Green synthesis of silver nanoparticles from coriander peel and its biological effect

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Abstract: Bio-nanotechnology has emerged as integration among biotechnology and nanotechnology for growing biosynthetic and environment-friendly technology for synthesis of nanomaterials. Different sorts of nanomaterials like copper, zinc, titanium, magnesium, gold, alginate and silver were diagnosed however silver nanoparticles have proved to be only because it has good antimicrobial efficacy against bacteria, viruses and different micro-organisms. Conventionally silver nanoparticles had been synthesized by the aid of using chemicals as reducing agents which later on become accountable for various biological risks due to their general toxicity; engendering the serious concern to develop eco-friendly processes. Thus, to remedy the objective; biological approaches are coming up to fill the void; for instance green synthesis using biological molecules derived from plant sources in the form of extracts exhibiting superiority over chemical and/or biological methods. These plant based biological molecules undergo highly controlled assembly for making them suitable for the metal nanoparticle synthesis. Silver nanoparticles possess unique properties which find vast of applications.
This paper aims to synthesis of silver nanoparticles from Coriender Peel and their applications in Pharmaceuticals.

Index Terms*:* Silver nanoparticles (AgNPs) Synthesis, Genotoxicity, Catalase Assay, Antioxidant activity, DPPH Assay.

I. INTRODUCTION:-

Bio-nanotechnology has integration among biotechnology & nanotechnology for growing environment-friendly technology for synthesis of nanomaterials. Due to different physiochemical properties of nanomaterials, they provide scientific and industrial applications. Its size ranges from 1to100 nm and shape vary from circular, pentagonal, icosahedra. Nanoparticles exhibits completely new or improved properties based on specific characteristic such as size, distribution and morphology. The parameters of nanoparticles vary their shape, size. Nanoparticles are presented as an aerosol (solid or liquid phase in air), a suspension (solid in liquid), or emulsion (two liquid phases). Inorganic nanoparticles have been examined for medical imaging as well as for treatment. In nature most of the nanoparticles are synthesized by biological process. The use of environmentally benign material like plant leaf extract, bacteria, fungi, and enzyme for synthesis of silver nanoparticles offers numerous benefits of eco friendly and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for synthesis protocol. Plant mediated nanoparticle synthesis is easy and safe with one step protocol. Biosynthesis of silver nanoparticles using plant extracts is an excellent approach in green nanotechnology. The metabolite obtained from the plants induces the production of silver nanoparticles in eco-friendly manner. There is a growing interest in inorganic nanoparticles due to their size features and advantages over available chemical imaging drugs agent and drug. Inorganic nanoparticles have been examined for medical imaging as well as for treatment. There are two approaches for nanoparticles. Synthesis namely bottom to up approach and top down approach. The top bottom approach, scientist tries to formulate nanoparticles using larger ones to direct assembly. The bottom top approach is process that builds towards larger and more complex system by starting at molecular level and monitoring precise control on molecular structure. Plant mediated nanoparticle synthesis is easy and safe with one step protocol.

Selection of plant -

Coriander sativum belongs to the family Apiaceae. The nutritional profile of coriander seeds is different from the fresh stems and leaves. Leaves are particularly rich in Vitamin, Vitamin C, Vitamin K.

X-ray diffraction of nanoparticles –

The AgNP solution was repeatedly centrifuged at 5000 rpm for 20 min, re-dispersed with distilled water and lyophilized to obtain pure AgNPs pellets. The dried mixture of AgNPs was collected to determine the formation of AgNPs.

Genotoxicity of roots -

Historically plants have been used as indicator organisms, in studies on mutagenesis in higher eukaryotes. Plant systems have a variety of well-defined genetic endpoints including alterations in ploidy, chromosomal aberrations, and sister chromatid exchanges (9). *Allium cepa* L. is the common onion and is widely used in all parts of the world as flavouring vegetable. The use of the *A. cepa* root length inhibition bioassay as a sensitive, cost effective and valid indicator of toxicity test. *Allium cepa* has been used for evaluating chromosomal aberrations since 1920s. There are still many unresolved issues and challenges concerning the biological effects of nanoparticles. Therefore, the present study indicates cytotoxic and genotoxic impacts of silver nanoparticles on *A. sativum*.

