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SPECTROPHOTOMETRIC ANALYSIS OF DEPROTEINISED SUPERNATANT MEDIATED SILVER NANOPARTICLES SYNTHESIS OBTAINED AFTER LEAF PROTEIN PRECIPITATION: THE MOLECULAR APPROACH

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ABSTRACT

In order to study the cellular nanoparticles, leafy tissues fractionated to juice during which the upper supernatant obtained is deproteinised juice. This supernatant consists of the majority of the disposed nutrients passed through the juice by heating and isolation of leaf protein of the green crop. Silver nanoparticles has attracted much attention because of their unique shape-dependent optical, electrical, and chemical properties, have potential applications in biomedical proteomic molecular research. An attempt has been made to the formation of the nanoparticles by reduction of the aqueous metal ions during exposure to the broth of *Colocasia*, fenugreek, *Basella* and cruciferous leaves by light microscopy and UV–Vis spectroscopy. In earlier research, deproteinised juice found inhibiting the growth of fungi *Trichoderma* and cell division in the meristematic root tissues of Allium sp and Celosia argentia. During present investigation, it indicates that the constituents responsible were nanoparticles. Attempts were experimented the detection of nanoparticles in the DPJ prepared from various species. *Colocasia* DPJ found having spherical, irregular, hexagonal and triangular shaped nanoparticles. DPJ nanoparticles binds enzymes for its activation and interacts with cellular soluble proteins.

Contribution/Originality: This study contributes presence of silver nanaoparticles in DPJ. It used novel methodology by microscop and spectroscopy. The formula is first attempt in leaf protein research. The contribution motivates to assess gold and nitrogen nanoparticles from fungi grown on DPJ.

1. INTRODUCTION

Mostly the sizes of the nanoparticles are 1 to 100 nanometers. 0.001 micrometer equals to 1 nanometer 0.1 micrometer equals to 100 nanometer. The size of the most penetrating particle size is ranges between 100 and 300 nm [1]. Fine particles are sized between 100 and 2,500 nm, and a coarse particle bears a range of 2,500 and 10,000 nm. *Allium cepa* root spherical nucleus is 0.3 to 0.8 µm in diameter when compared with size of nanoparticle. Silver nanoparticles are utilised in photographic processes, catalysis, and chemical reactions. Nanoparticles are solid dispersion particulates of size range 10-1000 nm. They cause enhancement of particle mobility, diffusion, thermal stability, storage capacity, greater surface area and also modulate catalytic activity of the attached enzymes via chemical linkages, by adsorption.The enzymes can be immobilized on nanoparticles [2].

The biosynthesis of Ag NPs was investigated using nitrate reductase enzyme available in the bacterium. The activity of the nitrate reductase enzyme was optimised by changing its cultural conditions, and the effects of silver nitrate concentration and enzyme amount on nanoparticles synthesis were studied [3]. It was found no definite correlation between the leaf proteinase levels and the grain yield, the integrated leaf Nitrate reductase (NR) activity was positively correlated with the yield of grain. The total nitrogen content per plant seems to be dependent on the levels of NR activity in leaves and root growth [4]. When DPJ from *Basella* was subjected to the soil, in order to reach roots of grown *Eleucine* seedlings, there was enhanced NR activity.

During the process of leaf protein from green crop fractionation, large amount of deproteinised juice obtained was disposed randomly. Therefore therefore to avoid pollution, its utilization for benefit was recommended. At appropriate concentrations or freshly obtained DPJ from various crops, when was utilised for growth of beneficial fungi viz., *Fusarium*, *Phoma*, *Cunninghamela*, *Alternaria*, etc, it was successful. It was because of the presence of nanoparticles [5]. The culture filtrates of lucerne DPJ on which the fungi were grown, also secreted the beneficial secondary metabolites like hydrolytic enzymes. The fungi viz., *Sordaria*, *Nigrospora* and *Pestalotia* found secreting high enzymes lipases when grown on liquid DPJ of Lucerne. Many microorganisms are able to reduce the Ag+ ions into spherical AgNPs. To determine magnetic nanoparticles, Transmission Electron Microscopy can be utilized [6]. Lipase from *Aspergillus flavus* was purified using magnetic nano - particles (MNPs) [7].

Nanoparticles can also be biosynthesized by yeast [8]. Yeast also showed single cell protein enhancement and secretion of the enzymes protease, cellulase, lipase and amylases when grown on DPJ from lucerne as well as from various cucubitaceous crops viz., *Cucubita maxima*, *Momordica charantia*, *Lagenaria ciceraria*, *Luffa acutangula* etc.

