The Pharma Innovation Journal 2023; 12(4): 1298-1302

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(4): 1298-1302

www.thepharmajournal.com
Received: 02-02-2023
Accepted: 04-03-2023

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Bio synthesis of silver nanoparticle using plant extracts

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Abstract
This Experiment was carried out in Department of Biotechnology at Mahatma Gandhi Mission College of Agricultural Biotechnology, Gandheli. The experiment was carried out in year 2014-15. The objective of the study was to establish suitable protocol for synthesis of silver nanoparticles using biological method. Different plant extracts were prepared and tested for their ability to synthesis nanoparticles. Biological mean of nanoparticle synthesis is easy, cost effective and eco-friendly. The experiment was laid out in factorial randomized block design (FRBD) with 65 treatment combinations of 13 different plants and 5 concentration of silver nitrate solution (1 mM, 2 mM, 3 mM, 4 mM and 5 mM). Each plant extract was added to different concentrations of silver nitrate solution in separate test tube. Formation of silver nanoparticles were primarily confirmed by color change followed by UV-VIS spectrophotometer. Among all the 65 treatments 13 were found positive for synthesis of silver nanoparticles. The UV-VIS readings are referred with the previous research paper readings. This study clearly indicate that synthesis silver nanoparticle by using plant extract is easily possible.

Keywords: Nanoparticle, plant extract, silver nitrate, UV-VIS spectrophotometer

1. Introduction
Nanoparticles (Nano-scale particles=NSPs) are atomic or molecular aggregates with at least one dimension between1 and 100 nm (Ball *et al*., 2002) [4], that can drastically modify their physico-chemical properties compared to the bulk material (Nel *et al*., 2006) [22]. Nanoparticles can be made from a fully variety of bulk materials and that they can explicate their actions depending on both the chemical composition and on the size and/or shape of the particles (Brunner *et al*., 2006) [6]. Because of its smaller structure, they trigger the chemical activity due to their distinctive crystallographic nature that increases surface area, hence the scope of reactivity (Osaka *et al*., 2006) [25]. The advance technology accepts that the concept of interdisciplinary research in the areas of engineering and sciences leads to creation of environmentally acceptable "green process", with special concern to nano-science and nanotechnology. In nano-biotechnology, silver nanoparticles are the most promising one. Silver nanoparticles are nanoparticles of silver, i.e. silver particles size in range of between 1 nm and 100 and because of its Nano size it has attracted intensive research interest. It is observed that silver nanoparticles do not affect living cells, so not able to provoke microbial resistance. It is believed that Silver nanoparticles can attach to the cell wall and disturb cell-wall permeability and cellular respiration (Singh *et al*., 2008) [32]. Silver containing particles also used in textile fabrics, as food additives, and in package and plastics to eliminate microorganisms. Because of such a wide range of applications, various methods concerning the fabrication of silver nanoparticles, as well as various silver-based compounds containing metallic silver (Ag0) have been developed (David *et al*., 2010) [36]. The special attention towards the silver nanoparticles because of their strong antimicrobial activity either in metallic nature and nanoparticles form also, so it is found that silver nanoparticles has different applications to the environment and human. It has been well studied that a variety of biological sources are able to produce silver nanoparticles of different shapes and nature. Nanoparticle production and applications have been extensively studied; studies related to drug delivery, tissue engineering have been undertaken for a great number of scientific publications and patents. Sometimes the synthesis of nanoparticles using various plants and their extracts can be advantageous over other biological synthesis processes which involve the very complex procedures of maintaining microbial cultures (Sastry *et al*., 2003) [30].
Many such experiments have already been started such as the synthesis of various metal nanoparticles using fungi like *Fusarium oxysporum* (Nelson *et al*., 2005) [23], *Penicillium* sp.

(Hemanth *et al*., 2010) [12] and using some bacteria such as *Bacillus subtilis* etc. There has also been several experiments performed on the synthesis of silver nanoparticles using medicinal plants such as *Oryza sativa* (Rice), *Helianthus annus* (Sunflower), *Saccharum officenarum* (Sugarcane), *Sorghum bicolour* (Jawar), *Zea mays* (Maize), *Aloe vera* (Korphad),*Capsicum annuum* (Chilli) and *Medicago sativa* (Alfalfa) in the field of pharmaceutical applications and biological industries (Prasad, 2014) [27]. synthesis of nanoparticles using plant extracts is the most adopted method of green, eco-friendly production of Nanoparticles and also has a special advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites. In the context of global efforts to reduce hazardous waste, the continuously increasing demand of nano-materials must be accompanied by green synthesis methods. Synthesis of nanoparticles by chemical route is much expensive tedious process. But synthesis by biological route is easy and needs more exploration.