Catalase assay -

The UV absorption of hydrogen peroxide can be measured at 240nm, whose absorbance decreases when degraded by the enzyme catalase. From the decrease in absorbance, the enzyme activity can be calculated.

Lipid peroxidation assay -

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process in which free electrons from the lipids in the cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene bridges that possess especially reactive hydrogens. Oxidative damage is a major source of mutation load in living organisms. Being highly reactive, the hydroxyl radical is the predominant ROS that targets DNA. DNA damage can result in single or double strand

breakage, base modifications and DNA cross linking. Cell death, DNA mutation, replication errors and genomic instability can occur if the oxidative DNA damage is not repaired.

The sensitivity of measuring Thiobarbituric Acid Reactive Substances (TBARS) has made this assay the method of choice for screening and monitoring lipid peroxidation, a major indicator of oxidative stress. This rapid, easy-to-use procedure has been modified by researchers for use with many types of samples including drugs, food products and human and animal biological tissues. Biological specimens contain a mixture of thiobarbituric acid reactive substances (TBARS), including lipid hydroperoxides and aldehydes, which increase as a result of oxidative stress. TBARS return to normal levels over time, depending upon the presence of anti-oxidants. In practice, TBARS are expressed in terms of malondialdehyde (MDA) equivalents. It has long been recognized that high levels of free radicals or reactive oxygen species (ROS) can inflict direct damage to lipids. The primary sources of endogenous ROS production are the mitochondria, plasma membrane, endoplasmic reticulum and peroxisomes.

II. MATERIAL AND METHODS:-

Plant material and preparation of extract –
Coriander were used as source of plant to make the use of aqueous extract. 30g of Coriander peels were crushed in 100ml sterile distilled water. Then the mixtures were filter through the Whatman paper.

Synthesis of silver nanoparticles –
1mM aqueous solution of Silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 25ml of filtered extract was mixed with 25ml of 1mM silver nitrate solution respectively and kept it in dark condition for 3 days.

UV spectra analysis –
The reduction of pure Ag+ and Cu^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after 3 days from 200nm to 600nm range. The 500ml solutions after reaction were centrifuge at 8000rpm for 15min. The resulting supernatant was again centrifuge at 8000rpm for 15min. Thereafter, the pellet was allowed to dry to obtain dried powder. The dried nanoparticles were not miscible in sterile distilled water therefore sonicate the particles using sonicator for 10min.

X-ray diffraction of nanoparticles –
X-Ray Diffraction of silver nanoparticles was done. The size of the nanoparticles was calculated using Scherer's formula -
$d= (0.91*\lambda)/ (B* \cos \theta)$.

Single stranded breaks in dna –
Following additions were done-

Reagent	Control	Test (Nanoparticles)
Plasmid (20ng/µl)	5µl	5µl
Sterile distilled Water	15µl	-
Nanoparticles	-	15µl
6X Loading dye	4µl	4µl
Total	24µl	24µl

The reaction was incubated at 37°C for 2 hours and 3 hours separately. The above samples were loaded on 1.2% agarose gel and subjected to electrophoresis to observe bands of plasmid.

Broth assay –
0.1 O.D. culture of *E. coli* was prepared in saline. 3ml of sterile LB broth was taken in sterile saline tube. 100µl of bacterial suspension was added in each tube. Then different volumes of nanoparticles were added in a tube. The growth pattern was observed after 24 hrs of incubation at 37°C.

Genotoxicity of silver nanoparticles –
The silver nanoparticles were suspended directly in deionised water and dispersed by ultrasonic vibration (100w, 30 kHz) for 5 min to produced two different concentration at 50ug/ml, 100ug/ml. *Allium sativum* placed in soil rite at room temperature. When roots reached 2 to 3 cm in length they were treated with different concentration of silver nanoparticles suspension for 2hrs and 4hrs.

