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ANTIOXIDANT ACTIVITY OF LYOPHILIZED SILVER NANOPARTICLES USING TAMARIND FRUIT SHELL EXTRACT AND ITS CHARACTERIZATION STUDIES

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Abstract

The present investigation reveals that, the green synthesis of silver nanoparticles and capability of the fruit shell of pharmacologically important tree Tamarindusindica. An ecofriendly, easy, one step, non-toxic and inexpensive approach is used for the synthesis of silver nanoparticles. The Silver Nanoparticles were characterized and Surface plasmon resonance of the Nanoparticles was observed at 447 nm in UV-Visible Spectroscopy. FTIR result confirmed that the aqueous fruit shell extract was acting as the reducing, as well as the capping agent of the Silver Nanoparticles. SEM indicated that the synthesized Nanoparticles are cubic in shape. Green synthesized (lyophilized) and characterized silver nanoparticles were assessed for its efficacy of antioxidant activity. Antioxidant assay was determined at different concentrations ranging from 10 µg to 50 µg / ml by DPPH scavenging assay. The percentage of inhibition of DPPH free radicals is dependent on the concentration manner. The percentage of inhibition reached upto 95.06 % and it was found at increased concentration of 50 µg / ml. IC50 concentrations of lyophilized silver nanoparticles were noted for DPPH scavenging as 15.18 µg / ml. acts as effective antioxidant agent.Thus, it concluded that silver nanoparticle acts as effective antioxidant agent.

Keywords: green synthesis, silver nanoparticles, lyophilized, DPPH, SEM, FTIR, Surface plasmon resonance.

1 Introduction

1974, Norio Taniguchi, a Japanese scientist, was the first to use and define term "nanotechnology," stating, "Nanotechnology mainly consists of the processing of separation. Nanotechnology is an area of science that deals with small objects. On general, while looking at materials in the nanoscale, between 1 and 100 nanometers. It is a science that operates at the molecular level and provides a variety of focal points for many topics of study. Dentistry, medicines and bioengineering are examples of science [1].

Nanotechnology is an emerging field in an area of interdisciplinary research, especially in biotechnology. In recent years, noble metal nanoparticles have attracted considerable attention because of their unique optical, electronic, mechanical, magnetic and chemical properties that are significantly different from those bulk materials [2]. Fruit, seed, stem, flower, leaves and skin of fruits were all used to make nanoparticles. In this study, the reduction of silver nanoparticle was accomplished using Tamarindusindica fruit shell extract. Limonene, geraniol, safrole, cinnamic acid, methyl salicylate, pyrazine and alkylthiazoles are among the volatile phytochemicals found in tamarind fruit. It's also high in thiamin, vitamin A, folic acid, riboflavin, niacin, and vitamin C [3].

Nanotechnology research and study have improved fast all around the world. Despite the potential for growth in the subject of nanotechnology, there is still a lot of work to be done. There are still some concerns about the potential dangers and consequences of Environmental and human health effects of nanoparticles have grown in importance as a research topic. Due to their wide range of applications in a variety of fields, they have become increasingly popular in recent years. Diagnostics, biomarkers, cell labelling, and other fields such as antibacterial agents, drug delivery and cancer therapy are all examples of this [4]. Nanoparticles were primarily employed in the development of a wide range of products. Carbon nanotubes, for example, are among the materials that have been created Carbon nanotubes (CNTs) [5], carbon quantum dots (CQDs), epoxy resin-coated carbon nanotubes, silver nanoparticles with a polymer coating and super magnetic iron oxide nanoparticles (SPION), mesoporous silica particles, catalytic metals, oxides, quantum dots (QD) [6], dendrimers, nanofilms, and nanofibers are all examples of nanomaterial [7] and nanocomposites with reinforcement [8].

