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Biological synthesis of silver nanoparticles from *Mentha piperita* and evaluation of its antimicrobial activity

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Abstract- **The synthesis of metal nanoparticles through plant extract is an important aspect of current nanotechnology. Biosynthesis of silver nanoparticles was carried out by using Mentha piperita plant extracts for the reduction of aqueous silver ions in short period. The biosynthesized silver nanoparticles were characterized by UV and FTIR spectroscopy techniques. The formation of silver nanoparticles was confirmed by the colour change of the sample and a strong absorption peak at 438nm. The synthesized nanoparticles was evaluated for antimicrobial activities against bacteria such as P.aeroginosa, H.influenzae, S.aureus, S.pyogenes,and fungi like A.nigar, C.albicans.**

Keywords: Mentha piperita, silver nanoparticles, UV, FTIR, P.aeroginosa, H.influenzae, S.aureus, S.pyogenes, A.nigar, C.albican.

1. INTRODUCTION

Silver is of great choice in the field of biological systems, living organisms and medicine among the various noble metals[1]. Silver has been not only proven as an effective tool for retarding and preventing the bacterial infections but also they are found to exhibit wound healing activity. Colloid silver nanoparticles (AgNPs) exhibits distinct properties such as catalytic, antibacterial[2], good conductivity, and chemical stability. The investigations on AgNPs have attained importance due to their use in the field of opto-electronics, anti-microbial activity and silver-embedded fabrics are now used in sporting equipment. However, there is still need for economic, commercially viable as well as environmentally clean route of synthesis for AgNPs[3]. With respect to the microbes, silver nanoparticles get attached to the cell wall, thereby disturbing the permeability of cell wall and cellular respiration. The nanoparticles may also penetrate deep inside the cell wall, thus causing cellular damage by interacting with phosphorus and sulfur containing compounds, such as DNA and protein, present inside the cell. The bacteriocidal properties of silver nanoparticles are due to the release of silver ions from the particles, which confers the antimicrobial activity [4]. Besides, the potency of the antibacterial effects corresponds to the size of the nanoparticle. The smaller particles have higher antibacterial activities due to the equivalent silver mass content[5].Green synthesis of AgNPs by microorganisms and plant extracts as an alternative feasible synthesis technique which has gained much attention and application these days. Various metabolites existing in plants including sugars, alkaloids, phenolic acids, terpenoids, polyphenols, and proteins play an important role in the bioreduction of silver ions to silver nanoparticles[6]. Synthesis of silver nanoparticles can be achieved through different methods such as chemical and physical[7]. Although these two are currently the most popular, they present some disadvantages such as the use of large amounts of toxic chemicals, high energy requirements,high expense, and not being suitable for biological purposes[8]. For these reasons, there has been seen in recent years a growing need to start focusing on and developing more environmentally friendly processes of nanoparticle synthesis[9].

2. MATERIALS AND METHODS

2.1. Preparation of mentha piperita leaf extract

20 g of mentha piperita leaves were washed thoroughly with distilled water and dried for 24hrs at room temperature. The extract solution was prepared by boiling dried leaves in Erlenmeyer flask with 100 ml of distilled water for ten minutes at 100◦C. Freshly prepared aqueous extract was then used for synthesis of silver nano particles (Figure:1).

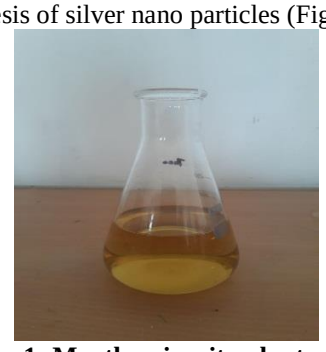

Figure 1: Mentha piperita plant extract

2.2. Preparation of Silver Nanoparticles

5 ml of fresh leaves extract was added to a conical flask containing 60 ml of 3 mM aqueous AgNO3 solution heated at 65ºC with continuous stirring. The silver ions were reduced to silver nanoparticles by mentha piperita leaves extract. The colour of the extract changed rapidly from greenish yellow to dark brown. (Figure: 2) Color change showed the positive test for synthesis of AgNPs.

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Figure 2 : Synthesized Silver nanoparticles

2.3. Characterizations of biosynthesized AgNPs:

2.3.1. Visual characterization:

Biosynthesis of AgNPs were confirmed by visual observations of the developed colour in the reaction mixture flask. The colour changed from pale yellow to brown was visually checked, which indicated the extracellular synthesis of AgNPs.

2.3.2. UV-visible spectroscopy analysis:

UV-Visible absorption spectra of the AgNP's were recorded on a JASCO variant 630 spectrometer. The colour change in reaction mixture (metal ion solution + leaf extract) was recorded through visual observation. The bio reduction of silver ions in aqueous solution was monitored by UV-visible spectrometry. UV spectrum were recorded between 200–900 nm wavelengths and the maximum range was recorded. All measurements were carried out at room temperature.