The design of novel nanoscale tools, may bring molecular systems back to normal operations after their function has been perturbed by disease. The first NP platform was the liposomes. Liposomes have to be applied for gene and drug delivery. Liposomes self-assemble in aqueous systems are spherical vesicles that contain a single or multiple bilayered structure of lipids. Their advantages imparted by liposomes are their diverse compositions, abilities to carry and protect biomolecules, as well as their biocompatibility and biodegradability. These advantages have wide use of liposomes as transfection agents of genetic material into cells (lipofection) in biotechnology research. Polymeric NPs have been formulated to encapsulate hydrophilic and hydrophobic drug molecules, proteins as well as nucleic acid macromolecules [9].

Nanoparticles are embraced to use as a main hormone carrier which have the great increase in ploidy numbers, embryogenesis, induction, and methylation level [10]. Jadhav gave the mycological review with reference to the fungal growth on DPJ by performing the amino acid tryptophan by glyoxylic test which proves the presence of hormone auxin in DPJ from some brassicaceae members. Tryptophan is a hydrophobic and soluble amino acid. Nanoparticles act as the carrier systems of auxins and gibberellins in the plants [11-13]. Leaf proteins are one of the essential components of nutritional food materials and an excellent source for food-grade nanomaterials. Protein solubility is a key factor in assembling protein nanoparticles with desired functional properties in even deproteinised leafy broth [14].

Cabbage, cauliflower, *Raphanus* and fenugreek DPJ contains the nitrogen, calcium and phosphorus content [15]. These mineral elements content were also analysed separately in the pulp, pressed crop, juice and in fraction of leaf protein obtained after the fractionation of 9 crops [16]. Chloroplastic and cytoplasmic proteins were prepared by this method [17].

DPJ also consists of carbohydrares. A significant increase in nitrogen and protein contents of harvested vegetation of *Pisum sativum* was recorded by the application of lucerne DPJ which might be the source of nanoparticles [18]. Inorganic nanoparticles of nitrogen are important due to their role as major plant nutrient. There can be the probability of the availability of synthesized nitrogen nanoparticles from fungal culture filtrates grown on various DPJ [19]. Silver Nitrogen Particles may be a promising novel antimicrobial agent against oral microorganisms as it was found onion DPJ inhibited growth of *Trichoderma* and its enzyme protease. Onion leaves DPJ seems have these nanoparticles [20]. Results contrasted with those of other studies demonstrating that Phosphorus addition decreases metal toxicity for phytoplankton, as DPJ is good source of phosphorus [21].

DPJ during present investigation is consisting of the nanoparticles, will be appreciable as the bacterial contamination inhibitor while using it as the medium for the tissue culture. *Cassia tora* DPJ found retarding the rate of transpiration in drought area crops. In earlier finding, DPJ of beet leaves found as antibiotic for *Penicillium chrysogenum* and *Aspergillus niger* of Ascomycetes growth by inhibiting its protease and amylase which might be because of its nanoparticles source despite the addition of substrates of casein and starch [22].

When the chromosomal studies elucidated in meristematic root tissues of *Celosia* plants, to which the DPJ from antibacterial *Eicchornia* was treated at concentration above 1 %, there were found aberrations because of the presence of nanoparticles [23]. Silver nanoparticles (AgNPs) bearing deproteinised extracts is reported as improving cell division mitotic index at low concentration and caused chromosomal abberetions at high concentrations revealed that there was significant decrease in mitotic index (MI) in *Allium cepa* root meristems which indicates even presence of gold nanoparticles [24-26].

Effects of DPJ stimulated the seed germination and growth rate of paddy seems due to its nanomaterials. It was concluded that smaller nanoparticles produced better germination rate as they are able to penetrate the seeds' pores better and subsequently increase the seeds water uptake in *Helianthus* etc [27]. In earlier findings, from treated, it caused phytotoxic influence on the crop, whereas in case of cowpea at diluted concentration of DPJ, it induced nitrogen content in plants [28]. Phytotoxicity might be because of presence of nanoparticles as seen in radish plants [29]. Silver and gold nanoparticles were founded by earlier reporters in the sprouts of Lucerne. Jadhav, et al. [30] founded the enzyme activity of protease in DPJ of *Colocasia* and radish which revealed the presence of hormone gibberrelin during the seed germination and seedling growth of *Abelmoschus*, pumpkin, *Cyamopsis*, *Cucumis* etc. The presence of enzyme protease indicates the soluble protein present in DPJ which is associated with binding of nanoparticles [31]. DPJ arrested the dormancy of the seeds and there was found appropriate germination.