Nanomaterials have recently piqued the interest of scientists due to their unique and significant features that distinguish them from their bulk counterparts. Noble metal nanoparticle, such as Ag, is among them. Au, Pt, and Pd nanoparticles are used in a variety of physical chemical and biological applications. Applications in biology AgNPs

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are a promising contender for carry out the antimicrobial part of the nanostructured applications with anticancer properties. The properties of nanoparticles are as follows [9], their shape, size and nature control them. AgNPs are abundant in the environment. Water is in high demand due to its numerous medical applications. Catalysis and treatment Colloidal dispersions, in general, result in AgNPs are formed differently and have different morphologies depending on what they are made of the synthetic procedures that were used [10].

The present investigation reveals that, green synthesis of lyophilized Silver Nanoparticles and the potential of the fruit shell of the pharmacologically essential tree Tamarindusindica.

1. To synthesize the Silver Nanoparticles by elaborating and standardizing the existing green methods of synthesis.
2. To synthesize and characterize the lyophilized Silver Nanoparticles by using different characterization techniques like UV-Visible, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM).
3. To investigate the antioxidant activity of the lyophilized Silver Nanoparticles.

2 Experimental

2.1 Taxonomical Classification of Tamarind

Kingdom : Plantae
Family : Fabaceae
Genus : Tamarindus L.
Species : T.indica,
Order : Fabales
Binomial Name: Tamarindusindica L

2.2 COLLECTION OF PLANT MATERIAL

Tamarindusindica fruit shells were collected from Koratti village, Tirupattur,VelloreDt, Tamil Nadu, India. The collected fruit shells were washed thoroughly with tap water followed by distilled water to remove all the unwanted materials. Then cut into small pieces and dried at room temperature. Then well dried shells are grounded into fine powder for further use (Fig. 1).

Fig. 1: Tamarindusindica Plant

2.3 PREPARATION OF PLANT EXTRACT

Aqueous extract of Tamarindusindica fruit shell was prepared by mixing 20 g fruit shell powder with 200 ml deionized water in the 400 ml Erlenmeyer flask. The mixture was then heated in the hot plate at 60°C for 20 minutes. The prepared solution was initially filtered through normal filter paper mesh so that the unwanted materials could be filtered out; then the extract was filtered through Whatman filter paper (Fig. 2). The filtered extract was used as the reducing and stabilizing agent in the Nanoparticles synthesis [11].

Tamarindusindica fruit shells

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Aqueous Extract of Tamarindusindica Fruit Shell

Fig. 2: Preparation of Plant Extract

2.4 GREEN SYNTHSIS OF SILVER NANOPARTICLE

40 ml silver nitrate solution was prepared in an Erlenmeyer flask using double distilled water. Then 180 ml of fruit shell extract was added to 40 ml silver nitrate solution and made upto 200ml with double distilled water. Then the mixture was stirred for 2 hours at 45 °C by hot plate method with magnetic stirrer [11] (Fig. 3).