2.3.2. FTIR analysis:

FTIR spectra data is commonly used for identifying biomolecules responsible for the reduction of Ag ions and capping of the biosynthesized AgNPs. FT-IR spectra were recorded on a Thermo scientific Nicolet iS5ATR-iD1 Spectrometer. FTIR spectra of the aqueous leaf extract and AgNPs samples were analyzed by FTIR spectroscopy. The peaks corresponding to various functional groups were identified.

2.3.3. Phytochemical screening : The phytochemical analysis were carried out for the AgNPs as per standard methods described by Brain, Turner and Evan.

1. Detection of flavonoids:

Extract was treated with few drops of sulphuric acid. Formation of orange colour indicates the presence of flavonoids.

2. Detection of phenols:

2mL extract was treated with few drops of ferric chloride solution. Formation bluish black colour indicates the presence of phenol.

3. Detection of saponins:

Extract was shaken well with 5mL of distilled water. Formation of frothing indicates the presence of saponins.

4. Detection of tannins:

An aqueous solution of the leaf extract was heated on a water bath. Then the solution was filtered and a few ml of ferric chloride was added to it. An appearance of dark green colour indicates the presence of tannins.

5. Detection of Steroids:

Few ml of acetic anhydride and sulphuric acid was added to the leaf extract. The colour changes from violet to blue or green indicates the presence of steroids.

6. Detection of Carbohydrates:

To 1 ml of barfoed's reagent, add an equal volume of the plant extract. Boil for 5 minutes, in a water bath and allow to stand. Formation of brick red precipitate indicates the presence of carbohydrate.

7. Detection of Protein & Amino acids:

The extract was mixed with equal volume of NaOH and 1% copper sulphate. The appearance of violet colour indicates the presence of protein.

8. Detection of Oils and Resins:

The leaf extract was applied over the filter paper. If it develops a transparent appearance on the filter paper,it indicates that the presence of oils and resins.

2.3.4. Antimicrobial efficiency of biosynthesized AgNPs

The synthesized AgNPs obtained from the leaf extract of M.piperita was tested for its antimicrobial potent against P. areoginosa, Hemophilus influenza, S. Aureus, S. Pyogenes, A. Niger and C. Albicans. The antimicrobial activity were determined by the agar well diffusion method using Mueller Hinton agar medium . The agar plates were seeded with freshly prepared 4 bacterial and 2 fungal suspension. The AgNPs concentration was 50µl, 75µl, 100µl and 125µl for each well. The plates were incubated at 37°C for 24 hours, and the zone of inhibition (ZOI; mm) appearing around the wells was recorded.

2.4. Results and discussion

2.4.1. UV–visible spectroscopy

The synthesis of silver nanoparticle had been confirmed by UV-visible spectroscopy. The UV-visible spectrum showed distinct absorption peak at 438nm. This was due to the excitation of surface plasmon resonance (SPR) by AgNPs. AgNPs have free electrons, which give rise to SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with the light wave. Thus the reduction of AgNPs in the aqueous solution of silver complex during the reaction with the leaf extract of Mentha piperita was confirmed by the UV–visible spectra.(Figure:3)

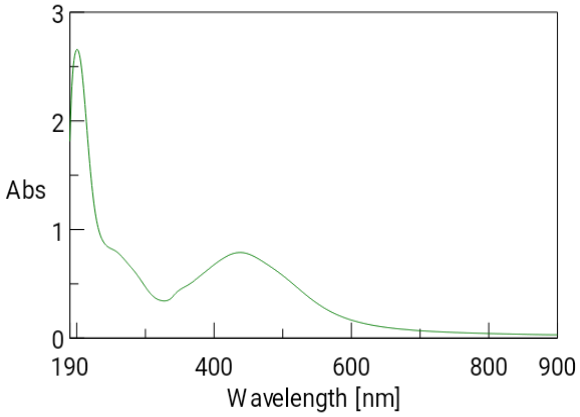

Figure:3 UV-Visible spectra of AgNPS

2.4.2. Fourier transform infrared spectroscopy:

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The aim of the FTIR analysis was to check the existence of functional groups. The FTIR analysis showed different stretches of bond as follows: It showed a broadband at 3287. 31cm^{-1} which was due to the stretching of -OH groups. A sharp absorption peak appeared at 1637.71cm^{-1} was assigned due to the bending mode of H_2O molecule present in the leaf extract. While a peak at 1442.57 cm^{-1} was related to CH_2bending mode. The peaks at 1397.63 cm^{-1}, 1279.35cm-1, 1146.33cm^{-1} was due to terminal CH_3, C-N, C-O groups, respectively. A peak at 1470.12cm-^{1} indicated the aromatic C=C stretching. Thus FTIR results confirmed the presence of $–CH_3$, –OH, C=C, C-O and CH groups, which indicated that the plant extract containing the hydroxyl and amine groups substituted flavonoids, saponins and tannins. (Figure: 4)

