SYNTHESIS AND CHARACTERIZATION OF PLANT MEDIATED SILVER NANOPARTICLE AS AN EFFECTIVE ANTIOXIDANT

R. Christynal Oliviya*[1]
Assistant Professor,Department of Biotechnology, Lady Doak College, Madurai
Dr. S. Krishnakumari[2]
Associate Professor, Department of Biochemistry, Kongu Nadu Arts and Science College, Coimbatore
Research and Development Centre[3]
Bharathiar University, Coimbatore

ABSTRACT -The present investigation was carried out to determine the effect of phytochemicals in the form of nano particle as an antioxidant representative. This includes using natural plants and herbal supplements as an alternative way to manage and control diseases caused by oxidative stress.The study focuses the efficacy of silver nano particle to impart the medicinal value of the plant extract Abelmoschusesculentus in the treatment of diseases caused by oxidative stress. Initially, the qualitative and quantitative analysis of phytochemcials present in Abelmoschusesculentuswas studied. Then silver nano particle was produced by using the extract of Abelmoschusesculentus and its characterization study was done.Then, the anti-oxidant activity of plant mediated silver nano particle was analysed by compared with the control and with the extract of Abelmoschusesculentus. This effort has been seen towards providing the evidence to nutritional properties of Abelmoschusesculentusin the form of nano particle that may be benefited by the modern medicine, thus suggesting new potential target for drug discovery.

***KEY WORDS*-Antioxidant,novel drug, FTIR spectra, phytochemicals, silver nano particle,**

INTRODUCTION

The recent growth in the knowledge of free radicals and reactive oxygen species (ROS) in biology is producing a medical revolution that promises a new age of health and disease management.[3] It is ironic that oxygen, an element indispensable for life, under certain situations has deleterious effects on the human body.Antioxidants are powerful free radical scavengers in the body, while free radicals are reactive chemical substances such as peroxide, hydroxyl radical, singlet oxygen etc. that travel around in the body and cause damage to the body cells (Alia et al., 2003). [1] Most of the potentially harmful effects of oxygen are due to the formation and activity of a number of chemical compounds, known as ROS, which have a tendency to donate oxygen to other substances. The increase in oxidative stress causing inflammation is a unifying hypothesis for predisposing atherosclerosis, carcinogenesis, and osteoporosis and for type 2 diabetes. [5,6,12]Medicinal and aromatic plants are an important source of raw materials for traditional as well as modern medicines. These plants synthesise variety of secondary metabolites with remarkable structural diversity, which have been subsequently exploited by humans for their beneficial role in a diverse array of applications. Secondary metabolites are classified as: alkaloids, glycosides, tannins, phenolic compounds, volatile oils, terpenoids, saponins, steroids, resins and bitter principles. Many of these secondary metabolites of plants are commercially important and find use in fragrance, flavouring and pharmaceutical formulations worldwide. [11]

Nowadays, research on medicinal plants has attracted a lot of attention globally; especially green synthesis of silver nanoparticles using plant sources.The noble metals (Ag, AU, Pb, Pt and Hg) are widely used for the synthesis of nanoparticles. Among the noble metals, silver is the metal of choice because it is used as a health additive in traditional medicine. The most important and distinct property of nanoparticles is that they exhibit larger surface area to volume ratio. This stands as a great application in the field of nano medicine. Medicinal property of the plant extract and nano silver could play vital role in treatment of many diseases.Green nanoparticle synthesis has been achieved using environmentally acceptable plant extract and ecofriendly reducing and capping agents. Plants and microbes are currently used for nanoparticle synthesis. The use of plants for synthesis of nanoparticles is rapid, low cost, eco-friendly, and a single-step method for biosynthesis process [10].

MATERIALS AND METHODS

SAMPLE COLLECTION AND AUTHENTICATION OF PLANT MATERIAL

The fruits of*Abelmoschusesculentus*were collected from Melur Village in Madurai District. Authentication of plant was carried out.