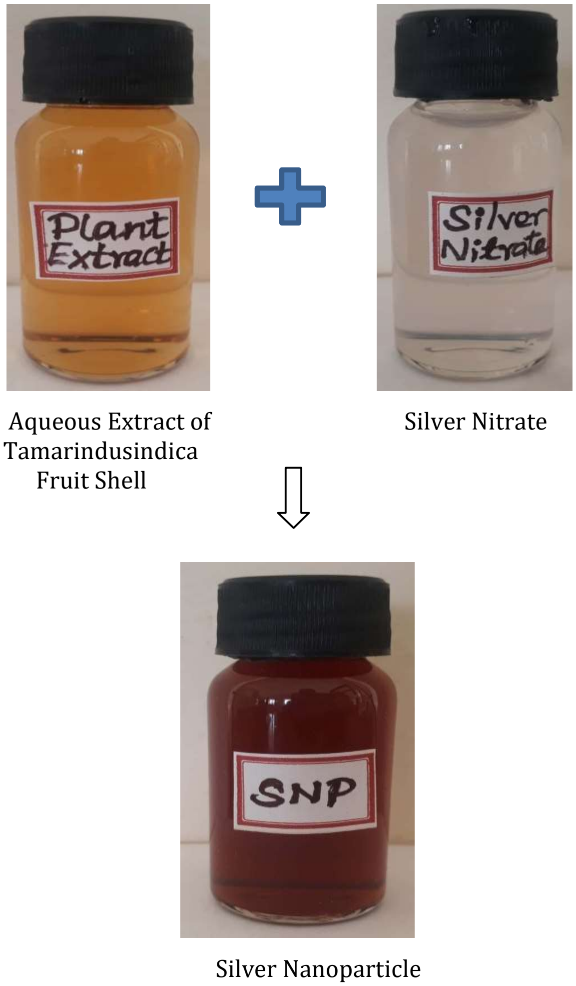

Fig. 3: Green Synthesis of Silver

2.5 LYOPHILIZATION

Lyophilization and Freeze drying are synonymous. Lyophilization is a water removal process typically used to preserve perishable materials, to extend shelf life or make the material more convenient for transport. Lyophilization works by freezing the material, then reducing the pressure and adding heat to allow the frozen water in the material to sublimate. Lyophilization occurs in three phases, with the first and most critical being the freezing phase (Fig. 4).

Fig. 4: Lyophilization Process

Proper lyophilization can reduce drying times by 30%.
1. Freezing phase
2. Primary drying (sublimation) phase
3. Secondary drying (absorption) phase

2.6 CHARACTERIZATION OF LYOPHILIZED SILVER NANO PARTICLE

The reaction mixture was centrifuged for 20 minutes with 4000 rpm and the precipitate was washed with water and centrifuged. Then the pellet was collected and dried in hot air oven with proper temp between 35 - 40 °C. Dried nanoparticle was collected and used for further characterization and application studies (Fig. 5). Nanoparticles are generally characterized by investing their size, shape and surface area. A homogeneity in these properties result in the advancement in applications of Nanoparticles. The characterization of lyophilized silver nanoparticle is done by using various instrumental tecniques.

Fig. 5: Lyophilized Silver Nanoparticle

2.6.1 UV-VISIBLE SPECTROPHOTOMETER

The reduction of silver ions to silver nanoparticles was characterized using double beam UV visible spectrophotometer at wavelength range from 300 nm to 700 nm. This analysis was performed in Perkin Elmer Spectrophotometer (Singapore) and the resolution of instrument is 1 nm. The maximum absorption peaks for lyophilized silver nanoparticles were recorded.

2.6.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY

The IR spectrum of lyophilized silver nanoparticles was performed by FTIR (SHIMADZU instrument). The samples were grinded with KBr pellets and scanned in FTIR spectrum in the range of 4000 – 400 cm-1.

2.6.3 SCANNING ELECTRON MICROSCOPE

A morphological character of lyophilized Silver Nanoparticles was analyzed by SEM (ZEISS). For this electron microscopic study, the sample was made into thin films by placing on a carbon coated copper grids and the images were recorded.

2.7 APPLICATION OF SILVER NANOPATICLE

2.7.1 ANTIOXIDANT ACTIVITY

Antioxidant activity of lyophilized Silver Nanoparticles was carried out by DPPH Radical Scavenging Assay

2.7.1.1 DPPH RADICAL SCAVENGING ASSAY

The antioxidant activity of lyophilized silver nanoparticles using aqueous fruit shell extract of Tamarindusindicawas assayed against DPPH free radical by following method [12]. This assay was performed at different concentration of lyophilized silver nanoparticles (10 – 50 µg / ml). 10 µl of each concentrations of Silver Nanoparticle sample was taken in different test tubes. 0.659 mM DPPH was dissolved in methanol solution. From this 50 µl was added to all the test tubes and the tubes were incubated at 25 ºC for 20 minutes. The experiment was carried out in triplicates under same condition. After incubation the absorbance of reduced DPPH was recorded at 517 nm using UV-Vis spectrophotometer (Shimadzu-1800). Ascorbic acid was used as standard. Same procedure was followed for standard. A test tube containing DPPH solution without sample is considered as control. The percentage of DPPH inhibition was calculated by using the following formula

% Inhibition = [Absorbance of Control – Absorbance of Test Sample / Absorbance of Control] ×100

The 50 % of reduction of free radicals was considered as 50% inhibition (IC50).

