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PHYTOSYNTHESIS OF SILVER NANOPARTICLES BY LEAF EXTRACT OF *LEEA CRISPA* AND ITS ANTIOXIADANT ACTIVITY

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ABSTRACT: The integration of successful nanoparticles into biomedical applications needs modifications to their surface morphology that govern their interaction with the biological network and reduce toxicity to the environment. The present investigation focuses on the biosynthesis of silver nanoparticles using aqueous leaf extract of *Leea crispa* L. Silver nanoparticles were characterized by UV-Vis spectroscopy, Atomic Force Microscopy, X-ray Diffractometry, Zeta-Potential, HR-TEM analysis. The synthesized AgNPs were showed dark brown color at pH 8 and further confirmed by the character study of UV-Vis spectrum showed the resonance peak λ max of 420nm. FTIR peaks at 1624^{-1} and 1384 cm^{-1} majorly involved in that reduction of Ag+ ions into Ag ^{0} ions. Secondary metabolites were present in the aqueous extract involved in the capping and stabilization of molecules during the synthesis of silver nanoparticles. The Atomic Force Microscopic studies revealed that polydispersed nanoparticles were 10-80 nm in size with spherical shape. The XRD data depicted the crystalline nature of nanoparticles and an average minimum size of particles is 5 nm. HR-TEM and Zeta Potential analyses were conducted to determine the shape and dispersion stability of the particles. Further, the antioxidant efficacy of silver nanoparticles was evaluated. Silver nanoparticles exhibited free radical scavenging activity with an IC50 is 33.31µg/ml as determined by the DPPH assay. It is found that the results showed that inversely proportional to the concentration. As a result, we have developed an approach to synthesize silver nanoparticles by the green route and evaluated their antioxidant activity.

Keywords: Silver nanoparticles, Leea crispa, Antioxidant activity, DPPH.

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1.INTRODUCTION

Nowadays, Nanotechnology has been proved that a promising field in biomedicine [1]. Nanotechnology represents the design, synthesis, and characterization of nanoparticles at 1-100 nm. Nanoparticles are the building blocks of nanotechnology [2-4]. In recent year study of nanoparticles have become an outstanding field in research by its myriad application in various science fields [5]. Nanoparticles were extensively synthesized done by chemical, physical, and biological methods by using inert metals like silver, gold, and platinum. Researches and studies in the field of nanotechnology have been rapidly developing towards accepting eco-friendly methods for the synthesis of nanoparticles because of its low-cost, short period. Green synthesis of nanoparticles was synthesized by using several inorganic metals. Among them, silver nanoparticles were reported as ideal particles because of their known several numerous biological applications [6-8]. Synthesis of silver nanoparticles still draws unprecedented attention due to its simplicity process [9-10], with the towering environmental concerns, researchers gaining interest in synthesizing the nanoparticles using biological systems [11], such as Microbes [12-14], Plants [15-21]. Plant extracts have been placed at a higher rank than other biological processes in nanoparticle synthesis as they are easy to collect, safe to handle, and more useful than the other processes[22]. Many researchers are reported various parts of plant extracts are used to synthesize nanoparticles such as; Root extract of *Astragalus tribuloides* [23], the leaf extracts of *Impatiens balsamina* and *Lantana camara* [24], Stem extract of *Caralluma fimbriyata* [25], fruit extract of *Averrhoa carambola* [26]. Several authors have used that aqueous extract of leaves for the synthesis of metallic naoparticles because of easily available in every season such as *Jasminum sambac* [27], *Petrea volubilis*[28], *Limonia acidissima* [29]. In 21st century also people believed in ethnic people medicines, in spite of tremendous advances in synthetic medicines [30]. *Leea crispa* L. Ridsdale (family: Leeaceae), is used for the treatment of diseases including worm infection, wound, eye diseases, bone fracture, diabetes and gastrointestinal disorders, Liver disorders [31-34].

Kingdom- Plantae

Phylum- Tracheophyta

Class- Magnoliopsida

Order- Vitales

Family- Vitaceae

Genus- Leea

Species- *Leea crispa* L.