2. Materials and Method

2.1 Experimental Site

The experiment was conducted in Biochemistry and Molecular Biology Laboratory of MGM College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra during year 2014 -15

2.2. Experimental Details

The experiment was carried out in Factorial Randomized Block Design (FRBD) Model with 65 treatment (13 medicinal plant extract in combination with 5 different concentrations of silver nitrate solution.) Three replications of each treatment were carried out.

2.2.1 Concentrations of $AgNO_3$

Table 1: Concentrations of silver nitrate ($AgNO_3$) were used for treatment

Sr. No.	Symbol	Concentrations of $AgNO_3$
1	A_1	1 mM
2	A_2	2 mM
3	A_3	3 mM
4	A_4	4 mM
5	A_5	5mM

Table 2: Aqueous leaf extracts of following plant were used

Sr. No.	Symbol	Plants
1	P_1	Adulsa (*Justicia adhatoda*)
2	P_2	Bamboo (*Bambuseae*)
3	P_3	Bramhi (*Bacopa monnieri*)
4	P_4	Durva (*Cynodon dactylon*)
5	P_5	Gavatichaha (*Cybopogon flexuous*)
6	P_6	Karanj (*Millettia pinnata*)
7	P_7	Kusalgrass (*Heteropogon contortus*)
8	P_8	Neem (*Azadirachta indica*)
9	P_9	Panfuti (*Bryophyllum pinnatum*)
10	P_{10}	Pipal (*Ficus religiosa*)
11	P_{11}	Rantulas (*Ocimum album*)
12	P_{12}	Samudrashosh (*Argyreia nervosa*)
13	P_{13}	Vad (*Ficus benghalensis*)

2.3 Collection of plant material

The leaves of the following plants were used for the preparation of silver nanoparticles. These plants were *viz*. Adulsa (*Justicia adhatoda*), Bamboo (*Bambuseae*), Bramhi (*Bacopa monnieri*), *Durva* (*Cynodon dactylon*), Gavatichaha (*Cybopogon flexuous*), Karanj (*Millettia pinnata*), Kusalgrass (*Heteropogon contortus*), Neem (*Azadirachta indica*),Panfuti (*Bryophyllum pinnatum*), Pipal (*Ficus religiosa*), Rantulas (*Ocimum album*), Samudrashosh (*Argyreia nervosa*) and Vad (*Ficus benghalensis*). The fresh leaves of above plants were identified and collected from the areas of Aurangabad and some of the plant leaves were obtained from Lords Nursery, Aurangabad.

2.4. Preparation of Leaf extracts

Plant leaves were rinsed thoroughly first with tap water followed by distilled water to remove all dust and visible particles. Plant leaves were cut into small pieces and dried at room temperature. About 10 g of these finely incised leaves of each plant was weighed separately and transferred into 250 ml beakers containing 100 ml distilled water and boiled for 15 to 20 m at 100 °C. The extract was then filtered thrice through Whatmann No.1 filter paper (0.45 um). Leaf extracts were stored at 4 °C (Kouvaris *et al*., 2012) [16].

2.5. Synthesis of Silver nanoparticles

1 ml of each plant extract was added to the 9 ml of 1 Mm, 2Mm, 3Mm, 4Mm, 5Mm aqueous solution of $AgNo_3$. Silver nitrate of analytical grade was used from Qualigen Company. Then the sample was incubated in dark for 24 h. After 24 h, the sample was measured for its maximum absorbance using UV-Visible spectrophotometry (Saikia *et al*., 2014) [29].