1.1. Colocasia, Basella and Fenugreek Phytochemistry

The phytochemical analysis of *Colocasia* foliage DPJ confirms the presence of amino acids and vitamins. Phytosterols and phenols are present in foliage extracts. The other phytochemicals are saponins, terpenoids, flavonoids, tannins, steroids and alkaloids [32].

Fenugreek forage have various carbohydrates, proteins, phenols, alkaloids, flavonoids as major active constituents. The antimicrobial activity of stem of plant has been evaluated to determine the Phyto-constituents present in plants namely tannin, terpene, steroid, saponin, carbohydrate [33]. *Basella rubra* study confirms having the potential antimicrobial activity of its ethanolic extract [34].

2. MATERIALS AND METHODS

2.1. Release of DPJ

Foliages were taken into consideration for making the tissue pulp. The tissue pulp is squeezed to form juice or extract and the residue obtained on muslin cloth called pressed crop residue (PCR). Various extracts from the crops viz., Cabbage *(Brassica oleracia var capititis* L.*)*, Cauliflower *(Brassica oleracia var botrytis* L.*)*, radish *(Raphanaus sativus* L.*)*, Dasheen *(Colocasia esculentus* L.*)*, Climbing spinach *(Basella alba* L.*)* and leguminous fenugreek *(Trigonella foenicum graceum* L.*)* were taken into consideration for heating at 95° C, and filtered by whatman filter paper after cooling. The liquid obtained as supernatant is deproteinised juice (DPJ). The precipitated residue obtained in whatman filter paper after filtration is Leaf protein concentrate (LPC).

2.2. Green Synthesis of Ag Nanoparticles in DPJ

2 ml of the DPJ broth was added to 1 ml of $AgNO_3$ solution and kept at 26°C till the reaction is completed. After 3-5 minutes, the solution developed the yellowish brown colour. The nanoparticles were observed under the light microscope by taking a drop of DPJ by the added silver nitrate solution on the slide.

The reduction of ions in solution in the aliquots of aqueous component was measured under UV-Vis spectrophotometer of the solution. The nanoparticle solution consists of high optical density therefore was diluted 20 times with distilled water to avoid errors. A UV-Vis spectrum of the solution is measured in 10-mm-optical-path length quartz cuvettes at a resolution of 1 nm between 300 and 600 nm for 2 min. Excited band was near 530 nm for silver. The solution was stored at 4° C for further analysis.

3. RESULTS AND DISCUSSION

The yellowish-brown color in water, arise due to excitation of surface plasma on vibrations.

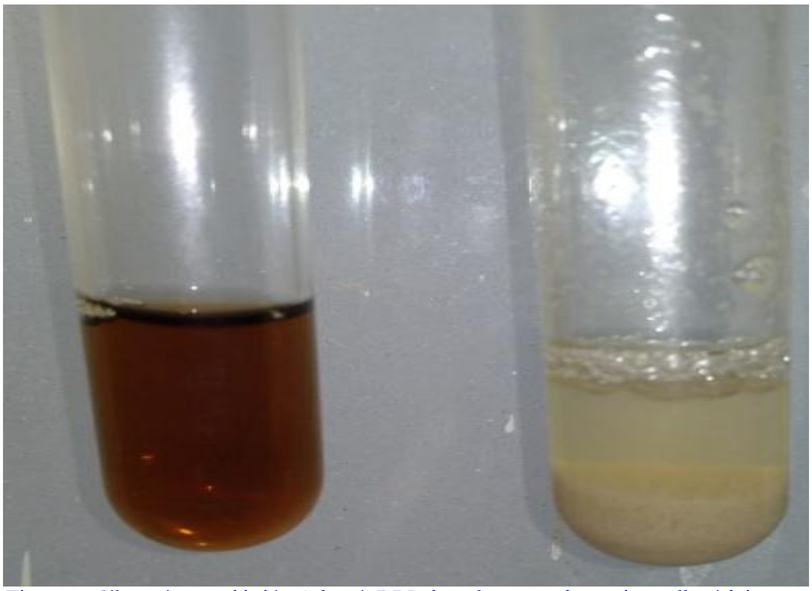

Figure-1. Silver nitrate added in *Colocasia* DPJ, the colour was changed to yellowish brown.