EXTRACTION OF MUCILAGE (RISHABHA MALVIYA, 2011)

*Abelmoschusesculentus*fruits were used for extraction of mucilage. The vegetables were washed with water to remove dirt. The seeds were removed and finely chopped and crushed into a mixer. The crushed material was soaked in warm water for 4 hours , boiled for 2 hours and kept aside for 2 hours for release of mucilage into water. The material was squeezed in a muslin bag to remove the mark from the filtrate. The filtrate was used for further study.

PHYTOCHEMICAL ANALYSIS (HARBONE, 1981)

(i) QUALITATIVE ANALYSIS:

Alkaloids, Tannins, Saponins, Steroids, Flavanoids, Glycosides and Amino acids were analyzed qualitatively.

(ii) CONFIRMATION OF PHYTOCHEMICALS BY HPLC

The sample was further analysed by using HPLC to confirm the presence of phytochemicals in the extract.

iii)QUANTITATIVE ANALYSIS

Once the presence of phytochemicals were confirmed, their amount in the plant extract was also analyzed by various standard methods. Such as Flavonoids-Libermamn by Burchard*et al.,* Saponins by Brunner 1984,Tannins by Van Burden and Robinson1981 .

GREEN SYNTHESIS OF SILVER NANO PARTICLE (GOVINDARAJU K *et al.*, 2010*)*

To 5ml of plant extract, 100ml of 1mM AgNo3 in 250 ml Erlenmeyer flask. Flasks were kept in a shaker at 200 rpm for 10minutes[pH of the solution was maintained as slightly acidic (6.5-6.8)]Incubated at room temperaturefor7hours.Colour change was observed.

RECOVERY OF SILVER NANO PARTICLE (SHAKEEL AHAMED *et al.*, 2016)

The silver nanoparticle solution was centrifuged repeatedly for 4-5 times at 10000 rpm for 10 minutes. Dispersion of the pellet in deionized water to get rid of any uncoordinated biological molecules.The process of centrifugation and dispersion in sterile deionized water was repeated three times.

CHARACTERIZATION OF SYNTHESIZED SILVER NANO PARTICLE

(i) UV-VISIBLE SPECTRAL ANALYSIS (KLULKARNI *et al.*, 2011)

To 0.2ml of the suspension, 2ml of double distilled water was added and measured at 200 nm in UV-VIS Spectrophotometer.

(ii) ATOMIC FORCE MICROSCOPY (ALAHMAD*et al.*, 2013)

A thin film was prepared on a glass slide by dropping 100µl of the sample on a glass slide and allowed to dry for 5 minutes.Slide was then scanned.Spectra were recorded at a resolution of 350-4500-cm-^{1} in UV- Visible spectrophotometer.

iii) FOURIER TRANSFORM INFRARED SPECTROSCOPY ANALYSIS (KUMAR*et al.*, 2014)

A drop of sample was mixed with a pinch of KBr powder. It was pelleted out after drying and the pellet was subjected to FTIR spectroscopy.

ANTIOXIDANT ASSAY OF PLANT MEDIATED SILVER NANO PARTICLE:

DPPH RADICAL ASSAY (MACDONALD-WICKS,2006)

To the 1ml of 0.2mM DPPH solution, 1ml of plant extract and 1ml of Silver nano particle solution was added separately. Both were incubated for 20 minutes in dark room at room temperature. Read at 515nm under UV -Visible spectrophotometer.

NITROUS OXIDE ASSAY (GARRAT, 1964)

To 2ml of 10mM Sodium nitroprusside,0.5ml Saline phosphate buffer was added.0.5ml of both the plant extract and the plant mediated silver nano particle samples were added separated. Incubated at 25˚C for about 90 minutes. After incubation add 0.5 ml of sulfanilic acid and tend to be diazotization for about 5 minutes. 1ml ofnaphthyl ethylene diaminedihyrochloride was added. Incubated at 25˚Cfor 30minutes. Read at 546nm under UV visible spectrophotometer.

