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Biosynthesis, Characterization and Evaluation of Silver Nanoparticles using Piper Betel Linn Leaves Extract.

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ABSTRACT:
Nanotechnology deals with the Nanoparticles having size 10-100 nm. Nanoparticles are used most widely due to its small size, physical properties and orientation etc. Nanoparticles can be prepared by physical, chemical and biological methods. The biological method (green method) for synthesis of nanoparticles is easy, efficient and ecofriendly. The present study deals with preparation of silver nanoparticles from betel leaves (Piper betel Linn) extract by green synthesis method. In this study we synthesized silver nanoparticles using 1mM silver nitrate solution into the plant extract. Three formulations were prepared having different concentrations (100, 150, 200mg) of plant extract. Out of the 3 formulations F2 and F3 was selected. The betel leaf extract has antimicrobial activity and it was carried out on Staphylococcus Aureus strain and the result showed that the F3 formulation has highest antimicrobial action. The betel leaf also shows cytotoxic activity/anticancer and it was observed by performing it on brine shrimp (A. salina) and LC_{50} value was calculated.

I. INTRODUCTION

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nano capsules can be obtained.[1]

Generally, nanomaterial can be fabricated through two main methods, i.e., "top-down" and "bottom-up".[2]. In producing nanoparticles using plant extracts, the extract is simply mixed with a solution of the metal salt at room temperature. The reaction is complete within minutes. Nanoparticles of silver, gold and many other metals have been produced this way.The nature of the plant extract, its concentration, the concentration of the metal salt, the pH, temperature and contact time are known to affect the rate of production of the nanoparticles, their quantity and other characteristics.[3]. Studies have shown that Ag-NPs have broad-spectrum antimicrobial properties against pathogens including bacteria, fungi and viruses. A variety of factors affect the antimicrobial activities of Ag-NPs, including size, shape, dose and stabilizer of Ag-NPs.[4]The chemical composition of betel leaf is associated predominantly with phenolic compounds in nature. Piper Betel Linn leaves extract have chemical compounds such as chavibetol, chavibetol acetate, allyl pyrocatechol, α-tocopherol, β-carotene, eugenol, hydroxy chavicol, piperol A, and piperol B, etc[5]Besides its antimicrobial effect, Ag-NPs are also known to induce toxicity. The toxicity of Ag-NPs has also been shown by a number of in vitro studies.

II. EXPERIMENTATION

MATERIALS:

1. Plant Extract:

The standardized extract powder and certificate of analysis of Piper Betel Linn was obtained from Shrisha Organics, Raebareli.

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Piper Betel leaf extract

2. Chemicals:

The silver nitrate (AgNO3) was obtained from Analab Fine Chemicals Mumbai. Distilled water was used for preparation of silver nitrate solution and the piper betel leaf extract solution.

3. Instruments

1. UV visible spectrophotometer – Jasco V-530, Japan.
2. FT-IR spectrophotometer – Bruker Alpha-T, Germany.
3. Particle size analyser - HORIBA SZ- 100
4. Zeta Sizer – HORIBA SZ-100

METHODS

1. Selection of plant

Plant material (Piper Betel) was selected depending upon the literature data and chemical constituents. The plant which has ability to reduce the silver nitrate and also has cytotoxic activity was selected.

2. Pre-formulation studies

The studies included solubility, phytochemical tests, UV, and FT-IR of extract of PBE.

3. Solubility of PBE

Solubility of PBE was determined in various solvents such as –

1. Distilled water
2. Ethanol

4. Phytochemical tests

Different tests of the PBE were performed such as –

1. Test for Alkaloids (Mayer's test)
2. Test for Glycosides (keller-killani test)
3. Test for Flavonoids
4. Detection of Saponins
5. Detection of Carbohydrates (Molisch's test)
6. Detection of tannins
7. Detection of terpenoids

5. UV studies

UV analysis of extract and PB-Ag-NPs was done by taking 1ml of the solution of extract and nanoparticles and diluted to 4ml with distilled water. The spectra is taken between 200-800nm.

6. FT-IR studies: FTIR of extract, silver nitrate and PB-Ag-NPs was done by taking the IR spectra with the help of Jasco FTIR – Japan. They were characterized in the range 4000-700cm-1. FTIR was used for functional group determination

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7. Synthesis of silver nanoparticles

The PB-Ag-NPs were prepared by using different concentrations of PBE (100, 150, 200 mg). The silver nitrate solution of 1mM was prepared in distilled water and 50ml for each concentration was used to prepare the nanoparticles. The reaction mixture was kept at room temperature for 24hrs for complete reduction and the colour change was recordedby visual observation. The synthesized NPs were dried at room temp as well as by evaporation method.

8. Characterization of silver nanoparticles:

1. Anti-microbial Activity:

The antimicrobial activity of synthesized PB-Ag-NPs was performed on Staphylococcus Aureus. The different concentrations of Ag-NPs (100, 150, 200mg) were used via well diffusion method. The zone of inhibition was observed for each concentration of Ag-NPs.

2. Particle size analysis:

Particle size was determined using HORIBA SZ100 particle size analyser. The PB-Ag-NPs was placed in a transparent polystyrene cuvette (path length=1cm) which was placed in a chamber. Mean particle size of the nanoparticles was determined by HORIBA SZ-100 analyser.

3. Zeta potential:

The zeta potential of the formulation gives the electrical charge present on the particles. It was determined by using HORIBA SZ-100 analyzer.