2.6 Characterization of nanoparticles

The synthesized silver nanoparticles were analyzed and confirmed by the UV-VIS Spectrometer. Ultraviolet-visible analysis was done by using PC Based UV-Probe software on Shimandzu UV-VIS Spectrophotometer 1800 series with resolution of 1nm between 300 to 600 nm. Reduction of Ag+ ions was monitored by measuring UV-Vis spectrum of mixture. Reduction of AgNO3 to Ag+ was confirmed by Colour change from colourless to brown. Formation of silver nanoparticles is easily detected by measuring the optical density of solutions/suspensions. The UV- VIS spectra of solution was recorded using Scimatzu spectrophotometer model UV-1800 (Dwivedi, 2013) [8].

3. Results and Discussion

3.1. Characterization of silver nanoparticles

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Gavhane *et al*., 2012) [9]. When the leaf extract was mixed in the aqueous solution of silver ion complex, the results obtained are given below in table no. These results are based on color change and U.V absorbance.

Table 3: Positive results obtained are indicated by "+" sign showing formation of nano particals

Sr. No.	Plants	1mM	2mM	3mM	4mM	5mM
1	Adulsa (*Justicia adhatoda*)			+		
2	Bamboo (*Bambuseae*)	+				
3	Bramhi (*Bacopa monnieri*)			+		
4	Durva (*Cynodon dactylon*)	+				
5	Gavatichaha (*Cybopogon flexuous*)					+
6	Karanj (*Millettia pinnata*)		+			
7	Kusalgrass (*Heteropogon contortus*)		+			
8	Neem (*Azadirachta indica*)			+		
9	Panfuti (*Bryophyllum pinnatum*)				+	
10	Pipal (*Ficus religiosa*)					+
11	Rantulas (*Ocimum album*)		+			
12	Samudrashosh(*Argyreia nervosa*)				+	
13	Vad (*Ficus benghalensis*)	+				

Change in color was visually observed in the silver nitrate solution incubated with aqueous leaf extract of above given plants. The bio reduction of precursor silver ions was monitored by sampling of aliquots (2ml). Absorption measurements were carried out on UV-VIS Spectrophotometer at a resolution of 1 nm. The scanning range for the sample was 200–800 nm.

Table 4: Solution showing colour change after addition of plant extract

Sr.no.	Plant leaves extract sample	Color change observed	Time required for color change (Approximate)
1	Adulsa 3 mM AgNP	Yellow to dark brown	1 h
2	Bamboo 1 mM AgNP	Colorless to dark brown	2 m
3	Bramhi 3 mM AgNP	Colorless to dark brown	6 m
4	Durva 1 mM AgNP	Light brownish to dark brown	10 m
5	Gavati 5 Mm AgNP	Yellow to dark brown	10 m
6	Karanj 2 mM AgNP	Light brownish to dark brown	18 m
7	Kusalgrass 2 mM AgNP	Light brownish to dark brown	16 m
8	Neem 3 mM AgNP	Light brownish to dark brown	9 m
9	Panfuti 4 mM AgNP	Colorless to dark brown	10 m
10	Pipal 5 mM AgNP	Light brownish to dark brown	3 m
11	Rantulas 2 mM AgNP	Light brownish to dark brown	1 m
12	Samudrashosh 4 mM AgNP	Light brownish to dark brown	3 m
13	Vad 1 mM AgNP	Light brownish to dark brown	2 m

Based on the absorbance readings taken on UV-VIS spectrophotometer the peaks were matched with the previous studies done on relevant to the silver nanoparticle preparations which are synthesized from plant leaves extracts. Perfectly matched peaks with references were given below in Table No.5

Table 5: Peaks of nanoparticle solution by UV-VIS spectrophotometer

Sr. no	Plant leaves extract sample	Matched Absorbance peaks (nm)
1	Adulsa 3 mM AgNP	609 (Devi *et al*., 2013) [7]
2	Bamboo 1 mM AgNP	461 (Banerjee *et al*., 2014) [5]
3	Bramhi 3 mM AgNP	453 (Devi *et al*., 2013) [7]
4	Gavatichaha 5 Mm AgNP	425 (Baishya *et al*., 2012) [3]
5	Durva 1 mM AgNP	489 (Puiso *et al*., 2014) [37]
6	Karanj 2 mM AgNP	460 (Banerjee *et al*., 2014) [5]
7	Kusalgrass 2 mM AgNP	426 (Azad and Banerjee, 2014) [2]
8	Neem 3 mM AgNP	445 (Saikia, 2014)
9	Panfuti 4 mM AgNP	343 (Devi *et al*., 2013) [7]
10	Pipal 5 mM AgNP	443 (Umoren *et al*., 2014) [38]
11	Rantulas 2 mM AgNP	437 (Kulkarni *et al*., 2011) [39]
12	Samudrashosh 4 mM AgNP	420 (Kulkarni *et al*., 2011) [39]
13	Vad 1 mM AgNP	498 (Devi *et al*., 2013) [7]