REDUCING POWER (YILDIRIM *et al.*, 2001)

To 1ml of both samples, 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5ml of potassium ferric cyanide were added. The mixture was incubated at 50°C for 20min. 2.5ml of 10% tricholoroacetic acid was added. Centrifuged at 3000 rpm for 20mins.The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and ferric chloride (0.5ml, 0.1%) The colour developed was observed at 700nm.

HYDROGEN PEROXIDE ASSAY (JAYAPRAKASHA *et al.*, 2004)

1ml of both samples were mixed with 3ml of phosphate buffer and 1ml of H_2O_2separately. Incubated for 10mins at 37°C. Observed at 230nm.

FERROUS ION CHELATING ACTIVITY (DECKER AND WELCH, 1998)

To 1ml of each sample (plant extract and silver nanoparticle), 0.3ml of 2mM of Ferric Chloride and 0.6ml of 5mM Ferrozine were added. Incubated at 60˚C and read at 562nm

RESULTS AND DISCUSSION

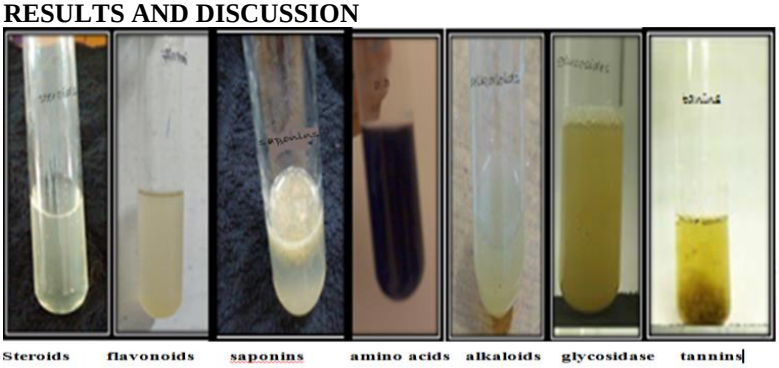


Fig: 1 Qualitative Analysis of Phytochemicals

TEST	RESULT
Steroids	-
Flavonoids	+
Amino acids	+
Alkaloids	-
Glycosides	-
Tannins	+
Saponins	+

