Green synthesis and anti- oxidant activity of Silver oxide nanoparticle prepared from *Carica papaya* root extract

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Abstract

The methods like bio-green are beneficial over chemical and physical methods due to biodegradable and cost-effective synthesis of nanoparticles. Current study was planned for green synthesis of silver nanoparticles (AgNPs) and their biological assessment. Methods: Methanolic extract of ***Carica papaya*** root prepared by maceration was used for the synthesis of AgNPs and confirmed by UVevisible spectroscopy. Further field emission scanning electron microscope (SEM) was used for the shape and size determination of the nanoparticles. The nanoparticles was spherical in shape having average particle size of 35 nm.. Green synthesized nanoparticles (AgNPs) showed the enhanced antioxidant properties compared to the crude extract..: Green synthesized AgNPs showed the improve the biological activities. Present study also support the importance of using bio-green method for the production of nanoparticles having the potential of anti-oxidant activity

key word : Uv-Vs spectroscopy SEM, anti oxidant activity

Introduction

The nanoparticles used for all the afore said purposes, the metallic nanoparticles considered as the most promising as they contain remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest for researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains . Among the all noble metal nanoparticles, silver nanoparticle are an arch product from the field of nanotechnology which has gained boundless interests because of their unique properties such as chemical stability, good conductivity, catalytic and most important antibacterial, anti-viral, antifungal in addition to anti-inflammatory activities which can be incorporated into composite fibres, cryogenic superconducting materials, cosmetic products, food industry and electronic components. For biomedical applications; being added to wound dressings, topical creams, antiseptic sprays and fabrics, silver functions as an anti-septic and displays a broad biocide effect against microorganisms through the disruption of their unicellular membrane thus disturbing their enzymatic activities**. (Ahmed *et al.*,2016)**

Nanoparticles synthesis in the size range of 1–100 nm have develop in to suit general research and concern due to their potential application in large areas of science and technology. Metal oxide nanoparticles have been broadly used for medicinal purposes in the past decades. Metal oxide nanoparticles has environmental applications as it can act as catalyst which is cooperative in reduction or elimination of the toxic unsafe chemicals from the environmentHence, the aim of the present study is to synthesis a rapid and eco-friendly metallic nanoparticles by biosynthesis from root of *Carica papaya* and to examine the potential role of *Carica papaya* root extract as antioxidant therapy through biosynthesized NPs. In vitro antioxidant activity have been investigated by different assays viz. DPPH, Beta Caroteen. The biological approach using root extract of *Carica papaya* is the first time used as a reducing material as well as surface stabilizing agents for the synthesis Ag_2O –NPs. Hence in the present study .We have explored the biological synthesis of Ag_2O NPs by using the root extract of *Carica papaya* . The synthesized Ag_2O NPs were evaluated for the antioxidant activity

MATERIALS AND METHODS

Preparation of *Carica papaya* root extract for synthesis of Ag NPs

The *Carica papaya* plant roots were washed several times with tap water and distilled water to remove dust and other contaminants, then cut into fine pieces, and dried under shade at room temperature. (5g) roots were added to 50 ml ultrapure water in 250 ml Erlenmeyer flask, heated in water bath at 50ºC for 30 min. The mixture was then cooled to room temperature and filtered using Whatman Grade 1 filter paper in order to obtain the aqueous root extract. The product thus obtained was stored at 4ºC for further analysis. **(Sreenivasulu *et al.*,2016)**

Fig 2.1: (A) Root of Carica papaya (B) Dried root under room temp.