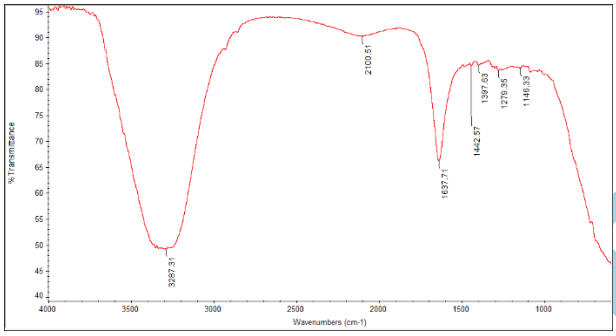

Figure:4 FTIR spectra of AgNPs

2.4.3. Phytochemical screening of AgNPs:

The present study was carried out on the M. Piperita revealed that the presence of few active phytochemical constituents. The phytochemical active compounds of M. Piperita were qualitative analysed and the results were mentioned in Table 1.

Table:1 Qualitative Phytochemical screening of M.piperita leaf extract

S.No.	Phytochemicals	*M.piperita* leaf extract
1	Flavanoids	Detected
2	Phenols	Detected
3	Saponins	Detected
4	Tannins	Detected
5	Steroids	Not detected
6	Carbohydrates	Not detected
7	Proteins & amino acids	Not detected
8	Oils & Resins	Not detected

2.4.4. Antimicrobial efficiency of biosynthesized AgNPs

The silver nanoparticles synthesized from M. Piperita exhibited the antibacterial activity against 4 bacterial strains such as P.aeroginosa, H.influenzae, S.aureus, S.pyogenes and 2 fungal strains like A.nigar, C.albicans (Table 1& 2) and the results were expressed as mean ± standard deviation (n=3).

Table : 1 Antibacterial activity of AgNPs

Bacterial pathogens	Concentration of AgNPs			
	50 µl	75 µl	100 µl	125 µl
	Zone of inhibition(mm)			
Pseudomonas aeroginosa	9±0.85	11±0.55	11±0.57	14±0.47
Hemophilus influenzae	6±0.91	11±0.51	13±1.1	14±0.72
Staphylococcus aureus	9±1.0	10±1.2	11±1.3	13±0.55
Streptococcus pyogenes	11±0.81	11±0.51	14±0.84	15±0.12

Table :2 Antifungal activity of AgNPs

Fungal pathogens	Concentration of AgNPs			
	50µl	75 µl	100 µl	125 µl
	Zone of inhibition(mm)			
Aspergilus niger	4±0.75	6±0.52	8±0.57	10±0.53
Candida albicans	6±0.36	7±0.79	9±1.0	12±0.66

From the above two tables the more concentrated AgNPs(125 µl)showed the maximum antibacterial activity against S.pyogenes (15± 0.12) and the less concentrated AgNPs (50 µl) showed the minimum antibacterial activity against H.influenzae (6± 0.91). Similarly the more concentrated AgNPs(125 µl)showed the maximum antifungal activity against C.albicans (12± 0.66).

2.5. CONCLUSION:

In conclusion, we report a green approach for the synthesis of silver nanoparticles using M. piperita leaf extract. This work offers a quick, simple and non-toxic method for the synthesis of silver nanoparticles without using any harmful reducing and stabilizing agent. The biosynthesis of AgNPs using M. piperita leaf extract results in the formation of nanoparticles. The leaf extract was found to possess flavonoids, phenols, tannins and saponins. From UV analysis, the formation of AgNPs was confirmed by the the maximum absorption at 438nm. The FTIR analysis showed the peaks for the presence of alcohol, Phenol O-H Stretch, Alkyl C-H Stretch, CH3 and C=C. IN FTIR analysis, the majority of bands showed the presence of characteristic functional groups such as water, alcohols, phenols,alkyl, alkene that were present in flavonoids, saponins and tannins. All of these phyto-constituents are greatly involved in the bioreduction and stabilization of the produced generate nanoparticles.

The nanoparticles were subjected to antibacterial and antifungal studies and were found to be effective against certain pathogens. Although there is no concrete information

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on the specific and exact mechanisms of the bactericidal actions of AgNP's against bacteria, several studies propose some possible mechanisms that may be responsible for the inhibitory effects that they present. One of the most accepted mechanisms is the release of highly toxic Ag+ ions, which are extremely attracted to the negatively charged cell wall of bacteria. It is well known that smaller particles exhibit stronger antibacterial effects against bacteria than do the bigger ones, because of the increase in their superficial area. This increase generates a larger contact surface which also allows higher Ag+ release, resulting in cell death. However, the loss in the effectiveness of antibacterial activity presented by the AgNP's synthesized using higher concentrations of extract against S. aureus (Table :1), could be due to the presence of larger nanoparticles at higher concentrations of the extract.

Consequently, it is concluded that AgNPs synthesized through this method could potentially be used in biomedical applications.

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