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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *BACOPA MONNIERI* LEAF EXTRACT AND ASSESSMENT OF THEIR DPPH ACTIVITY

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ABSTRACT

In this study, rapid, simple approach was applied for synthesis of silver nanoparticles using Bacopa monnieri leaf extract. The plant extract acts both as memory enhancer agent. To identify the compounds responsible for reduction of silver ions, the functional groups present in plant extract were investigated by FTIR. Various techniques used to characterize synthesized nanoparticles are DLS, and UVeVisible spectrophotometer. UVVisible spectrophotometer showed absorbance peak in range of 400-800nm. The silver Nanoparticles of Bacopa monnieri leaf extract showed *DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity*. Present study confirmed that it is a simple, rapid, one step, eco-friendly, nontoxic and alternative conventional physical/chemical methods, only 15 min were required for the conversion of silver ions into silver nanoparticles at room temperature, without the involvement of any hazardous chemical which shows prominent antioxidant property.

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INTRODUCTION:-

Nanotechnology is an emerging area in the field of science, with an increase in the development of new materials at the nanoscale level with the development of various sectors. Various chemical and physical methods are being invented for the production of silver nanoparticles, but most of the techniques involved are capital intensive. Silver nanoparticles (AgNPs) are assemblage of atoms that have sizes between 1- 100 nm. The prefix nano indicates a billionth of an mm or 10^{-9}m. The particle size of silver Nanoparticle is reduced when the relation between volume and surface increases. Silver nanoparticles are present in natural ecosystems [1].One of the important area of research in nanotechnology is the synthesis of silver Nanoparticles. Silver has long been endorsed as having an inhibition activity towards many bacterial strains and microorganisms. Antioxidant activity of the silver-materials containing Nanoparticles used in medicine to reduce oxidation of free radical which is mainly present in brain. Oxidative stress (OS) occurs when free radicals (chemical species with unpaired electrons, produced during normal metabolism) overcome the cell's homeostatic defense mechanisms [2]. Protective, free radical–quenching enzymes include superoxide dismutase, catalase, glutathione peroxidase (GPx), glutathione reductase (GSR), and others. Anti-oxidant compounds also play a key protective role, including vitamins A, C, E, and myriad phytonutrients (particularly phenols) [3-4]. OS plays a role in many diseases, even aging itself [5] , by degrading ligands, peroxidizing lipids, disrupting metabolic pathways, denaturing proteins, and breaking DNA strands [6]. Because of such wide range of applications, numerous synthetic methods have been developed.

Development of cost effective and eco friendly methods to synthesize nanomaterials seems to be a challenge for researchers [7-8]. Metal nanoparticles have grabbed the attention of researchers due to their unique as well as dynamic properties which do not exist in its bulk form[9-10]. In recent times, efficient nano silver materials are progressively used in diverse fields of biotechnology and medicine; having major focus of researchers over their biological applications [11--12]. Different types of nanoparticles available but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy [13]. The 'green' environment friendly processes in chemistry and chemical technologies are becoming increasingly popular and are much needed as a result of worldwide problems associated with environmental concerns. The Noble Silver Nanoparticles" are striving towards the edge-level utilities in every aspect of science and technology including the medical fields; thus cannot be neglected just because of their source of generation. Hence, it is becoming a responsibility to emphasize on an alternate as the synthetic route which is not only cost effective but should be environment friendly in parallel. Keeping in view of the aesthetic sense, the green syntheses are rendering themselves as key procedure and proving their potential at the top. Considering the vast potentiality of plants as sources this work aims to apply a biological green technique for the synthesis of silver nanoparticles as an alternative to conventional methods. A number of techniques are available for the syntheses of silver nanoparticles like ion sputtering, chemical reduction, sol gel, etc [14].

MATERIAL AND METHOD:

Preparation of Brahmi Extract

Leaves were thoroughly washed in running water to remove the dirt and dust on the surface of the leaves. Twenty g of finely chopped leaves were added to 100 ml of double-distilled water and boiled for 10 min. The extract was cooled and filtered and store for further use. This solution was used for green synthesis of silver nanoparticle (AgNP) or reducing the silver ions.