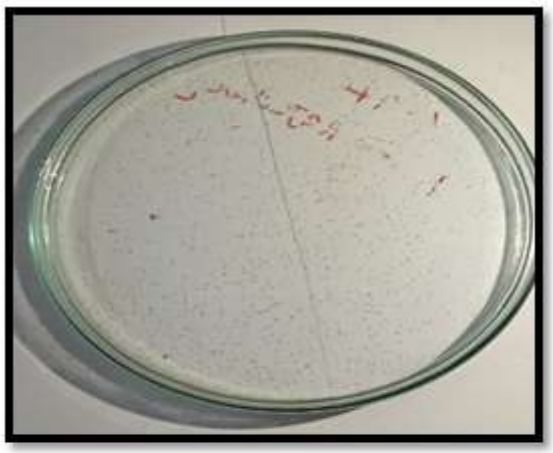
4. Stability studies:

The prepared PB-Ag-NPs were evaluated for its stability under different conditions of temperature for 15days.

- The stability study was carried out at room temp (37°C) and at 4°C.
- After 15days the PB-Ag-NPs were dissolved in distilled water and the absorption maxima was scanned by UV-visible spectrophotometer at wavelength 200-800nm (Jasco, Japan.)
- A graph was plotted.

5. Cytotoxic Activity on Brine Shrimp

Cytotoxic activity of the synthesized PB-Ag-NPs was performed on Brine shrimp Artemia salina. A. salina cysts were aerated in 1L capacity of glass jar containing saline water. The jar was aerated constantly for 48h at room temperature. After hatching, active free floating nauplii was collected from bright illumination and were used for the experiment. A standard solution was used to compare the result. 4 concentrations were prepared (5, 10,15, and 20ug/ml) and in each test tube 10 nauplii were added. This setupwas allowed to stand for 24hr under light and then the %mortality rate was calculated by the observations.[5]



Brine shrimps

III. RESULT

1. Selection of Plant

Piper betel Linn contains chemical constituents which has the ability to reduce the silver nitrate and therefore it was selected. Piper betel Linn has variety of activities such as – Antioxidant, Antidiabetic, Antineoplastic, Antiseptic etc…. It is also used to treat indigestion, cough, asthma, eye related problems.

2. Pre-formulation Studies –

2.1. Solubility of PBE

Solubility of PBE was found in the following

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SOLVENTS	SOLUBILITY
1. Water	Soluble
2. Ethanol	Slightly soluble

2.2. Phytochemical Tests –

Different phytochemical tests were performed of PBE as follows and result were seen -

Sr.No	TEST	RESULT
1..	Alkaloids	Present
2.	Glycosides	Present
3.	Flavonoids	Present
4.	Saponins	Absent
5.	Carbohydrates	Absent
6.	Tannins	Present
7.	Terpenoids	Present

Ag-NO3 Solution

Synthesis of silver nanoparticles

After adding extract to Ag-NO3 (Pale yellow colour)

1 hr. after the reaction (brown colour)

1. Synthesis of PB-Ag-NPs

The colour change of the formulation indicates the formation of Ag-NPs by the extract. The reduction of AgNO3 into Ag-NPs was visible from the colour change (brown colour). Silver nitrate solution was clear / transparent then when the extract was added to it within few minutes color change was observed, from pale yellow colour to dark brown colour after 1hour which indicates formation of nanoparticles.

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Time	Absorbance
15 min	0.89573
30 min	1.35632
45min	1.52685
1hr	1.53156