Leea asiatica (L.) Ridsdale Bot. Hist. Hort. Malabaricus 189. 1980.Saldanha, Fl. Karnataka 2: 174. 1996. *L. crispa* L. Nantiss. 1:124. 1767; Cooke, Fl. Bombay 1: 276. 1967 (Repr.),Gamble, Fl. Madras 1: 240. 1935, Talbot, For. Fl. Bombay 1: 327. 1909, Hook.f., Fl. Brit. India 1: 665. 1882, Saldanha *l.c. L. aspera* Edgeworth, Trans. L. Soc.20: 36. 1846; Saldanha *l.c. L. edgeworthii*

Santapau, Fl. Khandala 54. 1953;Saldanha *l.c. L. herbacea* Buch. – Ham., Trans., L. Soc. 14: 228. 1823; Saldanha *l.c.* Native: India, Nepal, Bangladesh, China, South-East Asia

Western Ghats: Maharashtra, Goa, Karnataka, Tamil Nadu, Kerala

It is commonly known as Vataal mara in Kannada, Banchalita in Hindi, it is an erect gregarious shrub with angular stem above the nodes and internodes. Petioles and peduncles usually have narrow crisped wings. Leaves are pinnately compound. Leaflets are lateral opposite, ovate, oblong, serrate, with sharp apex, Rounded base. Flowers are greenish white are borne in short, cymes at the end of branches, calyx united, cup-like, obscure often glandular- tipped, 5 petals, connate, ovate, acute, 5 stamens, united, 5 lobes stamina tube, Ovary is superior, short style, 2 lobed stigmas. The present investigation was undertaken to synthesize the silver nanoparticles and evaluate the efficacy of the antioxidant activity of aqueous leaf extract and silver nanoparticles.

2. MATERIALS AND METHODS

Silver nitrate, DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) was purchased from Hi-Media company, Dimethyl sulfoxide (DMSO). All glassware was cleaned with sterile distilled water and rinsed with deionized water.

Preparation of Leaf extract:

Healthy fresh leaves of *Leea crispa* were collected from the Botanical garden, Karnatak University, Dharwad, Karnataka. The leaves were washed thoroughly with Millipore water and dried in the shade until the droplets of water evaporated to remove dust impurities adhering to the surface. About 5 gm of healthy dried leaves were cut into small pieces and added to 100 ml of Milli-Q water taken in 250 ml of Erlenmeyer flask and kept in the water bath at 80˚C for 45 mins. The extract was filtered through Whatman filter paper No. 1. The filtrate was stored at 4˚C for further experimental analysis.

Synthesis of Silver nanoparticles:

For the synthesis of AgNPs, 5 ml of leaf extract was added to 95 ml of silver nitrate (AgNO3) solution in a 250 ml Erlenmeyer flask and incubated at room temperature for 30 min. During the reaction period, the color of the solution changed from colorless to dark brown color, which indicates the formation AgNPs and reduction of silver ions.

Characterization studies:

The aqueous broth-mediated leaf synthesized silver nanoparticles were characterized by UV-Vis spectrophotometer at a different wavelength from 300 to 660 nm with resolution at 1 nm. The phytochemicals present in the aqueous extract of leaf were involved in the reduction and capping of the synthesized silver nanoparticles and confirmed by FTIR analysis. The dried powder of AgNPs was used for X-Ray diffractometer (XRD) analysis at the 2θ range of 30 to 90 degrees to confirm the crystalline nature of the particles. Morphology, size, distribution, and stability of the nanoparticles were analyzed by using atomic force microscopy (AFM), High-resolution transmission electron microscope (HR-TEM) images, and zeta potential.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity:

Antioxidant activity was investigated by reducing the power scavenging activity of the DPPH assay. Silver nitrate, leaves extract and Silver nanoparticles were dissolved in distilled water separately and the concentration of the stock solution of each sample was 1mg/ml. 4 mg of DPPH was dissolved in 100 ml ethanol. The In-vitro antioxidant activity was measured by the method Zhang *et al.,* with minor modifications [35]. 2 ml of DPPH solution was added to the different concentrations of aqueous leaf extract of *L.crispa*, AgNps in the range 12.5-400 µg/ml. Then the mixture was vigorously mixed and kept it in dark for 30 mins at room temperature, absorbance was read at 517 nm. Ascorbic acid was measured as a standard. The DPPH radical scavenging activity was calculated by the following formula.

$$\text{Scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis:

Experiments were done in three replicates and then values were expressed as standard deviation ± standard error (SE) of six measurements. Statistical significance was evaluated by one-way analysis of variance (ANOVA) by SPSS 20 for Windows.