Synthesis of Ag NPs

Synthesis of Ag nanoparticles (AgNPs) We first prepare 1mM silver nitrate (AgNO3) solution. In order to prevent oxidation, the solution was stored in an amber-colored bottle.To synthesize silver nanoparticles, 10 mL of the *Carica papaya* aqueous root extract was added into 90 ml of 1 mM silver nitrate solution and the mixture continuously stirred for about 1hrs . The solution was then incubated for 1hrs at the room temperature. The colour of the resulting mixture was found to change from pale yellow to dark brown indicating the formation of silver nanoparticles (AgNPs) by *Carica papaya* root extract. The resultant solution containing the AgNPs was centrifuged at 4000 rpm for 10 minutes. The collected sample AgNPs were allowed to dry in a watch glass then stored for further characterization studies**. (Sreenivasulu *et al.*,2016)**

Fig. 2.2 (A) (B)
1 mM AgNO3 without *C.papaya* extract — **1mM AgNO3 with *C.papaya* extract after 10 hrs of incubation**

Charaterization Techniques

3.7.1 UV-Vis analysis - The optical property of metal oxide nanoparticles was determined via ultraviolet and visible absorption spectroscopy in the range of 420- 430 nm. After the addition of $AgNO_3$ to the plant extract, the spectra's were taken in up to 24Hrs. between 420 nm to 430 nm.

SEM analysis - The morphological features of synthesized zinc oxide nanoparticles from *Carica papaya* root extract were studied by Scanning Electron Microscope (Electron Probe micro Analyzer JEOL MODEL No JXA8100 from Allahabad University after 24 Hrs of the addition of $AgNO_3$ NPs the SEM Slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 25 KV

Anti -oxidant activity of silver oxide and zinc oxide nanoparticle –

The antioxidant activity of the metal oxides nanoparticle of *Carica papaya* root extract (n-hexane and methanol extracts) was determined by using DPPH and β-carotene method. The results were compared with the synthetic antioxidant BHT which is an efficient synthetic antioxidant agent metal oxide nanoparticle.

DPPH Free Radical scavenging assay-

(1,1-Diphenyl-2-picrylhydrazyl (DPPH) Free Radical scavenging potential of the metal oxide nanoparticles was determined . Different concentrations (1.0,0.50,0.25 and 0.125 mg/mL) of AgNPs and standard Butylated Hydroxytoluene (BHT) were taken in different test tubes. In the above samples, 3 mL of freshly prepared DPPH dissolved in methanol was added and vortexed thoroughly. Finally, the solution was incubated in dark place for 30 min. The absorbance of stable DPPH was recorded at 517 nm. The DPPH (containing no sample) was used as a control prepared using the same procedure. Free radical scavenging activity was expressed as the percentage of inhibition that was calculated using the equation . **DPPH radical scavenging activity(%)=(Ac−As)/Ac×100**

where Ac is the absorbance of control; As is the sample absorbance of DPPH radical + sample /standard BHT. **(Bhakya *et al.*, 2016)**

Beta Caroteen –linoleate bleaching (BCB) assay-

The antioxidant activity was assayed based on the β-carotene bleaching method. β-carotene (0.2 mg in 1 ml chloroform), linoleic acid (0.02 ml) and Tween 20 (0.2ml) were transferred into a round bottomed flask. Chloroform was removed at room temperature under vacuum at reduced pressure using a rotary evaporator. After evaporation, 50 ml of distilled water was added to the mixture, and then shaken vigorously to form an emulsion. 2 ml of emulsion was then added to 0.2 ml of methanolic extract or methanol (as control) into test tubes and immediately placed in a water bath at 50 °C. The absorbance was read at 30 min intervals for 2 h at 470 nm**. (Gupta *et al*.,2014)**

Degradation rate (DR) was calculated according to first order kinetics, using the following equation:

Lna/bx 1/t = DR sample or DR standard where Ln is natural loga is the initial absorbance(470 nm) at time 0,bis the absorbance (470 nm) at 20, 60, 90 or 120 min and t is the incubation time after which the absorbance was taken.

Antioxidant activity (AA) was expressed as percent of inhibition relative to the control, using the following formula:

AA = [(DR control- DR sample)/ DR control] x100

Where, DR control: Degradation rate in absence of extract and DR sample: Degradation rate in presence of extract.