- Microscopic Examination - The slides were analyzed for cytological changes. The mitotic index was calculated as the number of dividing cells per number of 1000 observed cells. The number of aberrant cells was noted per total cells scored at each concentration.

$$\text{Mitotic index (MI)} = \frac{T_{DC}}{T_C} \times 100$$

Whereas,
T_{DC} = Total number of dividing cell
T_C = Total number of cells observed

Catalase assay -
Treat the *A. sativum* roots for 4 hrs at 100ug/ml of coriander silver nanoparticles. Crush 5-6 treated roots from each concentration in 250ul phosphate buffer. Then centrifuge at 5000rpm for 5 min and kept it on ice. Take out the supernatant into fresh tube.

	Phosphate buffer (ml)	Test sample (ml)	Hydrogen peroxide (ml)
Control	1	0.05	0.01
Nanoparticles	1	0.05	0.01

Check the O.D of samples at 240nm.

Lowry assay –
The protein content is estimated by Lowry as follows:
Chemicals – 1) Lowry A – Alkaline sodium carbonate solution (2g) is dissolved in 0.1N sodium hydroxide and volume is made up to 100ml with distilled water.
2) Lowry B – Copper sulphate solution (0.5g) is dissolved in 1g% solution of potassium sodium tartarate and volume is made up to 100ml with distilled water.
3) Lowry C – This is prepared fresh before use by mixing 50ml of Lowry (A) and Lowry (B).
4) Reagent D – Folin – Ciocalteau.

5) Standard Bovine Serum Albumin (BSA) – 0.2mg/ml

Following additions were done to estimate the protein concentration in the sample:-

<table>
<tr><th>Concentration of BSA(ug)</th><th>Std.BSA (0.2mg/ml)</th><th>DW (ml)</th><th>Lowry C (ml)</th><th></th><th>FCR Reagent (ml)</th><th></th><th>Absorbance</th></tr>
<tr><td>0</td><td>0</td><td>1</td><td>3</td><td rowspan="8">Mix well and Keep it for 15min at RT</td><td>0.5</td><td rowspan="8">Mix well and Keep it In dark for 40min</td><td>0.04</td></tr>
<tr><td>40</td><td>0.2</td><td>0.8</td><td>3</td><td>0.5</td><td>0.17</td></tr>
<tr><td>80</td><td>0.4</td><td>0.6</td><td>3</td><td>0.5</td><td>0.32</td></tr>
<tr><td>120</td><td>0.6</td><td>0.4</td><td>3</td><td>0.5</td><td>0.41</td></tr>
<tr><td>160</td><td>0.8</td><td>0.2</td><td>3</td><td>0.5</td><td>0.55</td></tr>
<tr><td>200</td><td>1.0</td><td>0</td><td>3</td><td>0.5</td><td>0.64</td></tr>
<tr><td>Control</td><td>0.05</td><td>0.095</td><td>3</td><td>0.5</td><td>0.25</td></tr>
<tr><td>Coriander nanos</td><td>0.05</td><td>0.095</td><td>3</td><td>0.5</td><td>0.24</td></tr>
</table>

Lipid peroxidation assay -

The level of lipid peroxidation was determined by measuring the amount of MDA. About 0.1 gm of root tissues from the control and treated (100ug/ml) groups were cut into small pieces and homogenized by the addition of 1ml of 5% trichloroacetic acid (TCA) solution. The homogenates were then transferred into fresh tubes and centrifuged at 12000 rpm for 15 min at room temperature. Equal volumes of supernatant and 05% thiobarbituric acid in 20% TCA solution were added into a new tube and boiled at 96^0C for 25 min. The tubes were transferred to on ice-bath and then centrifuged at 10000rpm for 5 min. The absorbance of the supernatant was measured at 532 nm.