From above mentioned study data it is concluded that the silver nanoparticles can be easily prepared from the combination of silver nitrate solution and plant extract. The plant extract contains numerous phenolics and other bio-molecules which acts as nucleation centre for synthesis of nanoparticles.

Fig 1: Change in colour of silver nitrate solution after addition of kusalgrass extract

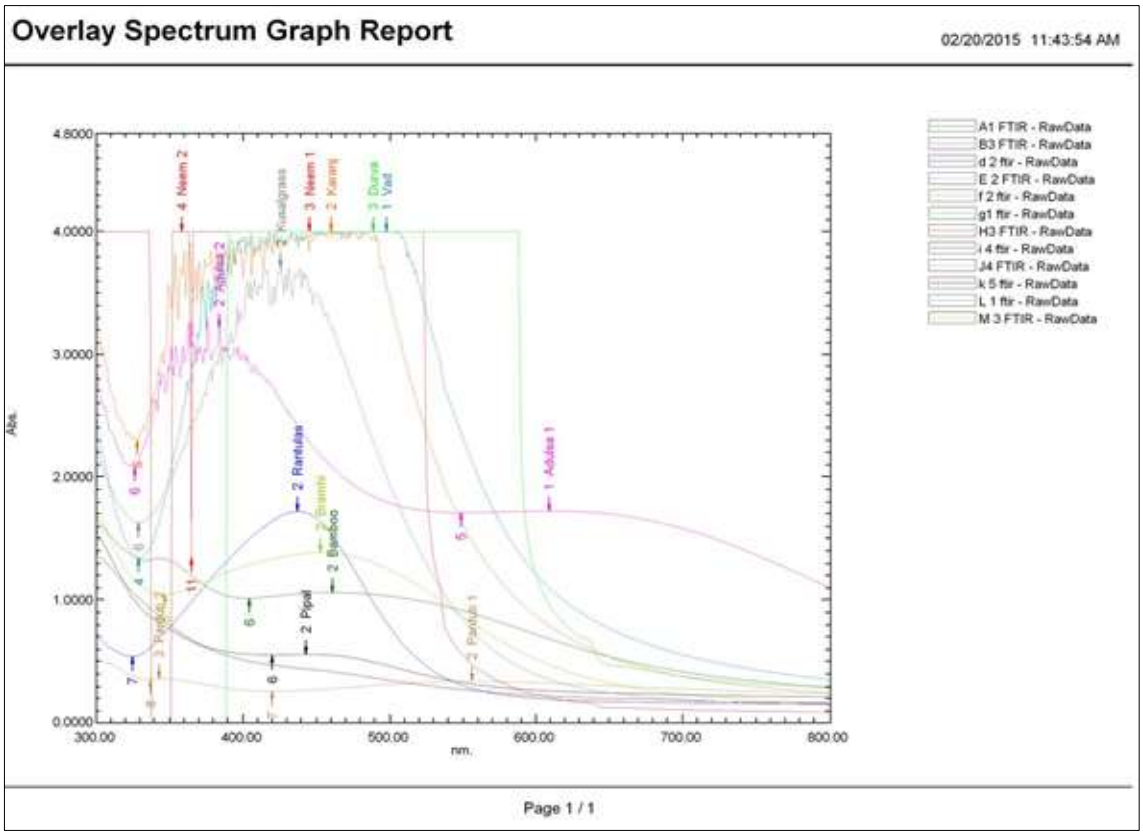

Fig 2: Overlay spectrum of UV VIS spectrophotometer

Acknowledgement:-
Authors are thankful to Management of Mahatma Gandhi Mission Trust, Aurangabad. Authors are also thankful to MGM College of Agricultural Biotechnology, Gandheli for providing all necessary material for this research work

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