3. RESULTS AND DISCUSSION

Visual observation:

The color of the reaction mixture changes from colorless to dark brown color in 30 mins. Fig 1, a & b shows the color changes in a different interval of time from 0 to 30 mins. Nanoparticles synthesis has begun at 10 mins of incubation of aqueous leaf extract with 1mM silver nitrate solution shows the appearance of light brown color. Colour intensity was increased with increasing the incubation time. The stable dark brown color appeared at 30 mins, oscillation of free electrons in the reaction mixture develops the color during the synthesis of nanoparticles. Fig 1, C. shows the formation of stable dark brown color at 30 mins of incubation [36-37].

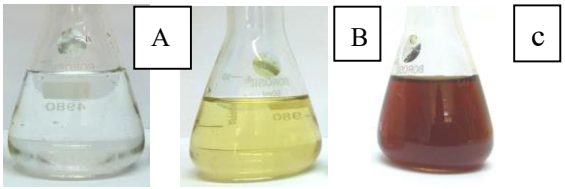


Fig 1. Visual observation of synthesized silver nanoparticles.

UV-Vis Spectroscopy:

The synthesis of nanoparticles was confirmed by the change in the color of the reaction mixture by visual observation. Followed by, silver nanoparticles were quantitatively examined through the measurement of the absorbance spectrum by using a UV-Vis spectrophotometer (Jasco V- 670 UV-Vis NIR spectrophotometer). 3 ml reaction mixture adjusted to pH 8 was used for UV-Vis

spectroscopic studies [38]. Synthesized silver nanoparticles by using leaf extract of *L.crispa* showed its maximum absorption spectrum at 420 nm due to surface plasmon resonance Fig 1. Hemalata *et al*. reported similar results silver nanoparticles synthesized by aqueous leaf extract of *Cucumis prophetarum* [39]. The pH of the solution was influenced by the formation of an intense peak. Simultaneously, it has been reflected on the size and shape of the nanoparticles synthesized by leaf extract of *L.crispa* [40].

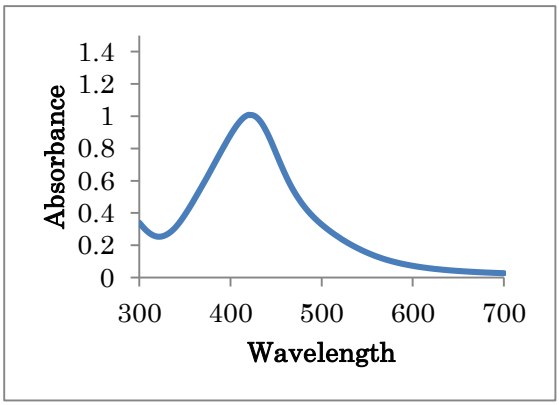


Fig 2. UV-Vis absorption spectra of Silver nanoparticles synthesised by aqueous leaf extract of *Leea crispa*.

FTIR:

The bioreduction of AgNPs is due to the reduction of Ag (I) to Ag(0) ions and is followed by secondary metabolites in leaf extract that were involved in the capping of the nanoparticles. The FTIR data revealed that phytochemicals were responsible for the capping of the silver nanoparticles Fig 3. The FTIR spectrum obtained from the AgNPs synthesized by *L.crispa* showed maximum absorption peaks at 3407, 2922, 2849, 1624, 1384, 1092, 870, 599 cm-1 signifies the capping and stabilization of the nanoparticles. The broadband at 3407 cm-1 in AgNps is attributed to the N-H stretching frequency arising from the secondary proteins of the extract of *L.crispa* [41]. The peak at 2922 cm-1 corresponds to the C-H stretching of methylene or aliphatic ring that is a characteristic of triterpenoid saponins [42]. The intense sharp peak at 1384 cm-1 is assigned to the C-C and C-N stretching vibrations co-related to water adsorption [43-44]. The peak at 1624 cm-1 is due to the bending of primary amines [45]. These results confirm that the functional groups of the afore-mentioned biomolecules play an important role in the size, capping of nanoparticles, and maintaining the stability of AgNPs synthesized from the leaf extract of *L.crispa*. These results are suggesting that amino acids have a strong ability to bind silver also, so it prevents agglomeration during the synthesis of nanoparticles.