Preparation of 0.1 N $AgNO_3$ Solution:

16.98 gm of $AgNO_3$ was dissolved in 1000 ml of distilled water.

Preparation of 0.1N HCL Solution:

8.18 ml of HCL was dissolved in 1000ml of Distilled water.

Preparation of 0.1N KOH Solution:

6gm of KOH was dissolved in 1000ml of distilled water.

Preparation of Silver Nanoparticles:

1 gm of Pure Brahmi Extract + 100ml Water

↓

Boiled properly and maintained the temperature between 40-50^0 C

↓

Pipette out 5ml Brahmi Extract add 45ml Silver Nitrate Solution.

↓

Change in colour

↓

Maintain the P^H

↓

Dilute small amount of sample 20 times

↓

Effect of time studied by taking 5,15,25,35 & 45 min time interval

↓

Effect of P^H studied

↓

0.1N KOH and 0.1N HCL add to adjust the ph of solution.

↓

P^H range 8-12

↓

Centrifuged at 10000 rpm for 15 min

↓

Precipitate washed with distilled water

↓

Purified pellet was then dried at 60^{0}C

↓

Reduction of silver salt studied using FTIR

CHARACTERIZATION:-

Particle size Analyzer

The size of the nanoparticles was measured using a Malvern Zetasizer (nano ZS-90) equipped with a 4 mW, 633nm He-Ne Laser (U.K) at 25^{0}C under the fixed angle in disposable polystyrene cuvettes.

UV-visible spectroscopy analysis:

The change in color of the reaction mixture was recorded by visual observation. The reduction of silver nitrate to silver nanoparticles was analyzed by measuring UV-vis spectra of the solution. The absorption spectrum of the sample was taken in the range of 300-500 nm using Thermo scientific UV–vis spectrophotometer.

FT-IR measurement:

FTIR analysis was performed using Jasco FT/IR Model-4700system. Apply one drop or a small micro-spatula portion (15-20 mg) to fill the dwell 60-80% full . Apply the press to the sample. Use only two fingers to screw the press and measure the IR spectra

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity:

The free radical scavenging activity of plant juice extracts of *Brahmi* and *Guduchi*, was carried out using a method described by Yen GC *et al*. with slight modification. 100 µL of the DPPH solution (0.1 M in methanol) was added to 150 µL of different concentrations of *Brahmi* and *Guduchi* extract (10, 20, 30, 40 and 50 µg/mL). The mixture was shaken and incubated under dark for 30 min at room temperature. Absorbance was taken at 517 nm. The percentage inhibition was calculated by using an equation:

$$\% \text{ Inhibition} = [(A0-A1)/A0] \times 100$$

Where, A0 is absorbance of the control, A1 is absorbance of extracts/standard.
Control = Ascorbic Acid

RESULT:

Particle Size analyzer:

The particle size of Sliver nanoparticles of Bacopa monnieri was found to be 73.5nm under the Malvern Zetasizer (nano ZS-90).

UV-visible Spectrophotometric Analysis:

The UV-vis spectroscopy is one of the most widely used simple and sensitive technique for the analysis of nanoparticle synthesis. The color exhibited by the samples is due to the excitation of electrons of the transition metals which affects the absorbance in the ultraviolet region. The silver nanoparticles synthesized by Bacopa monnieri leaf extract were confirmed by UV-vis spectrophotometer, it showed the peak at 325.6 nm which corresponds to the absorbance of silver nanoparticles.