DPPH-free radical scavenging activity of synthesized AgNPs

The free radical scavenging activity of AgNPs was evaluated on the potency to scavenge the synthetic DPPH. When DPPH free radicals accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The standard ascorbic acid, plant extract and biosynthesized AgNPs of Coriander peel showed concentration dependent radical activity i.e.100μg/ml respectively; similar findings were reported by Ramesh and Rajeshwari (2015). The synthesized AgNPs showed activity closer to control ascorbic and superior response compared to plant extract. The AgNPs can effectively sacavenge DPPH free radicals in a concentration dependent manner.

III. RESULT AND DISCUSSION:-

Synthesis of silver nanoparticles –

As coriander peels extract was mixed in aqueous solution of silver ions complex, it showed change in colour from orange to brown due to reduction of silver ions which indicates formation of silver nanoparticles. The absorption spectra of silver nanoparticles formed in coriander peel extract reaction has absorbance peak at 323 nm.

Fig 1:- Coriander silver NPs Change in colour

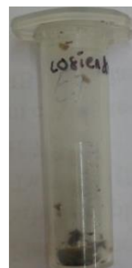


Fig 2:- Coriander silver NPs

Observation of UV spectra –

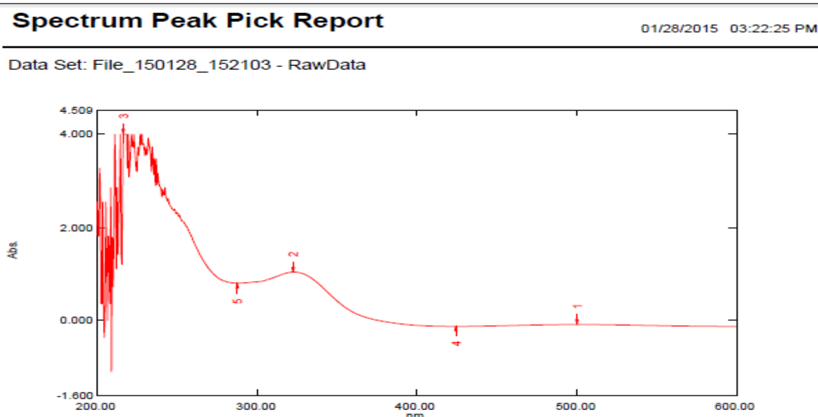


Fig 3:- Coriander peel in 1mM Silver nitrate solution

X-ray diffraction pattern of nanoparticles –

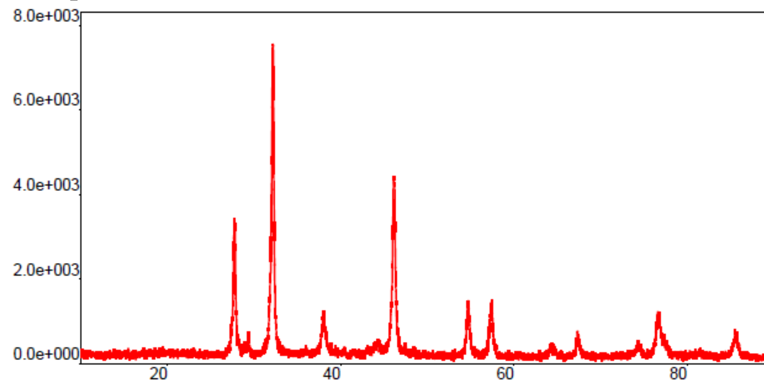


Fig 4:- X-Ray diffraction of Coriander silver NPs.

Calculation -

d = (0.91*λ)/ (B* cos θ)
= (0.91*0.154)/ (0.00480*0.8310)
= 35.13nm

Broth assay –

The O.D of the tube was taken at 600nm.

Table 1:- Broth assay of coriander NPs.

Volume of orange nanoparticles added	*E. coli*
0 (control)	0.91
0.02 ml	1.16
0.04 ml	1.16
0.06 ml	1.10
0.08 ml	1.52
0.10 ml	1.44

After 24hrs of incubation it is observed that Silver nanoparticles does not inhibit the growth of bacteria.