1.1. Indication of formation of nanoparticles

1. Colour Change – From pale yellow to dark brown colour.
2. UV after every 15 mins –

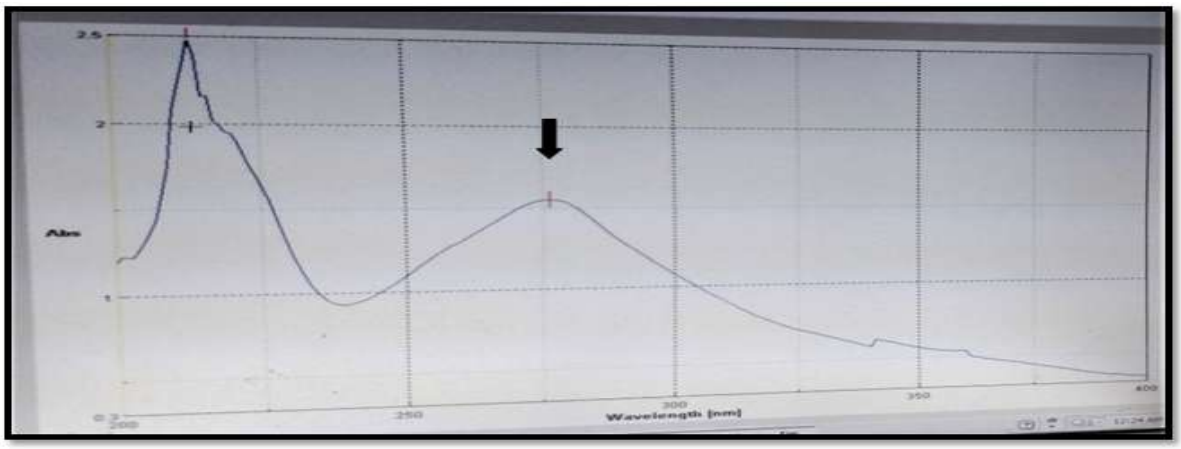
2. Characterization of PB-Ag-NPs

1. UV of Piper betel extract

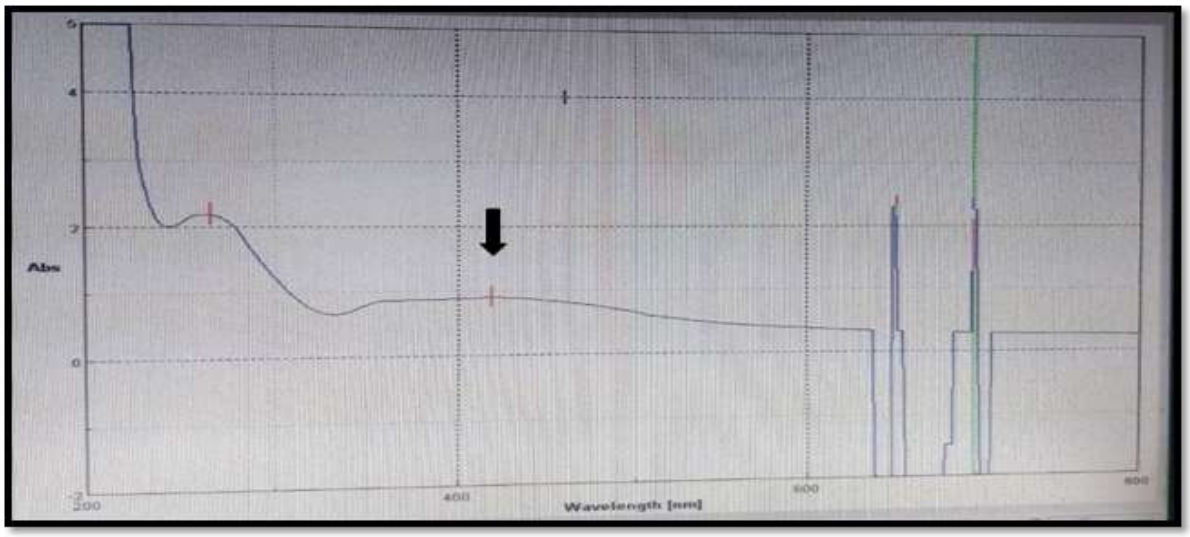
The UV of PBE was performed by dissolving the extract in distilled water and then using this solution for UV analysis.
The peak is observed at **276nm** having absorbance 1.55007.

2. UV of silver nanoparticles

The UV-visible spectra of PB-Ag-NPs (200mg) was performed and the result was recorded and the fig. shows that absorption spectra of PB-Ag-NPs has absorbance peak at **437nm.**



UV of piper betel extract



UV of silver nanoparticles

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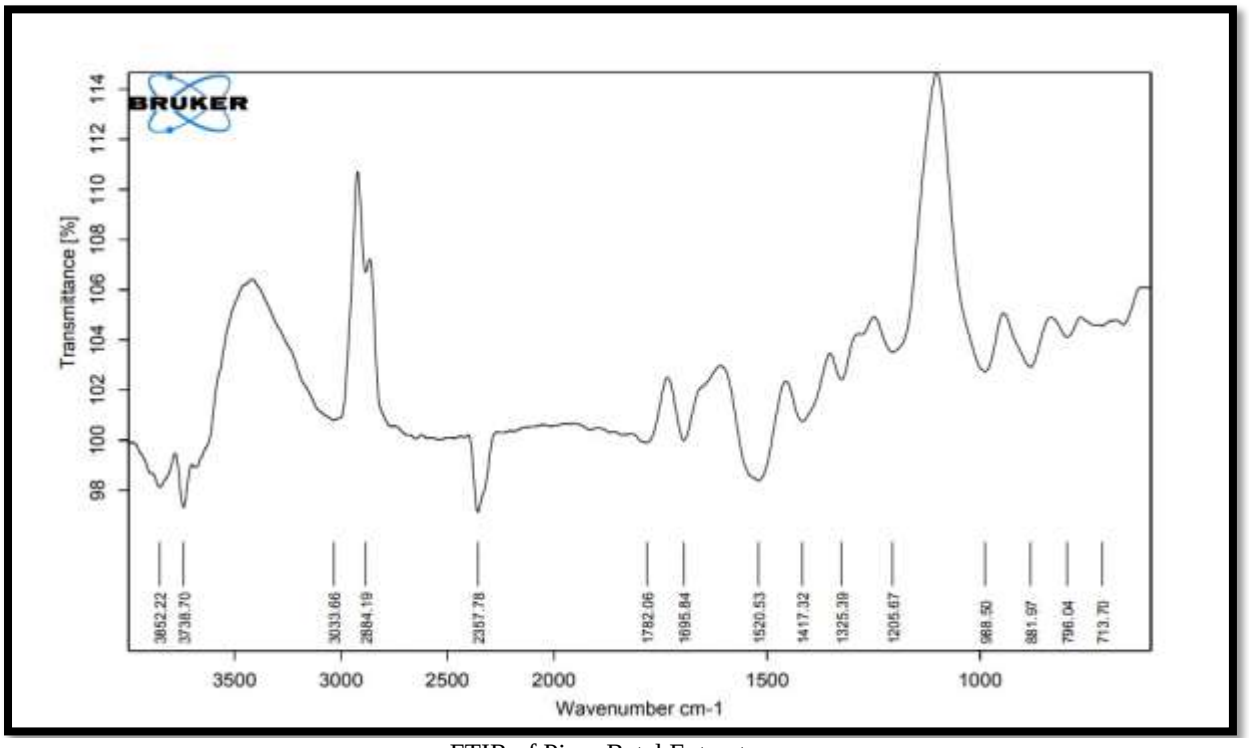
Peaks (cm^{-1})	Groups
3738.70	**O-H** Group
3033.66	**C=C-H** (Alkene) Group
2884.19	**C-H** Stretch
1695.84	**C=C** Bond
1205.67	**C-O** Bond