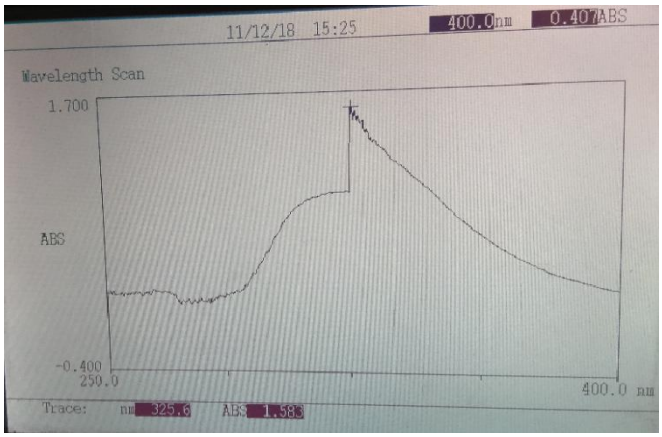

Figure 1: Graphical presentation of λ max of silver nanoparticles of Bacopa monnieri extract.

FTIR Analysis:

IR Spectra of pure Brahmi Extract.

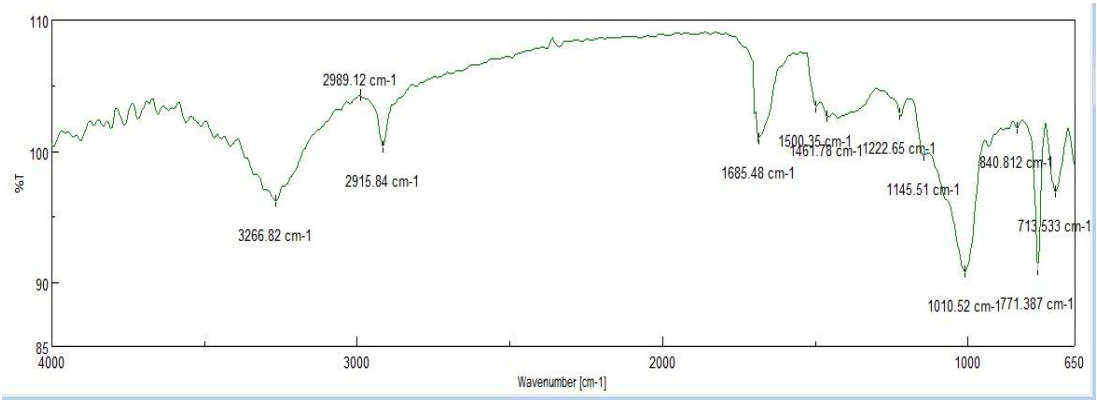

Figure 2: FTIR analysis of leaf extract of Bacopa monnieri. FTIR spectrum reveals prominent bands at 3266.82 cm-1, 2986.12 cm^{-1}, 2915.84 cm^{-1}, 1685.78 cm^{-1}, 1500.35 cm^{-1}, 1461.76 cm^{-1}, 1222.65 cm^{-1} and 1145.51 cm^{-1}, 1010.52 cm^{-1}, 840.812 cm^{-1}, 713.533 cm^{-1}.

IR Spectra of Silver Nanoparticles of Brahmi Extract:

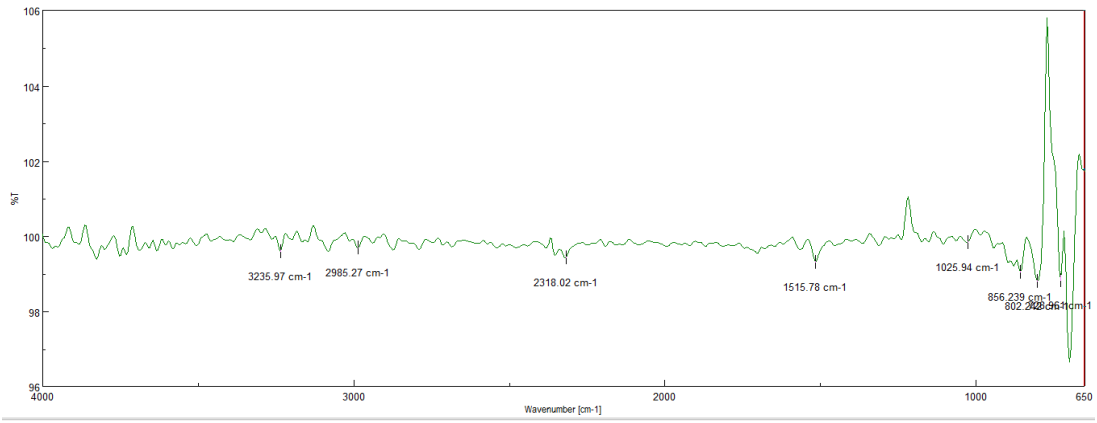

Figure 3: FTIR analysis of silver nanoparticles synthesized by using leaf extract of Bacopa monnieri.