Single stranded breaks in DNA –

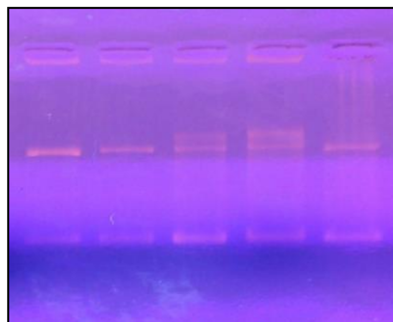


Fig 5:- Band Pattern Observed

Super coiled DNA plasmid convert to linear DNA.

Table 2:- Addition of coriander silver NPs into wells.

Well	1	2	3	4	5
Samples	Control	Coriander silver nanos R.T. (2 hrs incubation)	Coriander silver nanos R.T. (3 hrs incubation)	Coriander silver nanos 60^0C (2 hrs incubation)	Coriander silver nanos 60^0C (3 hrs incubation)

Genotoxicity on roots -

Silver nanoparticles induced chromosomal aberrations in root tip cells *A. sativum.*

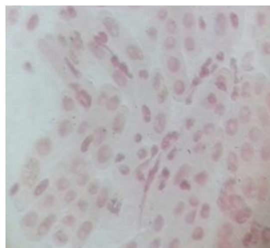


Fig 6:- Control

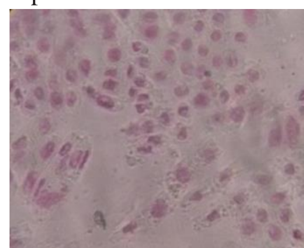


Fig 7:- Coriander NPs (100ug/ml) 2 hrs treated cells

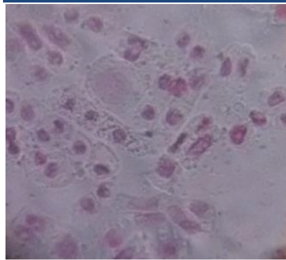


Fig 8:- Coriander NPs (50ug/ml) 4 hrs treated cells

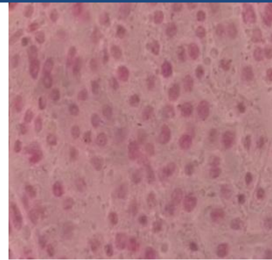


Fig 9:- Coriander NPs (100ug/ml) 4 hrs treated cells

Table 3:- The *A. sativum* roots were treated with NPs at 50ug/ml, 100ug/ml concentration for 2 hrs and 4 hrs. The following phases are observed.

Nanoparticles	Time	Concentration of NP's	Phases observed
Control	-	-	Prophase, Anaphase, Telophase
Coriander NP's	2 hrs	100ug/ml	Prophase, Abnormal
Coriander NP's	4 hrs	50ug/ml	Prophase, Telophase, Abnormal
Coriander NP's	4 hrs	100ug/ml	Prophase, Telophase, Abnormal

Table 4:- Mitotic index are as follows

Nanoparticles	Time	Concentration of NP's	Mitotic index (%)
Control	-	-	58.29
Coriander NP's	2 hrs	100ug/ml	38.10
Coriander NP's	4 hrs	50ug/ml	40
Coriander NP's	4 hrs	100ug/ml	28.34

No chromosomal aberration was observed in the control (untreated onion root tips) and the mitotic index (MI) value was 58.29%. Mitotic index was the lowest, 28.34% for coriander NP's at 4 hrs, 100ug/ml concentration. With increasing concentration of the nanoparticles, concentration dependent decrease in the mitotic index was noticed. The effect of silver nanoparticles concentration on mitotic index was significantly different for 50ug/ml, 100ug/ml concentration as compared to the control.

Catalase activity of treated roots -

The *A. sativum* roots were treated with coriander silver nanoparticles at concentration of 100ug/ml for 2 hrs and 4 hrs.

Table 5:- 2 hrs treated *A. sativum* roots at 100ug/ml concentration.

	Time in min	0 min	30 sec	1 min	1.30 min	2 min	2.30 min	3 min
Control	Absorbance	0.199	0.195	0.192	0.198	0.196	0.192	0.190
Coriander NPs	Absorbance	0.200	0.197	0.193	0.191	0.189	0.186	0.183

Table 6:- 4 hrs treated *A. sativum* roots at 100ug/ml concentration.