3. FT-IRof Extract



FTIR of Piper Betel Extract

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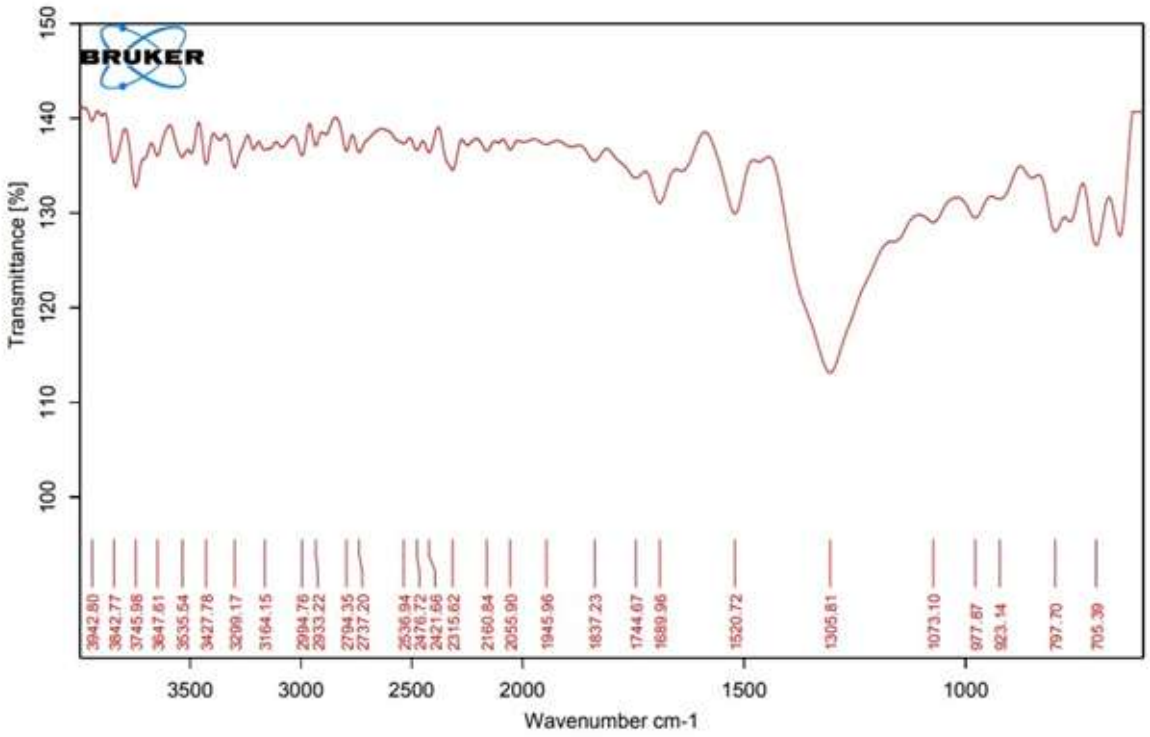
4. FT-IR Of Silver Nitrate



FT-IR Of Silver Nitrate

Peaks (cm^{-1})	Groups
1520.81	NO_2 Group
1305.81	NO_2Group

IR Interpretation of Silver nitrate

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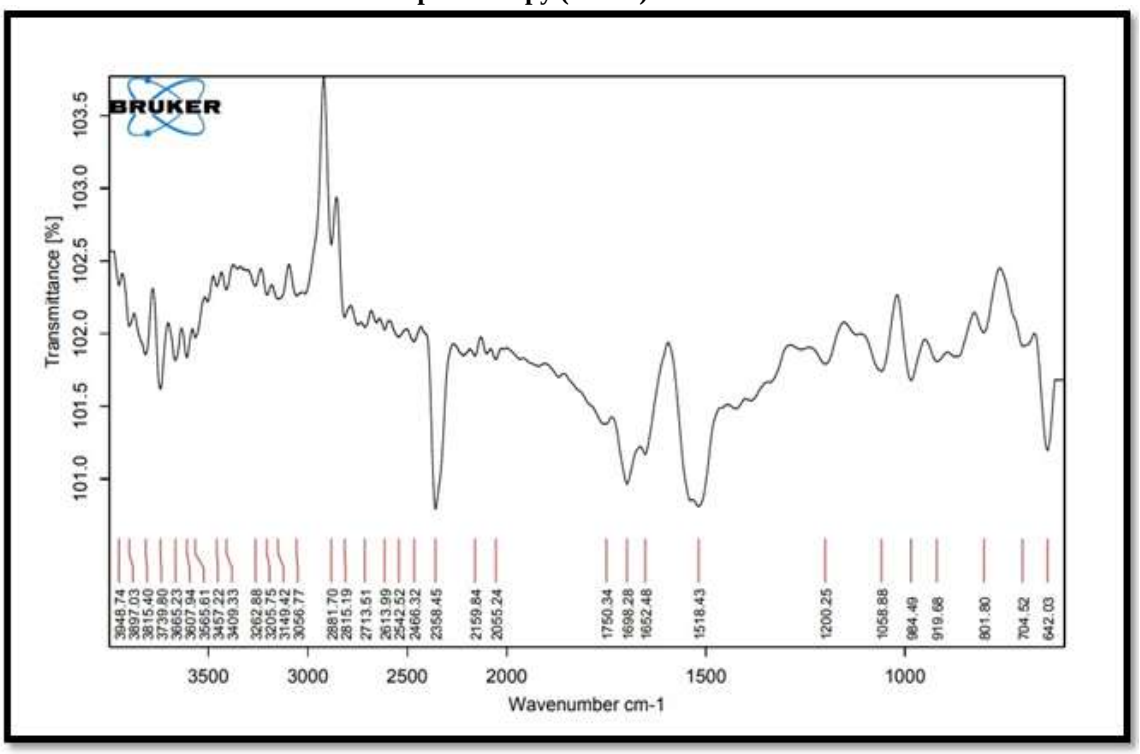
5. Fourier Transformed InfraredSpectroscopy (FT-IR)