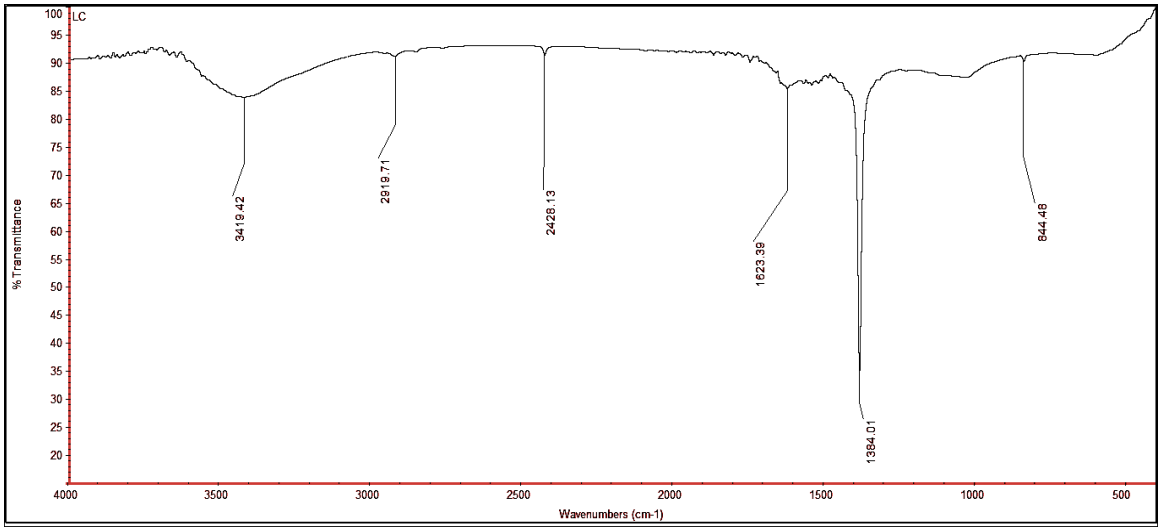


Fig 3. Fourier Transformed infrared spectroscopy of AgNPs synthesized by aqueous leaf extract of *L.crispa.*

XRD:

X-ray diffraction (XRD) analysis of the AgNPs synthesized by *L.crispa* leaf extract has been carried out using an X-ray powder diffractometer having Cu Ka (k= 1.54 A˚) radiation and a divergent slit with scanning range of 2h = 200–900 operating at a voltage of 40 kV. The presence of peaks located at 2θ angles corresponds to (111), (200), (220) and (311) shows intense peak that can be indexed as 38.45˚, 54.12˚, 64.26˚ and 78.48˚ planes of face cubic centered of silver, respectively (Figure-4). Thus, the XRD data confirmed the crystalline nature of AgNPs. The average size of silver nanoparticles can be calculated by using Braggs reflection D=Kʎ/cosθ. Where K is the Scherrer constant (K=0.94) λ is the wavelength of the X-ray, β is the FWHM (full width and half maximum) of the peak and θ is the half of the Bragg angle. The smaller size of the nanoparticle was found to be 6 nm.

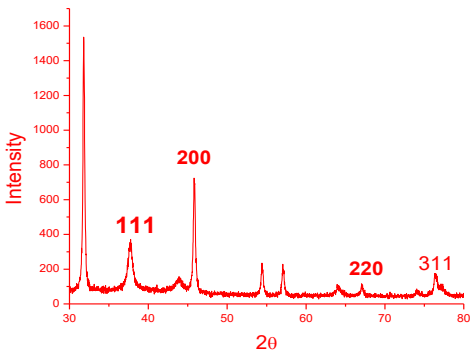


Fig 4. X-ray diffraction of silver nanoparticles

AFM and HR-TEM analysis:

The surface morphology and size of the silver nanoparticles synthesized by leaf extract of *L.asiatica* were recorded by using AFM and HR-TEM analysis. Atomic force microscopic data

revealed that nanoparticles show mono dispersion and spherical shape with an average size range that is said to be between 30 and 80 nm Fig (5). The surface morphology and size of the silver nanoparticles synthesized by leaf extract of *L.crispa* were recorded by using AFM and HR-TEM analysis. Atomic force microscopic data revealed that nanoparticles show mono dispersion and spherical shape with an average size range is said to be between 30 and 80 nm Fig (5). The HR-TEM micrographic images of the nanoparticle have depicted in fig 6. The TEM images of silver nanoparticles synthesized by an aqueous extract of a leaf revealed a spherical shape without any aggregation. These results are suggesting that silver nanoparticles correlated well with the XRD results. Many researchers reported that AFM and HR-TEM morphological studies suggested that the AgNPs synthesized from the leaf extract were of crystalline nature influenced in the formation of the spherical shape of nanostructures [46-47].

