

Green Synthesis and Characterization of Silver Nanoparticles (AgNPs) using Bacopa Monnieri leaf Extract

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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. Bacopa monnieri is an important medicinal plant which has been used in Ayurvedic system of medicine from very long period of time. It belongs to the Scrophulariaceae family and commonly known as Brahmi. The objective of this work is to prepare different crude extracts from the leaves of a medicinal plant, Bacopa monnieri through the maceration method and determine their total phenolics, flavonoids and other components and also the antioxidant activity by established methods. The crude extracts of Brahmi showed significant antioxidant activity; thus, these extracts could be used as natural antioxidants for the preparation of medicines to treat different diseases.*

Keywords: Silver Nanoparticle, Aqueous extract, Bacopa monnieri, Phytochemicals, UV-Visible

1. Introduction

Nanotechnology is an emerging area in the field of science, with an increase in the development of new materials at the nano scale level with the development of various sectors. Silver nanoparticles (AgNPs) are assemblage of atoms that have sizes between 1- 100 nm. The prefix nano indicates a billionth of an mm or 10⁻⁹m. The particle size of silver Nanoparticle is reduced when the relation between volume and surface increases. Silver nanoparticles are present in natural ecosystems.² One of the important area of research in nanotechnology is the synthesis of silver Nanoparticles. Oxidative stress (OS) occurs when free radicals (chemical species with unpaired electrons, produced during normal metabolism) overcome the cell's homeostatic defense mechanisms.³ 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).^{4, 5} OS plays a role in many diseases, even aging itself,⁶ by degrading ligands, peroxidizing lipids, disrupting metabolic pathways, denaturing proteins, and breaking DNA strands.⁷ Because of such wide range of applications, numerous synthetic methods have been developed. Development of cost effective and eco-friendly methods to synthesize nanomaterial's seems to be a challenge for researchers.^{8, 9} 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.^{10, 11} 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.^{12, 13} Different types of nanoparticles available but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy.¹⁴ 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. A number of techniques are available for the syntheses of silver nanoparticles like ion sputtering, chemical reduction, sol gel, etc.¹⁵



1.1 Pharmacological Activities of *Bacopa monnieri*

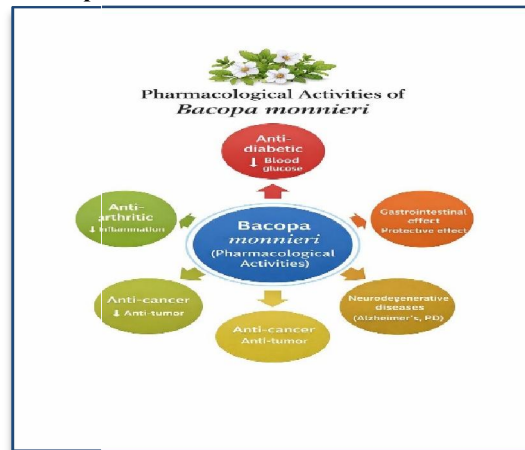


Fig 1:- Pharmacological Activities of *Bacopa monnieri*

A. Anti-cancer

Anticancer property of *B. monnieri* was demonstrated by Elangovan et al... It was found that *B. monnieri* induces dose and time dependent loss of cell viability. Effect of *B. monnieri* against the DNA damage in astrocytes and human fibroblast was also investigated. It was also found that a compound phenylethanoid glycoside of *B. monnieri* shows an inhibitory effect on human colon, breast and lung cancer.

B. Anti-arthritic

Several people suffer from the problem of rheumatoid arthritis. It was seen that the chances of rheumatoid arthritis in men is three times less than women, so women are more prone to this problem. Antiarrhythmic drugs are classified using the Vaughan Williams system, which categorizes them based on their mechanism of action to treat abnormal heart rhythms (arrhythmias).

C. Anti - Diabetic

Diabetes is a type of disorder which occurs due to the abnormal metabolic condition. It was reported that Anti-diabetic medications are designed to manage type 2 diabetes by reducing high blood sugar through various mechanisms, including enhancing insulin production, reducing hepatic glucose output, increasing sensitivity, and promoting sugar excretion.