FT-IR of 200mg PB-Ag-NPs

Peak (cm^{-1})	Groups
3739.80	**O-H** Bond
2881.70	**C-H** Bond
2358.45	**N-H/ C-O** Stretch
1698.28	**C=O** Bond
1200.25	**C-N/C=C** Bond

IR Interpretation OF 200mg PB-Ag-NPs

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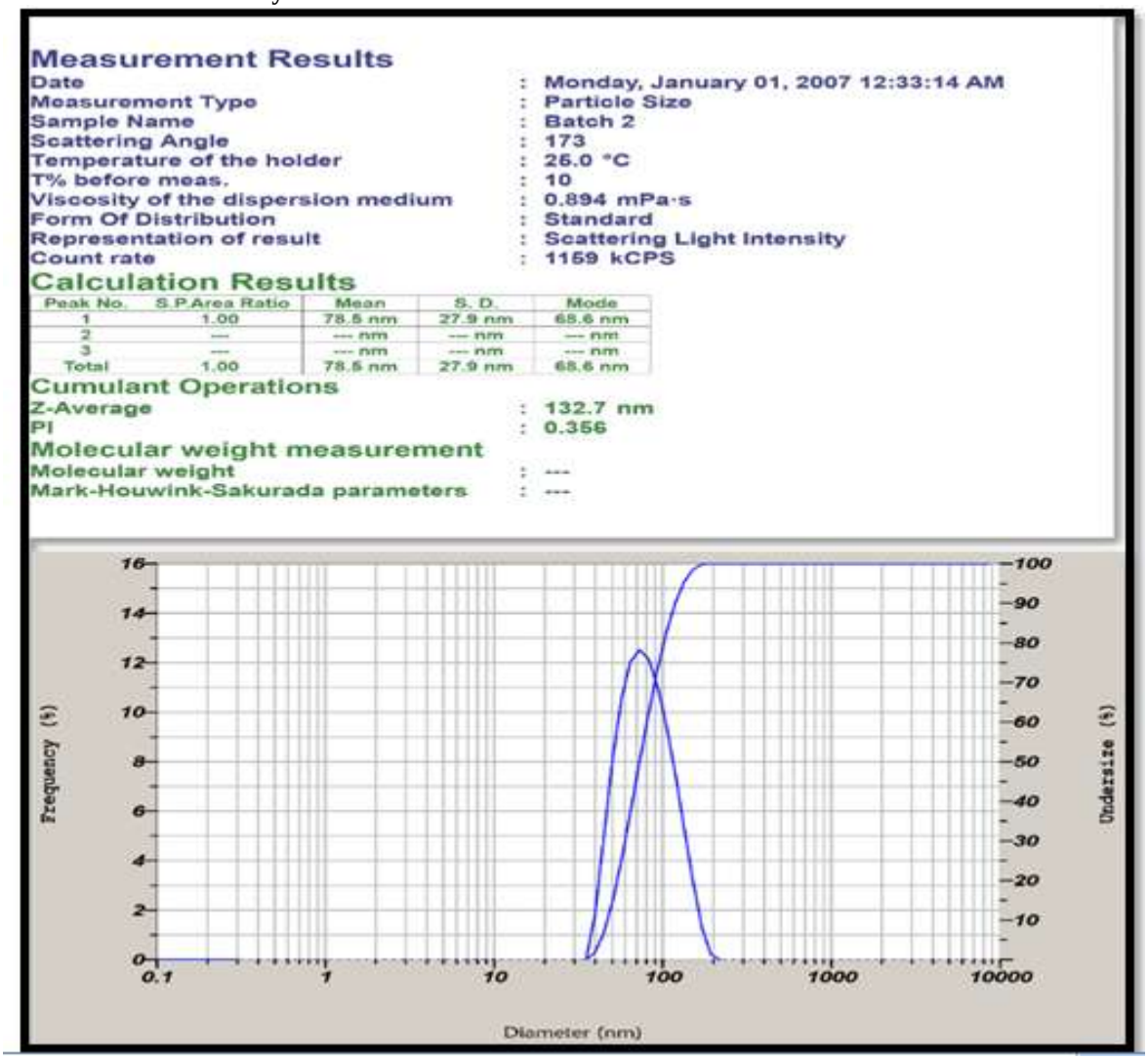
6. Particle size Analysis



Particle size of NPs

Particle size analysis of PB-Ag-NPs showed that the size distribution is within the nanometre range i.e., 1 to 100nm. The mean particle size of PB-Ag-NPs was found to be 78.5nm.