After half an hour, the silver nanoparticles was measured by UV-vis spectra in different samples of DPJ. *Colocasia* found serving as good source of synthesis of silver nanoparticles. There was formation of the metal nanoparticles by reduction of the aqueous metal ions during exposure of the DPJ broth. After adding the DPJ broth to silver nitrate solution, immediately a yellowish brown color forms indicating that silver nanoparticles are been formed by settling matter at the bottom shown in Figure 1. In previous experiment, *Colocasia* and radish DPJ when were treated for the source of phytohormones, or for enzyme source, it proved that retardation of the seed dormancy was taking place.

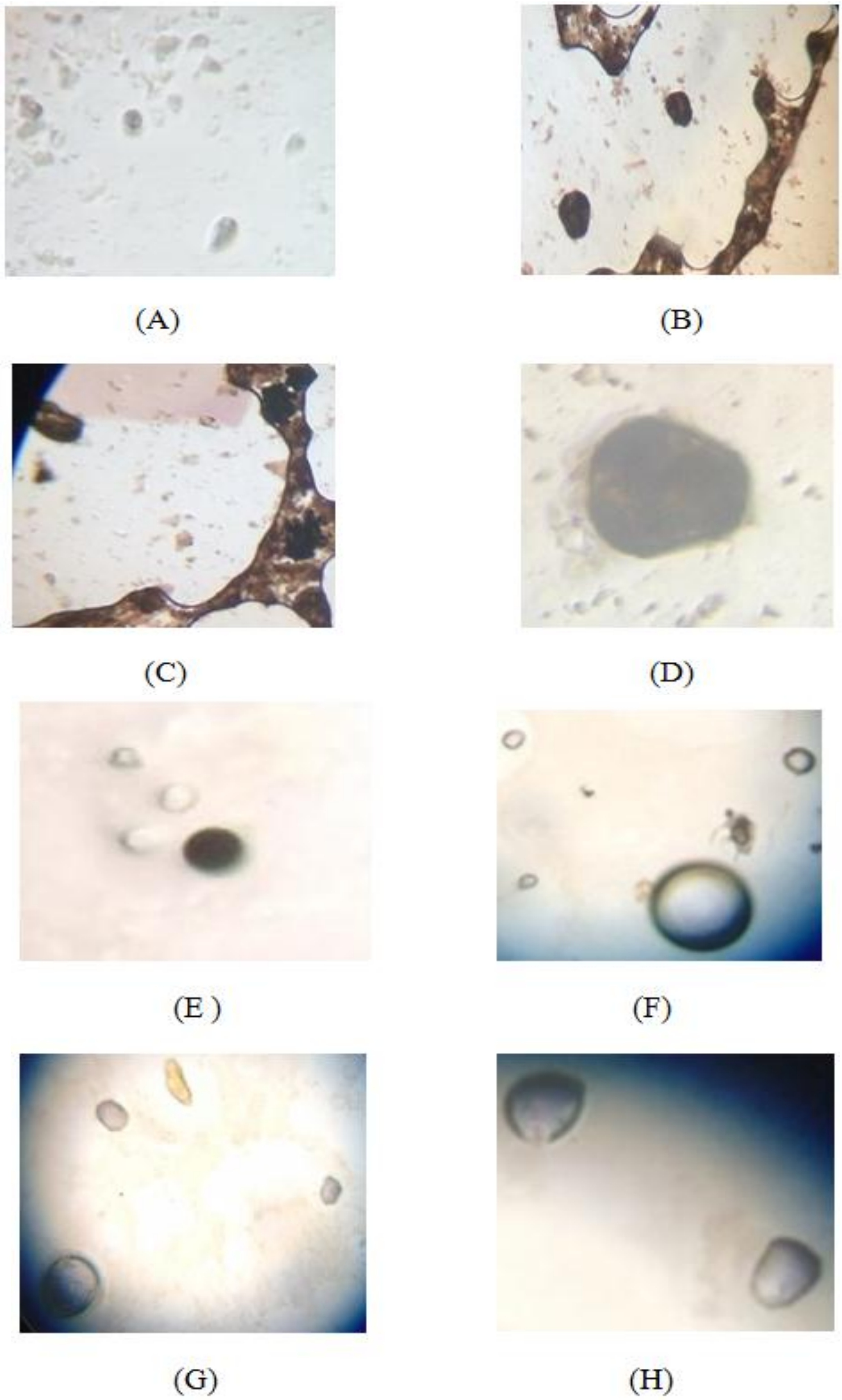

Figure-2. Microscopic images of silver nanoparticles found in the DPJ of various leaves (A) Irregular or triangular shaped nanoparticles from DPJ of cauliflower leaves (B) Irregular shaped NP from DPJ of cabbage leaves (C) Traingular shaped AgNP from cabbage foliage DPJ (D) Hexagonal or irregular shaped AgNP from foliage of radish DPJ. (E) Spherical shaped Silver Nanoparticles from the DPJ of *Colocasia*. (F) Spherical shaped NP in *Colocasia* DPJ (G and H) Spherical and irregular shaped NP in *Colocasia* DPJ.

Raphanus sativus var. longipinnatus deproteinised leaf extract can also be the source of Cobalt nanoparticles but result appeared its irregular and slight hexagonal silver nanoparticles [35].

Table-1. Shapes of the silver nanoparticles found under the microscope in the deproteinised leaf juice of various foliages by green crop fractionation.

No.	DPJ	Shape of the silver nanoparticles
1	Cabbage	Irreglar, triangular
2	Cauliflower	Irregular and triangular
3	Radish	Irregular and slight hexagonal
4	*Colocasia*	Spherical, hexagonal, irregular and triangular