D. Anti-oxidant

It was reported that *B. monnieri* has an antioxidant activity and anti-stress property. In rats morphine-induced liver and kidney toxicity were inhibited by *B. monnieri*. Also level of Superoxide dismutase increases when *B. monnieri* was used.

E. Gastrointestinal effect

Several studies showed that *B. monnieri* extract also had an effect on gastrointestinal tract. It was reported that *B. Monnieri* shows curative and protective effect on gastric ulcers due to its various mucosa offensive and defensive factors. *B. monnieri* releases pro-inflammatory mediator through which it inhibits inflammation.

□ Main Organs of Gastrointestinal System:-

- Mouth – starts digestion by chewing and saliva
- Esophagus – carries food to stomach
- Stomach – breaks down food using acid and enzymes

F. Neurodegenerative diseases

As *B. monnieri* is known for its treatment in memory loss, so it can be useful for even neurodegenerative diseases. A neurodegenerative disease is a condition in which the structure and function of neurons (brain cells) gradually



deteriorate over time. These diseases are usually progressive (they worsen slowly) and often cannot be completely cured, but their symptoms can be managed.

G. Parkinson's diseases

Parkinson's disease is a movement disorder in which aggregation of alpha synuclein protein and selective death of dopaminergic neurons occur and therefore in patients it leads to cognitive and motor impairment. In a study, it was found that pre-treatment with 50 µg/mL of *B. monnieri* significantly protected dopaminergic cell line against both 1-methyl-4-phenyl-pyridinium Iodide induced toxicity and Paraquat. *B. monnieri* also prevents the depletion of glutathione, preserved mitochondrial (MT) membrane potential and maintained MT complex activity.

H. Alzheimer's disease

B. monnieri also shows anti-Alzheimer activity. Alzheimer's disease is a neurodegenerative condition, which mostly affecting elder people. In vivo study of the effect of ethanol extract was done in a male albino Rat (225-250 g) where the oral dose of 100 mg/kg of body weight ethanolic extracts given for the fifteen days and it inhibits acetylcholinesterase activity in different brain regions According to Tembhre *B. monnieri* elicited acetylcholinesterase inhibition in cerebral cortex of rat and kinetic study showed that there was a competitive acetylcholinesterase inhibition in the brain regions. ^{16, 17}

1.2 Plant Profile:-

Bacopa monnieri has been used in Ayurvedic system of medicine from centuries and classified under medhyarasayana, i.e., medicinal plants which rejuvenate memory and intellect. It has been used by Ayurveda in India for almost 3000 years. It is a small perennial creeping herb which belongs to 'Scrophulariaceae' family that has 220 genera and 4500 species *Bacopa monnieri* has been used in Ayurvedic system of medicine from centuries and classified under medhyarasayana, i.e., medicinal plants which rejuvenate memory and intellect. It has been used by Ayurveda in India for almost 3000 years. It is a small perennial creeping herb which belongs to 'Scrophulariaceae' family that has 220 genera and 4500 species The name Brahmi is derived from the word 'Bramai' the mythical "creator" In the Hindu pantheon. Genus *Bacopa* includes more than 100 species of aquatic herbs. It is found in India, Nepal, Srilanka, Vietnam, China, Taiwan, Hawaii, Florida and some other Southern states of USA where it grows in wetland and muddy shores. ¹⁶



Fig 4:- *Bacopa monnieri*

- Scientific classification on *Bacopa monnieri* plant
- Kingdom: Plantae (Plants)
- Division: Magnoliophyta (Flowering plants)
- Class: Magnoliopsida (Dicotyledons)
- Family: Plantaginaceae (Plantain family)
- Species: *monnieri*
- Common Names: Brahmi, Water Hyssop



- Synonyms: *Bramia monnieri* (L.)
 - How to Herbal plant use on nanoparticles
- Herbal plants are used to make nanoparticles because they provide a green, safe, and eco-friendly method.
- Natural reducing agents: Plant extracts contain compounds like flavonoids, alkaloids, and phenols that help convert metal ions into nanoparticles.
 - Eco-friendly: No harmful chemicals are needed, so it is safe for the environment.
 - Cost-effective: Plants are easily available and inexpensive compared to chemical methods.
 - Benefits and Uses:
 - Eco-friendly & Cost-effective: No hazardous chemicals are used, making it safer and cheaper than traditional methods.
 - Applications: These plant-derived nanoparticles (e.g., from Tulsi, Neem) are utilized for antibacterial, antifungal, and antioxidant properties in drug delivery and medical treatments.