7. Zeta Potential

Nanoparticles are very small in size and are energetically very unstable. Therefore, the particles undergo agglomeration/ aggregation to stabilize themselves. The zeta potential analysis has direct relation with the stability of a form / structure as mentioned below. The zeta potential was found to be -31.4Mv i.e., Moderate stability.

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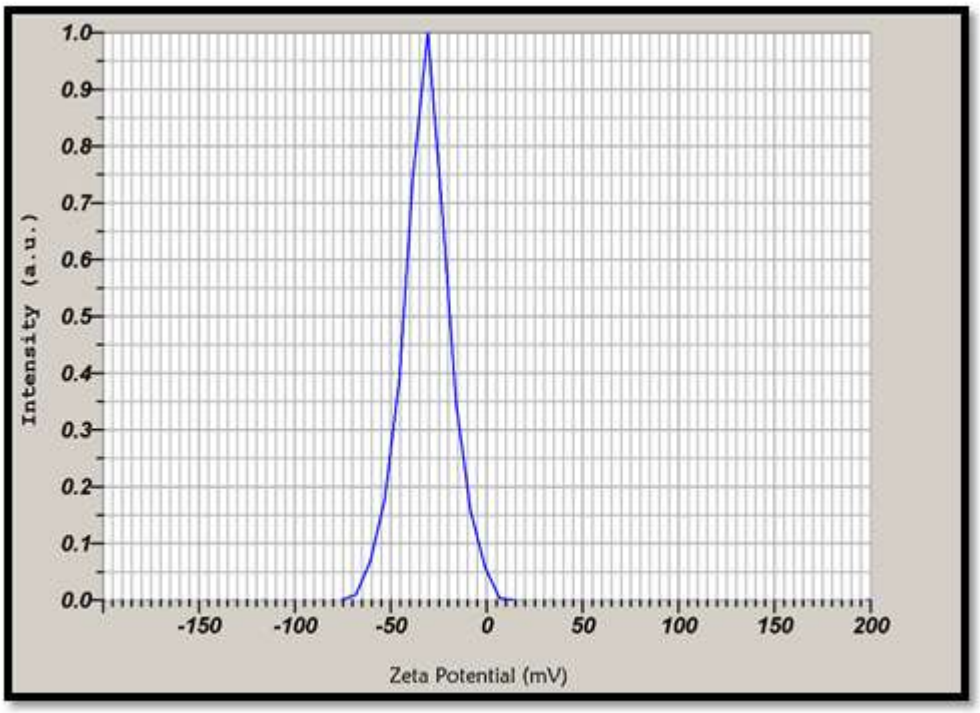

Zeta Potential of NPs

Zeta Potential (m V)	Stability behavior of the colloid
From 0 to ± 5	Rapid coagulation or flocculation
From ± 10 to ± 30	Incipient instability
From ± 30 to ± 40	Moderate stability
From ± 40 to ± 60	Good stability
More than ± 61	Excellent stability

Zeta potential and its relation with the stability

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8. In-Vitro activity of PB-Ag-NPs

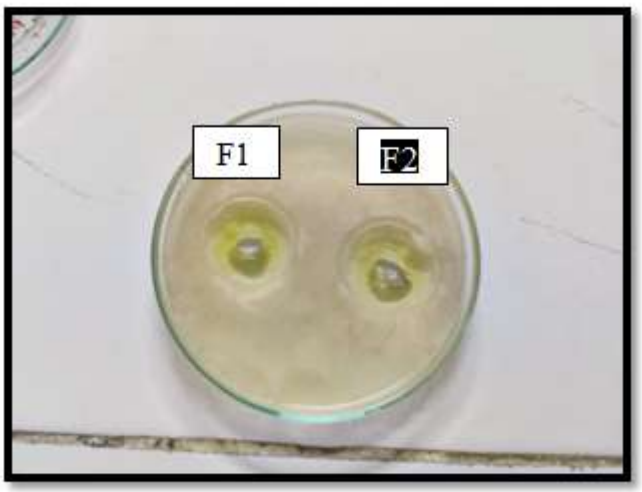
1. In-Vitro Antimicrobial activity

- **Zone of inhibition**

Antimicrobial activity was performed on Staphylococcus Aureus and as the production yield of F2 and F3 were high, antimicrobial activity was performed on these two formulations with standard one (extract), and the result obtained are shown in the table below.

Formulation	Zone of Inhibition (mm)
STD	12
F2 150 mg	16
F3 200 mg	20

Zone of inhibition



Antimicrobial activity

9. Stability studies

The synthesized PB-Ag-NPs (200mg) were stored at different conditions at room temperature and in the refrigerator for 2 weeks. The PB-Ag-NPs were then analyzed using UV-visible spectrophotometer. As there was no significant difference in the initial and final absorbance, it can be stored at any conditions.

10. Cyto-toxic activity on Brine Shrimp

Cytotoxic activity of the synthesized Ag-NPs was performed on A. salina nauplii. Different concentrations were prepared -10, 20, 30, 40, and 50 ug/ml. The lethality was found to be directly proportional to the concentrations. After 24 hours the count of living and dead A. salina nauplii was observed and LC50 value was calculated (%mortality). The LC_{50} value < 100 ug/ml is considered significant.

The result is shown in the following table.