	Time in min	0 min	30 sec	1 min	1.30 min	2 min	2.30 min	3 min
Control	Absorbance	0.150	0.147	0.141	0.140	0.137	0.136	0.133
Coriander NPs	Absorbance	0.172	0.170	0.167	0.165	0.164	0.161	0.157

Formula for catalase activity are as follows:

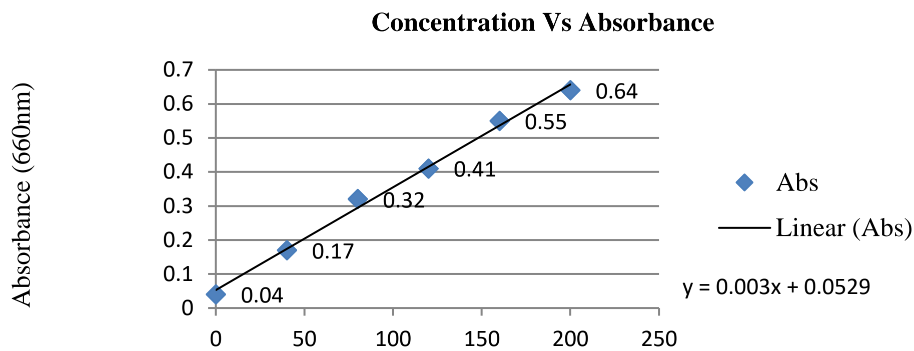
$$U/mg = \frac{(A_0 - A_{60}) \times V_t}{\mathcal{E}_{240} \times d \times V_s \times C_t \times 0.001}$$

Whereas,

$(A_0 - A_{60})$ = The difference between the initial and final absorbance.
V_t = The total volume of the reaction
$\mathcal{E}_{240}$ = The molar extinction coefficient for H_2O_2 at O.D. 240nm.
d = The optical length path of cuvette (1cm).
V_s = The volume of the sample in ml.
C_t = The protein concentration of the sample in mg/ml.

Table 7:- Standard graph of Lowry are as follows

Concentration of BSA (ug)	Absorbance
0	0.04
40	0.17
80	0.32
120	0.41
160	0.55
200	0.64



From the graph, equation of line is, y = Concentration of BSA (ug)

Table 8:- The concentration of protein are as follows

	Protein concentration (mg/ml)
Control	0.066
Coriander nanoparticles	0.062

Table 9:- The catalase activity for 2 hrs treated roots and control are as follows

Samples	**Catalase acitivity (U/mg)**
Control	69.23
Coriander nanoparticles	141.66

Table 10:- The catalase activity for 4 hrs treated roots and control are as follows

Samples	**Catalase acitivity (U/mg)**
Control	130.76
Coriander nanoparticles	125

The protein concentration decreases as compared to control but the catalase activity increases as compared to control.