3. Results and Discussion

3.1 UV –VISIBLE SPECTROPHOTOMETER

Based on the surface electron Plasmon oscillations, the ultraviolet-visible spectra were used to characterize the structure of the silver nanoparticles. The size and form of silver nanoparticles are reflected in the shifting wavelength [13]. The size of silver nanoparticles has a significant impact on their absorption [14]. Surface Plasmon band the greatest absorbance of the manufactured particles was 447nm. It represent silver nanoparticles characteristic peak (Fig. 6). Flavonoids and phenolic chemicals such as quercetin and caffeic acid, which can rapidly reduce Ag and act as both reducing and stabilizing agent [15].

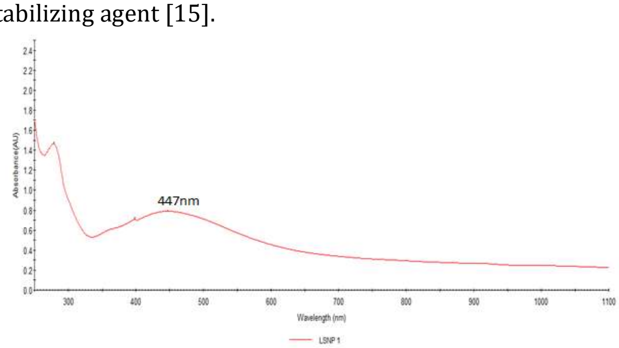

Fig. 6: UV Vis Spectra Analysis

3.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Fourier-transform infrared spectroscopy (FTIR) was carried out to identify the functional groups from pH involved in the reduction of Ag+ and capping of silver NPs. The spectrum bands were observed at 3436.28 cm−1 were assigned to O–H stretching in an alcohol, 2071.04 cm−1 were assigned tri C–H stretching in an alkane [16], 1634.89 cm−1 were assigned to C=O Amide I Band, 1117.87 cm−1were assigned to C-O-C polysaccharide and DNA and RNA back bones and 659.42 cm−1 assigned to CH out of plane bending vibration in an alkynes compound, respectively [17] (Fig. 7)

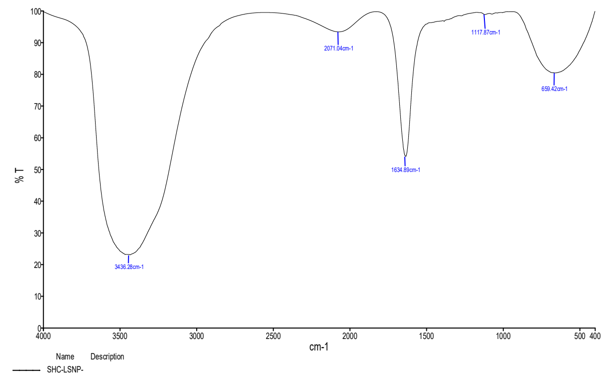

Fig. 7: FTIR Spectra Analysis

3.3 SEM ANALYSIS

The SEM images of the same lyophilized silver nanoparticles sample showed that most of the silver nanoparticles are predominately cubic in shape. The size of the lyophilized silver nanoparticle was observed about 78 nm to 87nm (Fig. 8). Silver nanoparticles with a cubic form from plant extract were also known in 15 and 17. The cubic Ag-NPs synthesized in [18] had a size of about 80 nm.