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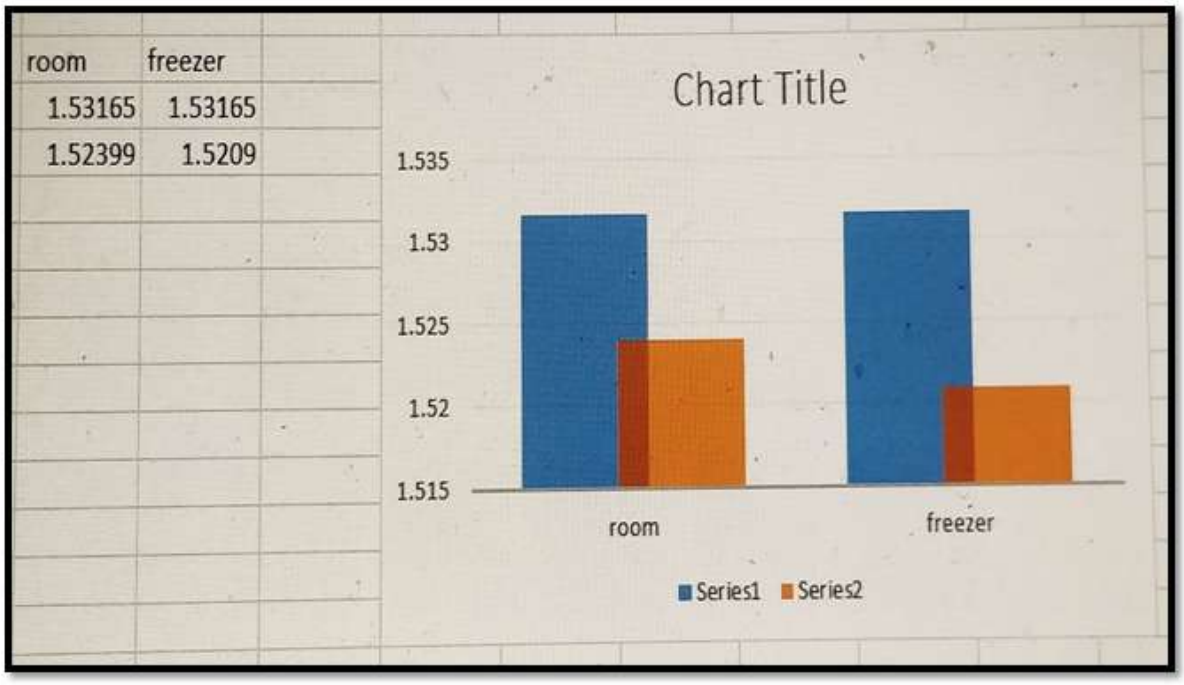
Sr.NO	Temp (°C)	Λ-max	Absorbance	
			Initial	Final
1	4	405	1.53165	1.52090
2	37	439	1.53165	1.52399



Stability studies

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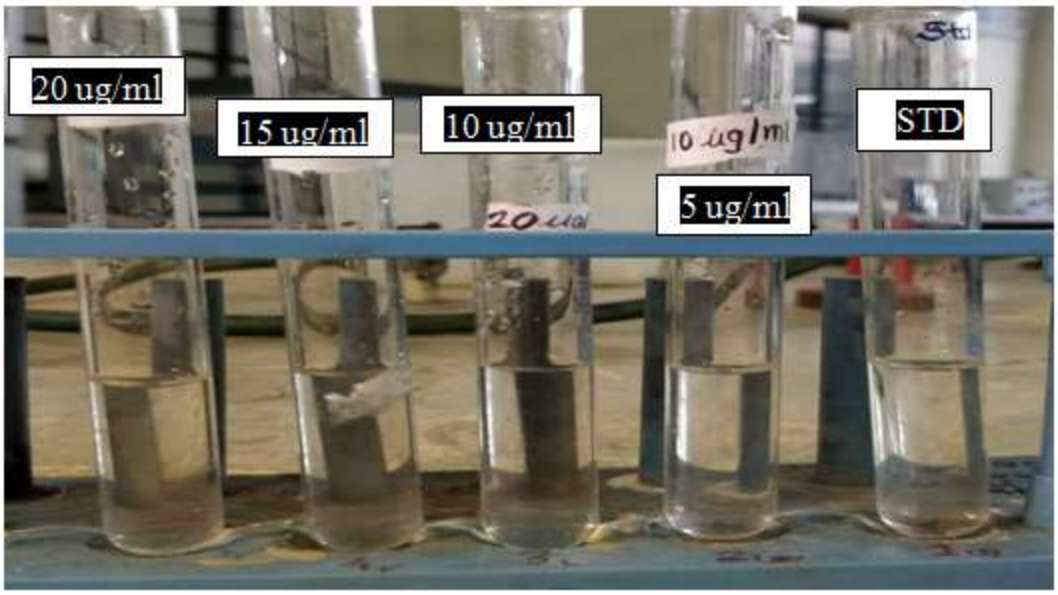
Test-tubes containing A. Salina brine shrimp and PB-Ag-NPs solution.

Concentration (ug/ml)	No. of living A. salina nauplii	No. of dead A. salina nauplii	% Mortality
5	7	3	33%
10	5	5	55%
15	2	8	88%
20	1	9	100%
STD	9	1	---

Formula:
% Mortality = no of dead nauplii/ no of live nauplii x100

RESULT -The lethality was found to be directly proportional to the concentration of PB-Ag-NPs; the number of deaths increased as concentration increased. Maximum mortality was observed at 20ug/ml concentration. 50%mortality was observed at 10ug/ml i.e., LC_{50} value is 8.44ug/ml that means 10ug/ml which indicates significant cytotoxic activity against A. salina.