Cabbage, *Colocasia* and cauliflower leafy DPJ were already used as the liquid broth medium for fungal growth but their antifungal properties yet not investigated/ reported during present status. There can be inhibition of fungal growth at high concentration of DPJ. But, there are the reports that the silver nanoparticles synthesized by using the vegetable cauliflower and cabbage analysis, which found consisting of its antibacterial property [36-38].

Table-2. Silver Nanoparticles in DPJ from various crops resonance at various wavelengths by UV visible spectrophotometry.

No	DPJ	Wavelengths (nm)			
		300	**400**	**500**	**600**
1	Cabbage	0.096	0.612	1.902	1.987
2	Fenugreek	0.260	0.488	1.578	0.088
3	Radish	0.207	0.471	0.835	0.868
4	Cauliflower	0.256	1.867	1.789	1.960
5	*Colocasia*	0.159	0.551	1.715	0.226
6	*Basella*	0.094	1.898	1.982	1.272
	Mean	0.178	0.981	1.633	1.066
	Standard deviation	0.074	0.700	0.415	0.823

DPJ of various foliages followed for quantification of nanoparticles by UV–vis spectroscopy. Silver nanoparticles were formed Figure 2 A to H. The nanoparticles observed were mostly of spherical, irregular, triangular and very less hexagonal sized as illustrated in Table 1. It is observed that as depicted in Table 2, the overall occurred 500 and 600 nm. Surface plasma on resonance band of cabbage occurred at the wavelength of 600 nm, fenugreek on 500 nm, radish on 600 nm, cauliflower on 600 nm, *Colocasia* on 500 nm and *Basella* on 500 nm respectively. *Basella* DPJ found lowering the proline content as compared with control in wheat crop seedlings. Because of the presence of the nanoparticles, *Basella* extract is used as antibacterial for humans. Graphical illustration shown in Figure 3 A to F. *Colocasia* serves as a good source of synthesis of silver nanoparticles Figure E to H. The UV vis spectra of silver nanoparticles taken after 30 minutes are analyzed Table 2. It was found that the DPJ of fenugreek and *Basella* contains the presence of amino acids tryptophan and phenylalanine which are precursors of IAA and salicylic acid. Enzyme productivity found at appreciable amount in earlier research without adding any substrates when various fungi were grown on DPJ broth medium for industrial purpose [39].