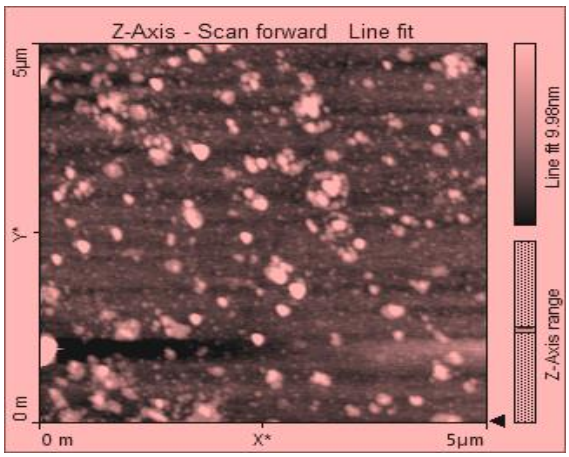


Fig 5. Atomic Force Microscopy (AFM) images.

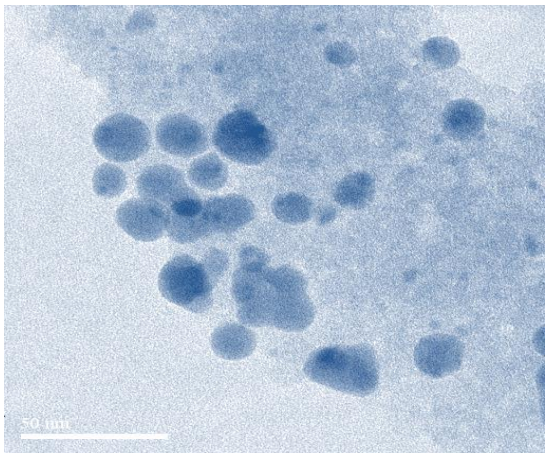
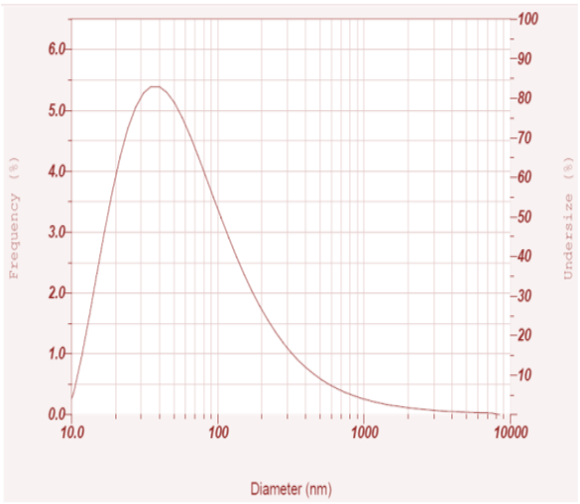


Fig6. HR-TEM image of silver nanoparticles.

Zeta Potential:

Zeta potential is an important tool that is used for examining the stability of the aqueous suspension of nanoparticles. Zeta potential values of synthesized nanoparticles were -41.6 mV. The negative value of the zeta potential confirms the repulsion among the nanoparticles and shows good stability [48].



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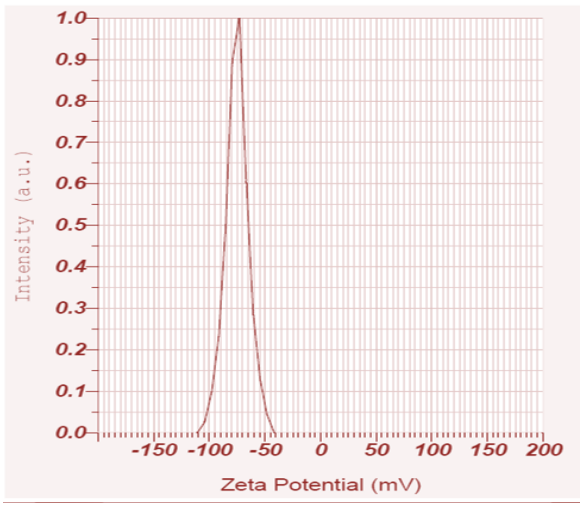

B

Fig7. Zeta potential images of silver nanoparticles A) Size distribution of AgNP B) Zeta potential of AgNP.