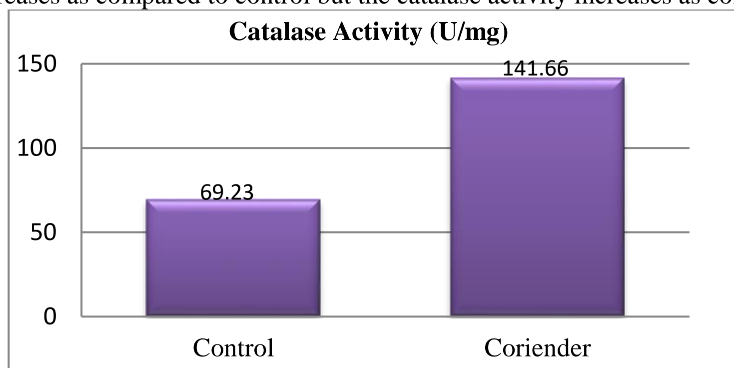


Fig 10:- Bar diagram of catalase activity for 2 hrs treated roots

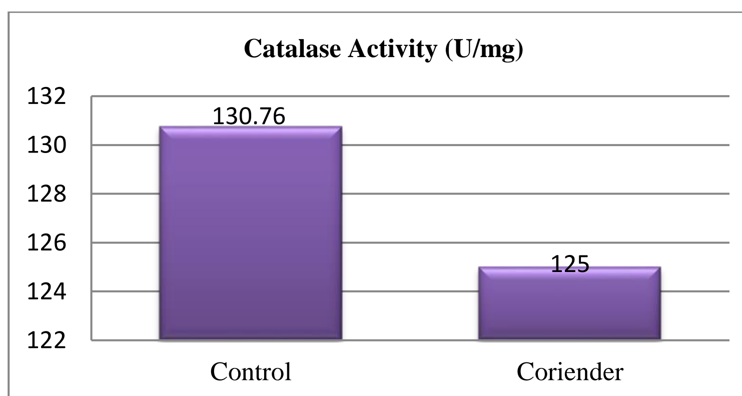


Fig 11:- Bar diagram of catalase activity for 4 hrs treated roots

Lipid peroxidation of treated roots -

The *A. sativum* roots were treated with coriander NPs having concentration 100ug/ml for 4 hrs.

Table 11:- Lipid peroxidation of coriander NPs.

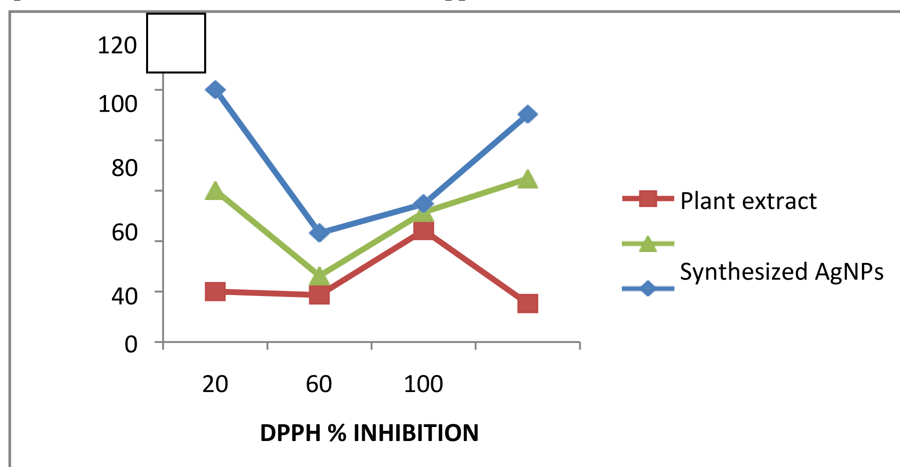
Samples	**Absorbance at 532nm**
Control	0.075
Coriander nanoparticles	0.264

DPPH-free radical scavenging activity of synthesized AgNPs –

Table 12:- DPPH-free radical scavenging activity of synthesize AgNPs of Coriander Peel.

Plant Name	Concentrations µg/mL	Plant extract	Synthesized AgNPs	Ascorbic acid
Coriander sativum	20	18.70^{c}	44.20^{c}	15.23^{c}
	60	26.23^{b}	51.50^{b}	64.77^{b}
	100	31.00^{a}	63.87^{a}	78.90^{a}

***Note-**All the value are calculated as mean. Means followed by different alphabet within a column are significantly different ($p<0.05$). For more information refer to appendix.



IV. CONCLUSION:-

The reduction of silver ions present in aqueous solution of silver nitrate during reaction with coriander peel extract observed by UV spectrophotometer revealed the presence of silver nanoparticles. The study showed a simple, rapid, economical route to synthesized silver nanoparticles. The formation of orange silver NPs was confirmed by XRD analysis and the size was calculated to be 35.13 nm. Super coiled DNA plasmid converts to linear DNA. With increasing concentration of the nanoparticles, concentration dependent decrease in the mitotic index was noticed. The silver nanoparticles exhibited cytotoxicity by decreasing the mitotic index in a dose dependent manner. Silver nanoparticles could penetrate plant system and may interfere with intracellular components, causing damage to cell division. The extracted Silver nanoparticles have free radical scavenging potential.