It was found that the nanoparticles possessed definite surface morphology. FTIR spectrum reveals prominent bands at 3235.97 cm-1, 2985.27 cm^{-1}, 2318.02 cm^{-1}, 1515.78 cm^{-1}, 1025.96 cm^{-1}, 856.236 cm^{-1}, 802.242 cm^{-1} and 728.96 cm^{-1}. These groups might be responsible for synthesis and stabilization of generated silver nanoparticles.

DPPH Activity

Free radical scavenging activity of Silver nanoparticles of Bacopa monnieri extract Plant was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). In brief, 0.1 mm solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of Silver nanoparticles of Brahmi extract in ethanol at different concentration (10, 20,30,40,50 µg/ml). Here, only those extracts are used which are Solubilise in ethanol and their various concentrations were prepared by dilution method [15]. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm. by using spectrophotometer (UV-VIS Shimadzu)[16]. Reference standard compound being used was ascorbic acid and experiment was done in triplicate [17].The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity [18].The percent DPPH scavenging effect was calculated by using following equation: DPPH scavenging effect (%) or Percent inhibition = A0 - A 1 / A0 × 100.

Tab No 1:- DPPH scavenging effect (%) or Percent inhibition.

Concentration	Absorbance (nm)	% Inhibition
10 µg/ml	1.14	55.11
20 µg/ml	1.36	46.45
30 µg/ml	1.80	29.13
40 µg/ml	1.940	23.62
50 µg/ml	2.140	15.74

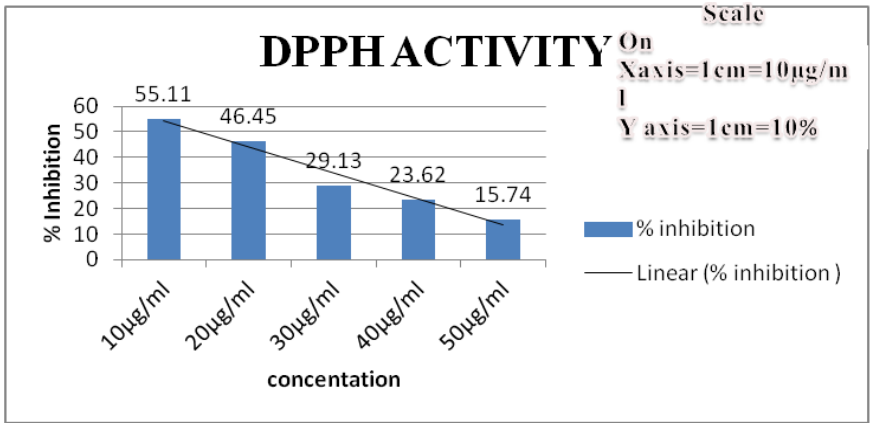

Figure 4: Graphical representation of dpph activity of Bacopa monnieri extract loaded silver nanoparticals.

DISCUSSION

The particle size of Sliver nanoparticles of Bacopa monnieri was found to be 73.5nm. it is been also confirmed with the help of spectroscopic characterization. These particles with differential concentration showed measurable dose dependent antioxidant activity.

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CONFLICT OF INTERESTS

We don't have conflict of interests.

ABBREVIATIONS

- % - Percent
- λ - maximum absorbance.
- P^H - Hydrogen ion concentration.
- AgNPs- Silver nanoparticles.
- OS - Oxidative stress.
- GPx - glutathione peroxidase.
- GSR - glutathione reductase.

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