Fig: 2 Confirmation of Phytochemicals by HPLC

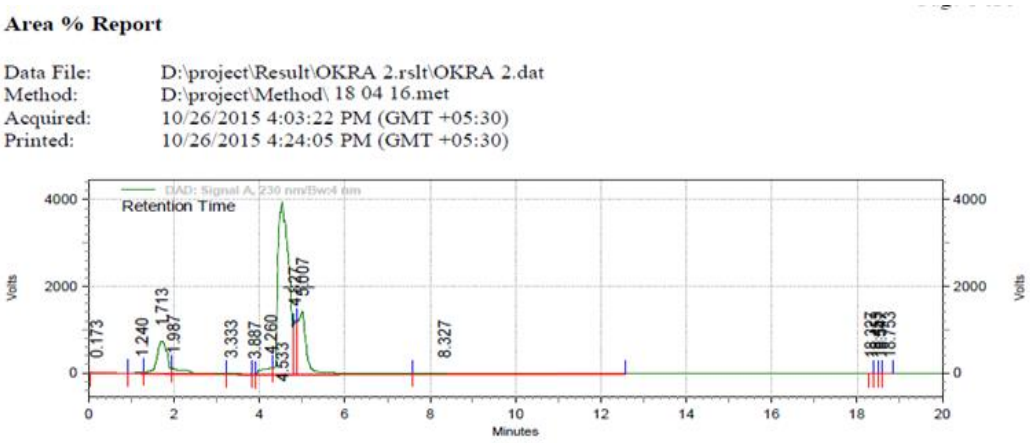


Fig: 3 Quantitative Analysis of Phytochemicals

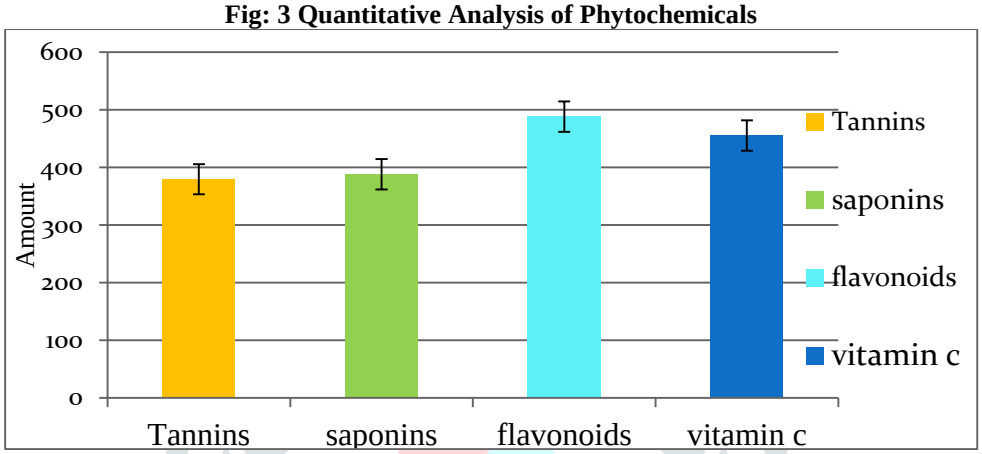


Fig: 4 Synthesis of Silver Nano Particle

Plant extract along with silver nitrate

~~7 hours~~ → incubation at room temperatur e