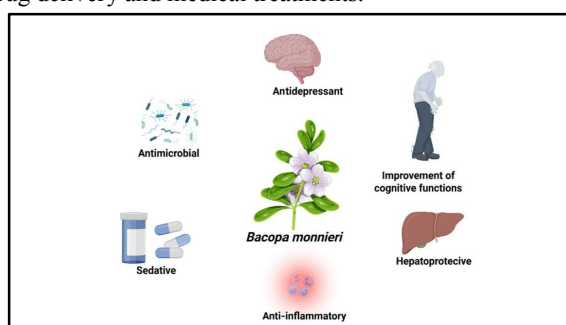


Fig 5:- Schematic representation of the main neuropharmacological effects of *Bacopa monnieri*.

Bacopa monnieri has traditionally been used to enhance memory, and this study aims to review the potential use of the plant in the treatment of Alzheimer's disease, Parkinson's disease, ADHD, and depression which differ in composition and concentration of active compounds.¹⁷

2. Material and Methods

- Chemicals
 - Silver Nitrate (AgNO_3)
 - Methanol
 - Ammonia solution
 - Mayer's reagent
 - Ferric Chloride
 - Sodium hydroxide
 - Ninhydrin reagent
 - Dil. Sulphuric acid
 - Sodium Nitroprusside
 - Distilled water

All the chemicals and reagents used were of analytical grade.

Collection of Plant Powder Material

The herbarium of *Bacopa monnieri* (L) was prepared and has been obtained from Botanical survey of India. The whole *Bacopa Monnieri* (L) plant material was shade dried at room temperature and kept in oven at 40°C to remove moisture. The dried plant was then finely ground by mechanical grinder. The powder obtained was then sieved and kept in air tight containers for further extraction process.¹⁸



Extraction process of Bacopa Monnieri leaf extract

Extraction of Bacopa monnieri (L)

The extracts of Bacopa monnieri (L) were prepared by Traditional maceration method. 250gm of Bacopa monnieri (L) dry plant powder was dissolved in methanol. Each mixture was kept separately for 72 hrs at room temperature with occasional shaking. After completion of the maceration, the supernatant was decanted and the mixture was filtered. The extract was concentrated to dryness by keeping filtrate for complete evaporation of the solvent. To calculate the amount of dry matter in the Aqueous extract, the bacopa monnieri aqueous extract earlier prepared as above (100ml) of the filtrate was evaporated on water bath (30°C) over 24 hours, and 15gm of dry extract was obtained.¹⁸.



a. Maceration process



b. Filtration process



c. Evaporation process



d. Leaf Extract on bacopa monnieri

Fig 6:- Extraction process of Bacopa monnieri leaf

Preparation of silver nano particle

Synthesis of silver nano particle: 5 mg of bacopa monnieri aqueous extract was taken in a beaker and paced on a magnetic stirrer with hot plate. Then this 50 ml of 1 mM silver nitrate solution was added drop wise with constant stirring at 120 rpm. The colour change of the solution was checked periodically.



Fig 7:- Preparation of silver nano particle



Separation of silver nano particle

The nano particle were separated by centrifugation using REMI centrifuge at 500 rpm for 15 minute. Then pellets were collected and store.¹⁹