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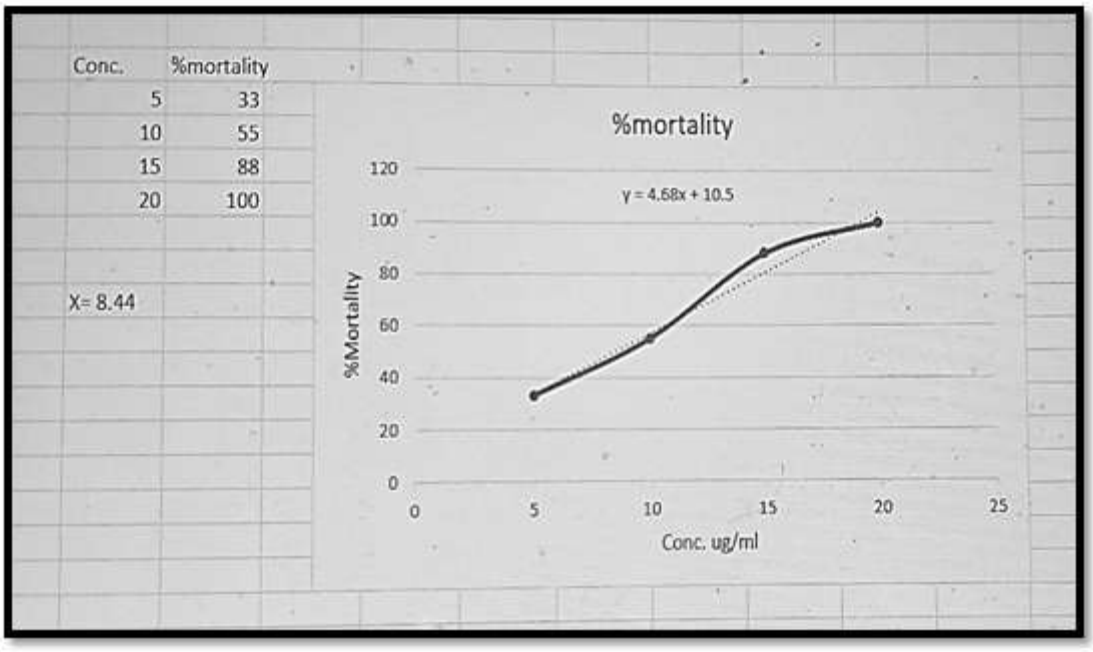
Cytotoxicity of certain compound does not always suggest its outright toxicity but may also suggest its potential anticancer activity



%Mortality rate

IV. CONCLUSION

The rapid biological synthesis of silver nanoparticles using Piper Betel leaves extract provides eco-friendly, simple and efficient route for synthesis of nanoparticles. The synthesized nanoparticles were spherical shaped having particle size between 70-100nm. The primary confirmatory for the silver nanoparticles was color change of solution from yellow to dark brown. The silver nanoparticles formed were confirmed by UV-visible spectroscopy, FT-IR, Particle size and zeta analysis. FT-IR spectrum of the nanoparticles suggested that Ag-NPs were surrounded by different organic molecules such as alcohols, ketones, aldehydes, terpenoids, and carboxylic acids. The zeta potential (surface potential) of PB-Ag-NPs was found to be -31 m V. The in-vitro anti-microbial activity was carried out on staphylococcus aureus. The PB-Ag-NPs at 200mg showed maximum zone of inhibition (20mm). In-vitro evaluation of stability of PB-Ag-NPs at different temperature was evaluated and was found to be stable at both 4°C and 37°C.The cytotoxic study was carried out on brine shrimp and the % mortality was observed and calculated.

REFERENCES

[1]. Mohanraj, V.J. & Chen, Y. (2007). Nanoparticles - A Review. Tropical Journal of Pharmaceutical Research. 5. 10.4314/tjpr. v5i1.14634

[2]. .Keat, C.L., Aziz, A., Eid, A.M. et al. Biosynthesis of nanoparticles and silver nanoparticles. Bioresour. Bioprocess. 2, 47 (2015). doi.org/10.1186/s40643-015-0076-2

[3]. Mittal AK, Chisti Y, Banerjee UC. Synthesis of metallic nanoparticles using plant extracts. Biotechnol Adv. 2013 Mar-Apr;31(2):346-56. doi: 10.1016/j.biotechadv.2013.01.003. Epub 2013 Jan 12. PMID: 23318667.

[4]. Xu L, Wang YY, Huang J, Chen CY, Wang ZX, Xie H. Silver nanoparticles: Synthesis, medical applications and biosafety. Theranostics. 2020 Jul 11;10(20):8996-9031. doi: 10.7150/thno.45413. PMID: 32802176; PMCID: PMC7415816.

[5]. Pradhan, D., Suri, K.A., Pradhan, D., &Biswasroy, P. (2013). Golden Heart of the Nature: Piper betle L. Journal of

Pharmacognosy and Phytochemistry, 1, 147-167.

[6]. Ponnuchamy, Kumar &Selvi, S. & Prabha, Lakshmi & Selvaraj, M & Rani, Lata &Palanisamy, Suganthi&Boominathan, Sarojini Devi &Munisamy, Govindaraju. (2012). Antibacterial activity and in-vitro cytotoxicity assay against brine shrimp using silver nanoparticles synthesized from Sargassum ilicifolium. Digest Journal of Nanomaterials and Biostructures. 7. 1447-1455.