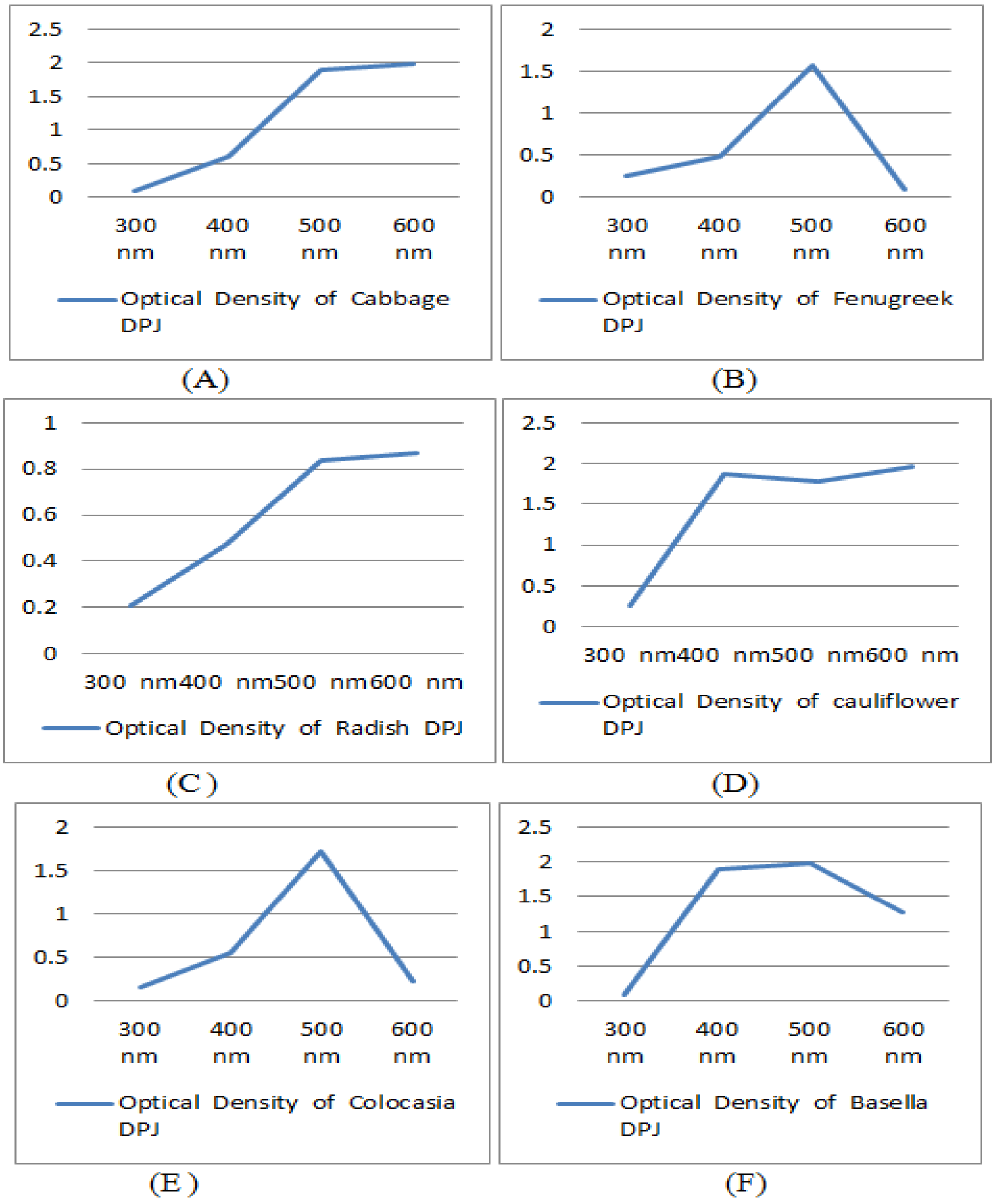

Figure-3. Calibration plot curves of the Silver surface plasma on resonance band (Optical Density) optimization of different DPJ made up of Foliages occurred at 300 to 600 nm wavelengths (A) Cabbage occurred at 600 nm, (B) Fenugreek on 500 nm, (C) Radish on 600 nm, (D) Cauliflower on 600 nm, (E) *Colocasia* on 500 nm and (F) *Basella* on 500 nm.

4. CONCLUSION

Various deproteinised leaf extracts were optimistic and served as a good source for synthesis of silver nanoparticles, which can be separated from the solution and used commercially. It was the unanswered question that, which constituent responsible to cause aberrations in plants by the treatment of DPJ. This experiment proved that there was some contribution of nanoparticles because of DPJ treatment at different or higher doses. The fungi grown on DPJ and their culture filtrates were taken into consideration for NP purpose work is in progress. DPJ from *Allium cepa* inhibitited protease enzyme of *Trichoderma* reveals presence of more nanoparticles. Therefore it is a novel attempt which reveals that DPJ contains nanoparticles. DPJ had been utilised for the tissue culture and organogenesis, as nanoparticles could inhibit contaminations. As the DPJ already used as the medium broth to cultivate fungi, yeast and as the manure for plant growth, therefore the process of GCF is proved as more economical because of the presence of NPs.

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