DPPH scavenging activity:

DPPH radical scavenging assay was investigated for the evaluation of the antioxidant potential of the synthesized nanoparticles using standard Ascorbic acid. The purple solution containing DPPH turns yellow on the addition of formulation, which indicates the scavenging of free radicals and the presence of antioxidant activity [49-50]. Antioxidant activity of silver nanoparticles, aqueous leaf extract of *L.crispa* was studied at different concentration (12.5, 25, 50,100,200 and 400 µg/ml). Phytogenic silver nanoparticles have shown the highest zone of inhibition of scavenging activity than aqueous leaf extract of *L.crispa. In-vitro* antioxidant activity was increased with the lowest concentration of AgNps while the highest concentration was in the leaf extract. In-vitro antioxidant activity was increased with the lowest concentration of AgNps while in leaf extract it shows at the highest concentration. Ascorbic acid was used as a control sample prepared with the same concentrations without any extract and as a reference. The results of *In-vitro* antioxidant activity were shown in fig (8) and table (1). We observed that the IC50 value of the control sample ascorbic acid was 6 µg /mL [51]. IC50 reading of aqueous leaf extract was 111.11 µg/ml and nanoparticles were 33.33 µg/ml. It is observed that results are shown that significant antioxidant activity in AgNPs than aqueous crude leaf extract of *L.crispa* when compared with the standard ascorbic acid. So, it depicts that the reducing power increased with the decrease in concentration. These results are strongly suggested that the antioxidant activity of silver nanoparticles synthesized by leaf extract of *L.crispa* is inversely proportional to the concentration. Parameshwar *et al.* reported that the scavenging activity of nanoparticles increases the surface area of antioxidant activity due to the presence of bioreduction molecules on the surface of the nanoparticles [52].

Table 1: Radical scavenging activity of silver nanoparticles. Readings are presented in SD±SE.

Concentration in µg/ml	Aqueous leaf extract	Silver nanoparticles
12.5	0.421±0.243	0.150±0.866
25	0.436±0.251	0.396±0.228
50	0.203±0.117	0.456±0.263
100	0.472±0.272	0.304±0.175
200	0.345±0.199	0.250±0.144
400	0.444±0.256	0.356±0.205

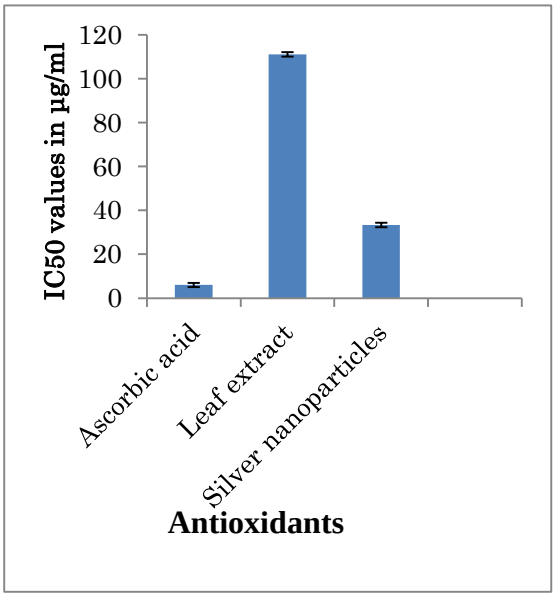

Fig 8. Antioxidant activities of AgNPs synthesized by leaf extract of *L.crispa.*

4. CONCLUSION

This investigation reveals that the phytosynthesis of silver nanoparticles by using an aqueous extract of *Leea crispa* leaf is an ecofriendly, green approach. These nanoparticles were spherical in shape and crystalline nature. Silver nanoparticles exhibit the highest antioxidant activity at lower concentrations when compared with the leaf extract. Antioxidant properties may further widen its utility in biomedical also.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the

article.

FUNDING

None.

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CONFLICT OF INTEREST

No conflict of Interest

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