Fig 8:- Separation of silver nano particles

Phytochemical Screening Test of Extract:-

1. Tannin Test: In a beaker 1 gm of leaf powdered samples were taken and 1 ml of ferric chloride was added. A brownish-black color observed, indicating the presence of tannin.
2. Amino Acid Test: In 1 ml of each crude extract 2-3 drops of ninhydrin reagent was added. A purple color produced, indicating positive results.
3. Anthraquinones Test: 1 ml of each crude extract was taken in a test tube and hydrolyzed by diluted sulphuric acid; it was then extracted with benzene. Then a dilute ammonia solution was added to the benzene layer. The formation of pink color indicates the presence of anthraquinones.
4. Cardiac Glycosides (Legal's Test): To the 3 ml of extract 1 ml pyridine was added by frequent shaking followed by 1 ml sodium nitroprusside. Pink to red color appeared. It indicates the presence of cardiac glycosides.
5. Alkaloids Test: In a beaker containing 3ml of ammonia solution 1 gm of powdered samples were added and kept for 5 min. Then to the beaker 10 ml of chloroform was added and mixed. It was then filtered to remove the solid particles. The filtrate was then evaporated using a water bath, then 2 ml of Mayer's reagent was added to it. A cream-colored precipitate appeared, indicating the presence of alkaloids.
6. Saponin Test: To every 1 ml of crude extract 20 ml of distilled water was added. Then it was stirred for a few minutes. The formation of foam indicating the presence of saponin.^{20, 21, 22, 23}

Characterization of AgNPs:-

UV-visible spectroscopy analysis:

The change in colour 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 336.0 nm using Thermo scientific UV-vis spectrophotometer. UV-Visible Spectroscopy (UV-Vis) is the most critical visual tool used in the green synthesis of nanoparticles. It serves as the primary "optical fingerprint" to prove that metal ions have successfully transformed into solid nanomaterials.¹

Visualization analysis:

Visualization test means observing the size, shape, and structure of nanoparticles using advanced microscopes. It confirms that silver nanoparticles (AgNPs) are properly formed. Which are usually spherical and 10–100 nm in size. Visualization analysis in the green synthesis of nanoparticles refers to the systematic interpretation of the images



and graphical spectra gathered during characterization. Researchers look at specific visual cues, peaks, and shapes on these charts to evaluate if the process worked and to measure the properties of the resulting materials.¹

3. Result

Screening Test Result:-

The Brahmi leaf samples collected from the EKW area and extracted by the maceration method with methanol and the semisolid masses were obtained. This crude extract was extracted with hexane, chloroform, ethyl acetate, butanol, methanol, and distilled water shown in Table 1.

Table 1: Total Amount Of N-Hexane, Ethyl Acetate, Chloroform, Butanol, Methanol And Water Crude Extract From Bacopa Monnieri Leaves

Extract	Amount of crude leaves (gm)
N-Hexane	0.21
Ethyl acetate	0.22
Chloroform	2.43
Butanol	2.26
Methanol	10.26
Water	2.33

Table 1:-Total amount of crude extract

The crude extracts from Brahmi leaf were used for different biochemical studies. The screening was performed according to previously described methods. The results showed that alkaloids, steroids, flavonoids, and saponins were present in all polarities of crude leaf extracts.

However, tannin is present in all polarities except n-hexane. Amino acids, anthraquinone, and triterpenoids were absent in all polarities shown in Table 2.

Table 2: Phytochemical Analysis Of N-Hexane, Ethyl Acetate, Chloroform, Butanol, Methanol And Water Crude Extracts From Bacopa Monnieri Leaves.

+ = presence; - = absence

Secondary metabolites	Inference					
	N-Hexane	Ethyl acetate	Chloroform	Butanol	Methanol	Water
Alkaloids	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Tannin	-	+	+	+	+	+
Amino acids	-	-	-	-	-	-
Anthraquinone	-	-	-	-	-	-
Triterpenoids	-	-	-	-	-	-

Table2:-Phytochemical Analysis



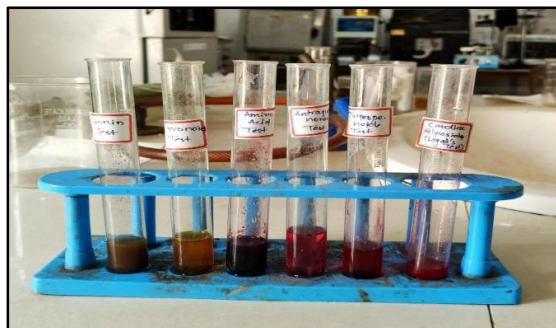


Fig 9:- Phytochemical Screening Test

Characterization Result:-

• 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 336.0 nm which corresponds to the absorbance of silver nanoparticles.

