	1.595

Bioassay of different components used in synthesis of AgNPs

Antibacterial activity of all component involved in formation of AgNPs was tested against Escherichia coli, Bacillus spp. and Salmonella spp. On the basis of evaluation of zone of inhibition (Photoplate-2) and observation recorded in Table-1 revealed that AgNPs significantly inhibited growth of these organisms. Plant extract didn't inhibited growth so no zone was observed. AgNO3 solution showed zone of inhibition of diameter 08mm, 09mm and 07mm and AgNPs showed zone of inhibition diameter 12mm, 11mm and 09mm against Escherichia coli, Bacillus spp. and Salmonella sp. respectively. Indicating that after formation of NPs, the efficiency of AgNO3 antibacterial agent is enhanced considerably.

TABLE - 2: Bioassay of different components used in synthesis of AgNPs

Sr. No.	Name of Microorganism	Diameter of zone of inhibition(mm)		
		Plant extract	AgNO3	AgNPs
1	E. coli	0.4	0.3	12
2	Bacillus subtilis	0.5	0.2	11
3	Salmonella spp.	0.3	0.2	9

Assessment of antibacterial activity of AgNPs by using different concentration of $AgNO_3$

The effect of AgNO3 concentration with 0.5mM, 1mM, 1.5mM, 2mM and 2.5mM were separately used for synthesis. On the basis of OD and zone of inhibition recorded in reveales that increase in concentration increased OD and zone of inhibition.

The significant effect of siver nanoparticle on zone of inhibition was recorded with 1mM AgNO3 concentration where OD was found (1.212) and zone of inhibition (12mm). Followed by 2.0mM, 2.5mM and 0.5mM concentration, optical densities were recorded (1.097), (1.261) and (1.191) and zone of inhibition were 13.5 mm, 12 mm and 10 mm respectively. That is optimum concentration of AgNO3 for effective antimicrobial activity was 1 mM.

TABLE – 5: Influence of $AgNO_3$ Synthesis of AgNPs

Sr. No.	$AgNO_3$ (mM)	OD(429 nm)	Diameter of zone of inhibition (mm)
1	0.5	1.191	10
2	1	1.212	12
3	1.5	1.200	11.5
4	2	1.097	10
5	2.5	1.2	11.2

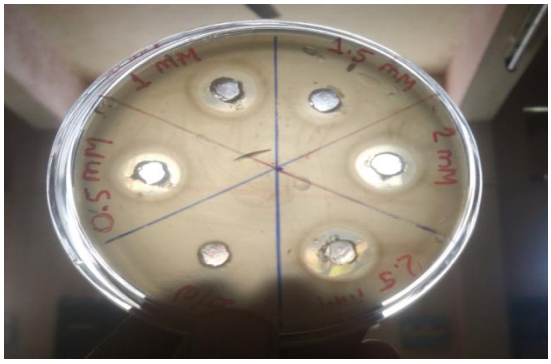


Photo plate -2: Bioassay of silver nanoparticles on Bacillus Subtilis.

Conclusion: -

Green synthesis of silver nanoparticles (AgNPs) by using leaf extract of Azadirachta indica is a cost effective and simple method. It is a rapid, ecofriendly and commercially economic method for synthesis of silver nanoparticles. The neem leaves proved significant role in reduction and stabilization of silver nanoparticles as it shows the pith at 429nm with 1.2 stable optical density on uv spectrophotometer (UV-1800 Shimdzu).

Silvernanoparticles demonstrated excellent activity against nonpathogenic and pathogenic bacteria which was determined by diameter zone of inhibition against E.coli B.subtilis and Salmonella spp. That is 12mm,11mm and 9mm respectively.

In the present work it is confirmed that silver nanoparticle synthesis by using Azadirachta indica leaf extract has high antimicrobial activity so that it can be used in medicinal field due to its great potential against bacteria.

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