Colour changes

Fig: 5 UV-Visible Spectroscopy

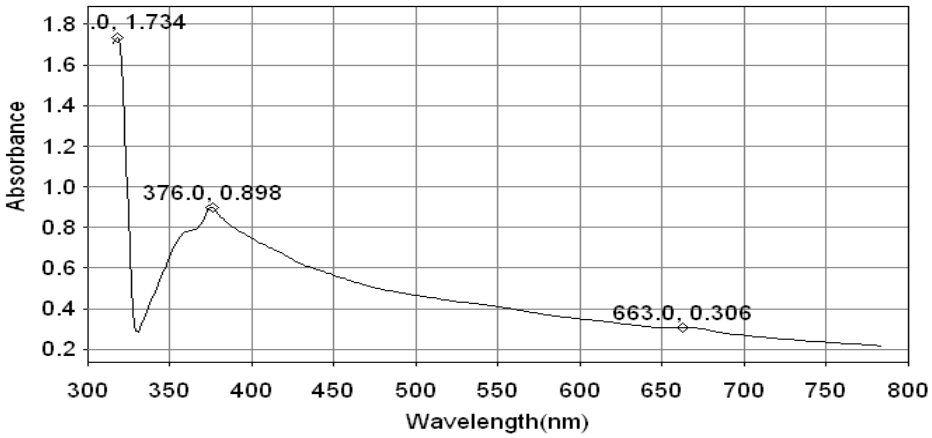


Fig: 6 AFM image of silver nanoparticle

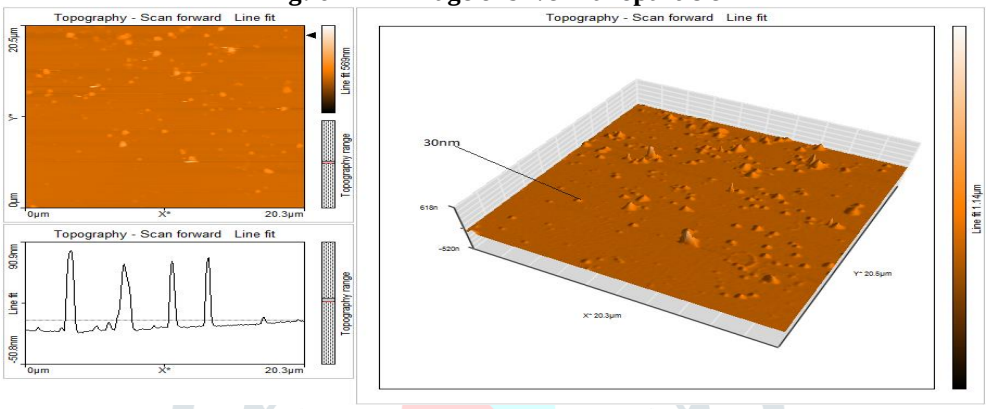


Fig: 7 FT-IR Analysis

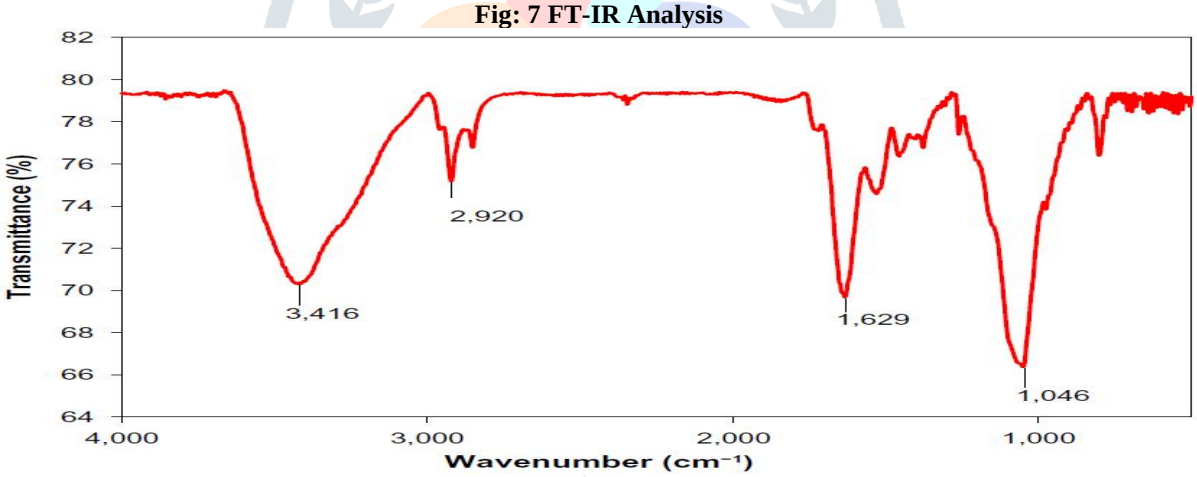
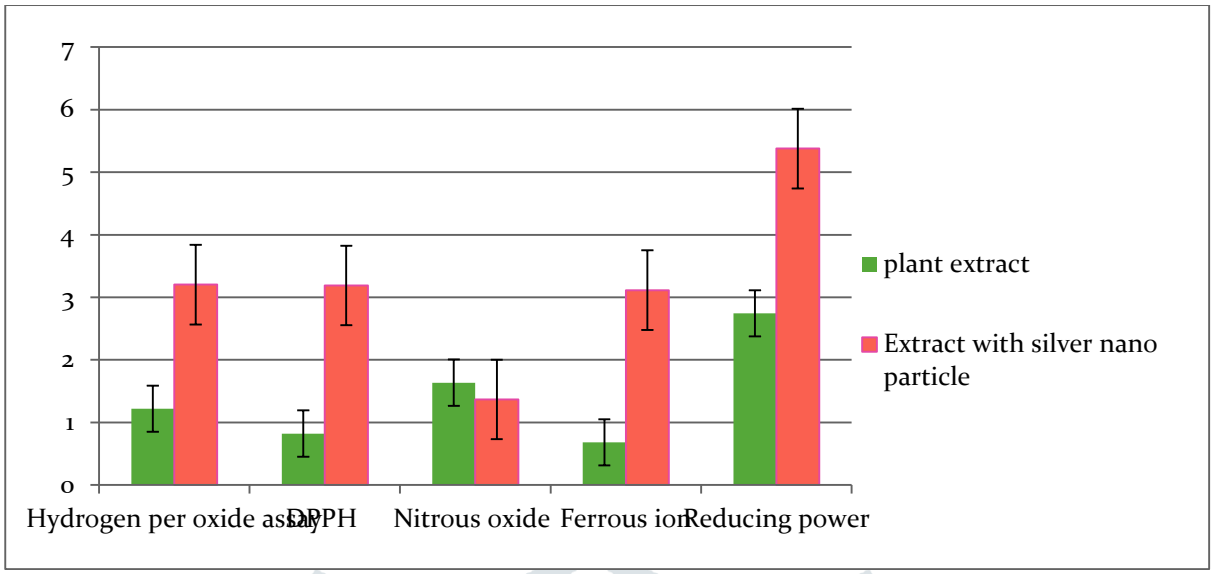