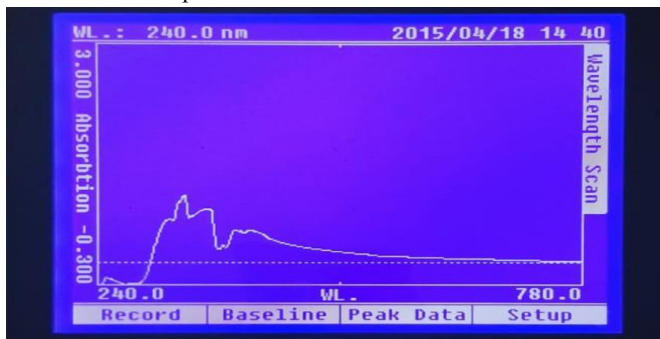


Fig10:- Graphical presentation of λ max of silver nanoparticles of Bacopa monnierextract.

• Visualization Result

Visualisation results for green synthesis rely heavily on the type of metal being reduced. Because silver and gold are the two most common and highly researched metallic nanoparticles synthesized via green routes, their physical results are highly predictable. A direct comparison of their standard visual and physical outcomes is detailed below:

Property Silver nanoparticles (AgNPs)

Visual colour Shifts from clear/light yellow to dark brown

Dominant Shape Spherical is most common, but also triangular (Microscopic)

Property	Silver nanoparticles (AgNPs)
Visual colour	Shifts from clear/light yellow to dark brown
Dominant Shape	Spherical is most common, but also triangular (Microscopic)

Table 3:- Result of Visualization

4. DISCUSSION

The Herbal Plant (Bacopa monnieri) Also known as Brahmi, Bacopa monnieri is a traditional, creeping, herbaceous plant widely utilized in Ayurvedic medicine for its neuroprotective and cognitive-enhancing properties.Extraction



Process The healthy leaves are thoroughly washed, shade-dried, and pulverized into a fine powder. The powdered leaves are then extracted using a solvent (such as aqueous/water or ethanol) via boiling or continuous magnetic stirring. The resulting mixture is filtered to obtain a clear, concentrated leaf extract, which is kept in a cool environment for subsequent nanoparticle fabrication. Screening Test of Extract Before synthesis, the extract undergoes preliminary phytochemical screening to confirm the presence of beneficial secondary metabolites. Characterization of AgNPs to confirm the successful synthesis and structural properties of the *Bacopa monnieri*-mediated AgNPs (BM-AgNPs), several analytical techniques are used: UV-Vis Spectroscopy: typically showing a distinct absorbance peak between 336.0 nm, confirming nanoparticle formation. The visual color change of the reaction mixture from pale yellow to dark brown indicates the successful bio-reduction of silver ions. Characterization results consistently demonstrate that the plant's biomolecules act as excellent stabilizing and capping agents, preventing unwanted aggregation. Furthermore, the biosynthesized AgNPs exhibit exceptional biological efficacy.

5. Conclusion

The Herbal Plant (*Bacopa monnieri*) *Bacopa monnieri*, commonly known as Brahmi, is a perennial herb renowned in traditional medicine for its cognitive-enhancing properties. The leaves are rich in bioactive compounds like bacosides, polyphenols, and flavonoids. These phytochemicals serve as natural reducing and stabilizing agents, making the plant an ideal "green" factory for producing silver nanoparticles without the need for toxic chemicals. Extraction and Screening Process the process begins by washing and drying the leaves, followed by boiling them in deionized water to create an aqueous extract. Phytochemical screening of this extract confirms the presence of alkaloids, tannins, and saponins. These specific metabolites are crucial because they provide the electrons necessary to reduce silver nitrate (AgNO_3) into metallic silver nanoparticles (Ag^0). Characterization of AgNPs Successful synthesis is confirmed through a battery of tests. UV-Vis spectroscopy typically reveals a sharp absorption peak around 336.0 nm, indicating the formation of nanoparticles. In conclusion, the green synthesis of silver nanoparticles using *Bacopa monnieri* is a cost-effective, eco-friendly, and rapid method.

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