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Competing Interests: The authors declare that they have no competing interests.

V. REFERENCES:-

1) Ali R Vahdati and Babak Sadeghi, A study on the assessment of DNA strand-breaking activity by silver and silica nanoparticles, Journal Of Nanostructure in Chemistry, 3:7, 2013.
2) Anamika Mubayi et al, Evidence based green synthesis of nanoparticles, Advanced Materials Letters,Vol. 3(6), 519-525, 2012.
3) Cavusuglu et al, Investigation of Toxic Effects of the Glyphosate on *Allium cepa,* Journal of Agricultural Sciences, 131-142, 19 August 2011.
4) Daniel et al., Effect of Cassava Processing Effluents on Antioxidant Enzyme Activities in *Allium Cepa* L., Nigerian Society for Experimental Biology, Vol. 23, No. 2, 49 – 61, 30June , 2011.
5) Gregor P Jose et al, Singlet oxygen mediated DNA degradation by copper nanoparticles: potential towards cytotoxic effect on cancer cells, Journal of Nano-biotechnology, 9:9, 2011.
6) Juarez et al, Accumulation of silver nanoparticles and its effect on the antioxidant capacity in *Allium cepa* L, International journal of experimental botany, 82: 91-97, 2013.
7) Kyung et al, Oxidative DNA Damage from Nanoparticle Exposure and Its Application to Workers' Health: A Literature Review, Safety and Health at Work, Vol. 4, 177-186, 26 July 2013.
8) L S Sosenkova, E M Egorova, The effect of particle size on the toxic action of silver nanoparticles, Journal of Physics, IOP Publishing, 2011.
9) Mamta Kumari et al, Genotoxicity of silver nanoparticles in Allium cepa, Science of the Total Environment, 5243–5246, 17 July 2009.
10) Manal A. Awad et al., Silver nanoparticles biogenic synthesized using an orange peel extract and their use as an anti-bacterial agent, International Journal of Physical Sciences, Vol. 9(3), pp. 34-40, 9 February, 2014.

11) Mukesh Tiwary & Ashok Kumar Jha, Biosynthesis of Silver nanoparticles using plant extracts; New approach in Agriculture & Pharmaceuticals: A Review, Int J Innov Res Multidiscip Field 3 (6), 1-7,May2017.
12) Odjegba VJ, Adeboye RO, Oxidative stress and DNA damage to root cells of *Allium cepa* L. by Copper and lead induction, Indian Journal of Innovations and Developments, Vol. 2 ISSN 2277 – 5382, March 2013.
13) P. Balashanmugam1 et al, Biosynthesis of Silver Nanoparticles from Orange Peel Extract and its Antibacterial Activity against Fruit and Vegetable Pathogens, International Journal of Innovative Research in Science & Engineering, ISSN (Online) 2347-3207.
14) Paulina Soto et al, Assessment of catalase activity, lipid peroxidation, chlorophyll-*a*, and growth rate in the freshwater green algae *Pseudokirchneriella subcapitata* exposed to copper and zinc, 39(2): 280-285, 2011.
15) Raghavendra. et al, Dignostics and therapeutic application of gold nanoparticles, International Journal of Pharmacy and Pharmaceutical Sciences, Vol 6 (2), ISSN- 0975-1491, 2014.
16) Rekha Dunpall, An *in vitro* assessment of the potential toxicity of Cadmium Selenide nanoparticles, February 2012.
17) Sebastian Ahlberg et al., PVP-coated, negatively charged silver nanoparticles: A multi-center study of their physicochemical characteristics, cell culture and in vivo experiments, Journal of Nanotechnology, 5, 1944–1965, 2014.
18) Talger Shaymurat et al, Phytotoxic and genotoxic effects of ZnO nanoparticles on garlic (Allium sativum L.): A morphological study, Nanotoxicology, Early Online, 1–8, 7 March 2011.