Fig: 8*In vitro* Antioxidant assay



DISCUSSION

When the plant extract was screened for phytochemicals,(fig:1) it was identified there is a presence of flavanoids, amino acids, tannins and saponins and they were confirmed by performing HPLC(fig:2). The amount of identified phytochemicals were estimated and it shows that the amount of flavanoids and vitamins were higher than saponins and tannins (fig:3). Then the plant extract was treated with $AgNO_3$ and the production of sliver nano particle was identified by colour change of the extract from yellow to brown (fig:4). This colour change was due to the property of quantum confinement which is a size dependent property of nanoparticle.

This was confirmed by UV-Visible spectrophotometer.The spectra shows the formation of spherical AgNP of the mucilage (fig:5), The frequency and width of the surface plasmon absorption depends upon the size and shape of the nanoparticles as well as the dielectric constant of metal and its surrounding metal.

Fourier tansform-infrared (FT-IR) analysis was performed to identify the possible biomolecule responsible for the reduction of Ag^+ ions. (Fig: 6) Strong IR bands were observed at 3,416 and 2,920 cm^{-1} corresponds to –OH stretching and aliphatic -C-H stretching respectively. The bands at 1,629cm^{-1} is due to the C = O and C = C stretching, respectively.The spectrum reveals that the carbonyl groups and –OH stretching and the aliphatic group are involved in the reduction of Ag^+ to Ag. Therefore, it may be concluded that flavonoids, saponins, tannins,are responsible for capping and efficient stabilization.AFM was used to analyse the particle morphology (shape,size). (Fig: 7)AFM image of synthesized AgNP shows that they were uniformly packed surface with the height of 54nm. Itindicatesthat, the size of the synthesized nanoparticle was about 30nm and shape of the particle is spherical.

Antioxidant activity:

DPPH is a stable radical that has been used to evaluate the anti-oxidant activity of plant extract(fig: 8). The effect of antioxidant on DPPH radical scavenging was due to their hydrogen donating ability.

Nitric oxide is a potent pleotropic mediator of physiological process such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is diffusible free radical which plays many roles as an effector in diverse molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and anti-tumor activities.

Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Thus, the removing of H_2O_2 is very important for antioxidant defence in cell or food systems.

The reducing power of the samples wasstudied, and it might be due to their hydrogen-donating ability. Possibly, both the (extract and silver nanoparticle) could react with radicals to stabilize and terminate radical chain reactions.

Ferrous ion chelating activity also has been studied. Ferrous iron can initiate lipid peroxidation by the fenton reaction as well as accelerating per oxidation by decomposing lipid hydro peroxides into peroxyl and alkoxyl radicals. The result shows that the chelating activity of both (plant extract and plant mediated silver nanoparticle) the samples was found.

In this study, the DPPH, the nitric oxide and the hydrogen peroxide scavenging activities, reducing power and ferrous ion chelating activitieswere found both in plant extract and plant mediated silver nanoparticle and it was noted that the plant mediated silver nano particle has shown significant anti-oxidant activity when compare to plant extract.

CONCLUSION

The aqueous extract of*Abelmoschus esculentus* (L) Moenchcan be a good source for the synthesis of silver nanoparticle which shows potent anti-oxidant than the plant extract. These activities of *Abelmoschus esculentus* (L) Moenchmight be due to the presence of flavonoids, vitamins,tannins, saponins in plants. The important outcome of thisstudy will be the development of value added products from the medicinal plant for biomedical and nanotechnology based indrustries. (Prasanth *et al.*, 2005).

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