Result and discussion

Visual observation of Ag_2O NPs

The present study elucidates the green synthesize of AgNPs from root extract of *Carica papaya* and their biological activity. NPs are generally characterized by their size, shape, surface area, and dispersity. Homogeneity of these properties is important in many applications. When the root extract was mixed with $AgNO_3$ and incubated at room temperature, within 30 min of the reaction, its color changed from brown to dark brown indicating the formation of AgNPs. It is an efficient and rapid method, which was very well explained by other researchers who worked with different plant systems (Muthukrishnan et al. 2015; Kanipandian et al., 2014; Kalaiselvi et al., 2015). Change in color was due to the excitation of surface plasmon vibrations in metal nanoparticles (Ahmad et al. 2003). Our results are in conformed to Muthukrishnan et al.. (2015), who reported the formation of AgNPs within 30 min of incubation. The variation in the rates of bio reduction observed may be due to the differences in the activities of the enzymes present in the plant root extract.

4.3: UV–Visible SpectroscopyUV–visible spectroscopy is an important technique used to confirm the formation of metal nanoparticles in an aqueous solution. As shown in **Fig.4.1** UV–Vis absorption spectrum of the produced AgNPs showed an absorbance peak at 420 -430 nm due to excitation of surface plasmon vibrations in nanoparticles. One of the most important features in the optical absorbance spectra of metal nanoparticles is surface plasmon band, which is due to collective electron oscillation around the surface mode of the particles .The presence of a single surface plasmon resonance band in the absorption spectra of the produced AgNPs gives an indication to their spherical shape.

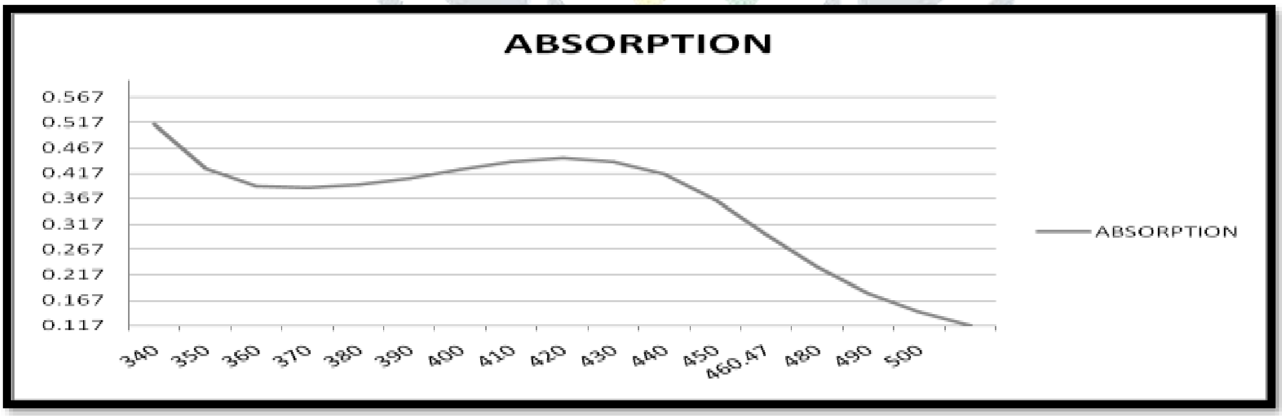

Fig : 3.1 UV–Vis absorption spectrum of the AgNPs

This analysis showed the sharp absorbance at around 420 -430 nm **(Fig. 3.1)**which was specific for AgNPs. The UV–Vis absorption band in the current visible light region (420–430 nm) is an evidence of the presence of surface plasmon resonance (SPR) of AgNPs. A single SPR band resembles to the spherical nanoparticles, where as two or more SPR bands correspond to the anisotropic molecules . The intensity of the SPR peak increased with reaction time indicating the increasing concentration of AgNPs. The reduction was ascribed to the steroids, terpenoids, alkaloids, carbohydrate and phenolic compounds present in the extract.