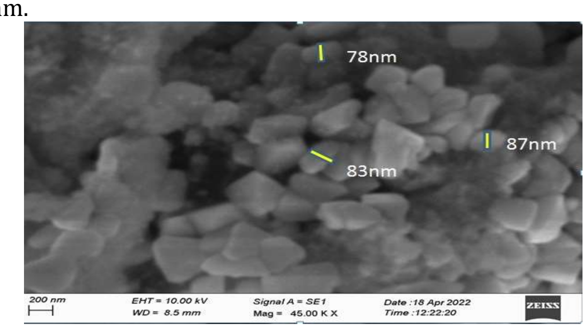

Fig. 8: SEM Analysis

3.4 ANTIOXIDANT ACTIVITY

3.4.1 DPPH Free Radical Scavenging Activity of Lyophilized Silver Nanoparticles

DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, is a direct and reliable method for determining radical scavenging action. Ascorbic acid was chosen as the reference antioxidant for this test. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple color (Fig. 9). When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which quantitatively measured from the changes in absorbance.

This study has revealed that the in vitro DPPH radical scavenging activity of lyophilized silver nanoparticles. The IC50 value of lyophilized silver nanoparticles was found to be 15.18µg/ml. Then standard value was found to be Ic50=11.44µg/ml (Table:1).

Similarly, Anantharaj Tamilselvan, et al.,, 2017 [19], Plant driven to synthesis of silver nanoparticle using Crataevareligiosa hook and frost and its utility in detaching antimicrobial and antioxidant assay Ic50=26.54.

Fig. 9: Antioxidant Activity of Lyophilized Silver Nanoparticles using DPPH Assay

Table: 1 Antioxidant Activity of Lyophilized Silver Nanoparticles using DPPH Assay

S.NO	SAMPLE	CONCENTRATION (µg/ml)	DPPH ACTIVITY: ABSORBANCE 517nm	DPPH ACTIVITY: % INHIBITION	DPPH ACTIVITY: IC 50 µg/ml
1	LSNP	10	0.657	33.77	15.18
2		23	0.346	65.12	
3		30	0.236	76.20	
4		40	0.132	86.69	
5		50	0.049	95.06	
6	CONTROL	0.992			

4. Conclusions

The presence study was made of antioxidant of lyophilized silver nanoparticles using tamarind fruit shell extract and its characterization studies. The lyophilized silver nanoparticles were characterized by UV, FTIR and SEM. The UV-Vis spectrum shows the absorption peak for silver nanoparticles at 447 nm. FTIR study was carried out to detect the possible functional groups responsible for imparting stability to nanoparticles and its synthesis. SEM micrograph images shows that the nanoparticles synthesized were cubic form and the average size of about 78 nm to 87nm. Morphological characters such as size, shape and distribution were provided by SEM studies. It revealed that the size ranges from 78 nm to 87 nm and cubicl shaped nanoparticles. Then the silver nanoparticles showed the antioxidant activity.Antioxidant assay of silver nanoparticles and standard ascorbic acid were determined at different concentrations ranging from 10 µg to 50 µg / ml by DPPH assay. IC50 concentration of lyophilized silver nanoparticles was noted for DPPH as 15.18 µg / ml. The standard ascorbic acid value was found to be Ic50=11.44µg/ml

As metal nanoparticles seems to fascinate for the future diverse industry due to their enriched chemical, electrical and physical properties. Metal nanoparticles are synthesized predominantly by wet chemical methods, where the chemical used are toxic and flammable. So there is need of ecofriendly nanoparticles synthesis approach.

From the scientific point of view, the potential application of these green synthesized silver nanoparticles in the biomedical field was observed and this simple procedure has several advantages and industrial applications as well as large scale commercial production. Wide ranging applications of silver nanoparticles have encouraged researchers to synthesize silver nanoparticles and to know more about these alluring tiny particles. Plants serve as one of the promising sources for the biosynthesis of silver nanoparticles as compared to other biological entities. They offer an eco-friendly, cheap, time saving, non-toxic way to achieve silver nanoparticles. Among the various applications we have studied a few of them in this research work. It gives scope for further research

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