SEM Analysis of AgNPs-

Scanning electron microscope (SEM) analysis was carried out by Electron Probe micro Analyzer JEOL MODEL No JXA8100.Thin film of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5min SEM provided further insight into the morphology and size details of the silver oxide nanoparticles. Comparison of experimental results showed that the diameters of prepared nanoparticles in the solution have sizes between 1-100 nm of in case of 25 mints at 5000 rpm. The size of the prepared nanoparticles was more than the size of nanoparticle which should be; i.e.; between 1-100 nm. The size was more than the desired size as a result of the proteins which were bound in the surface of the nanoparticles. The result showed that the particles were of spherical shape and sheet shape.

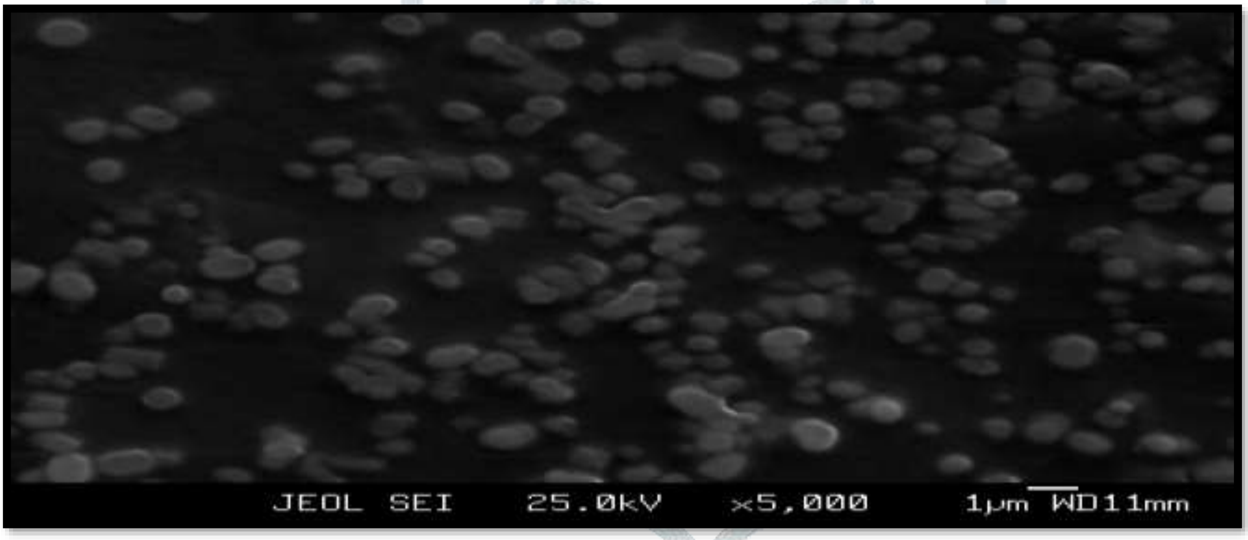

Fig: 3.2 SEM Image of AgNPs prepared from root extract of *Carica papaya*

DPPH Radical Scavaenging Analysis

DPPH (1,1-diphenyl-2picrylhydrazyl) DPPH is a more stable and well-known free radical based on the reduction of accepting hydrogen or electron from donors. The DPPH reducing ability of the metal oxide NPs was assessed by observing color change and the control does not show any color change. The DPPH scavenging assay exhibited effective inhibition activity of metaloxide NPs when compared with the standard BHT .The DPPH activity of the AgNPs was found to increase in a dose-dependent manner. However, the Metal Oxide NPs exhibited more inhibition with 90 % scavenging activity of DPPH. When adding metal oxide NPs in the DPPH solution color change was occur which is due to the scavenging of DPPH due to donation of hydrogen atom to stable the DPPH molecule which is responsible for the absorbance of 517 nm. The antioxidant potential of metal oxide nanoparticle could be attributed to functional groups adhered to them which were originated from the root extract. In this test, the Ag_2O nanoparticle exhibited good antioxidant activity.

Concentration (mg/ml)	%inhibition of Ag_2O NPs	% inhibition of Standard BHT
0.125	21.11	30.45
0.25	28.35	45.59
0.50	33.34	55.67
1.0	47.56	60.5

TableNo.3.1% inhibition of Ag_2O NPs

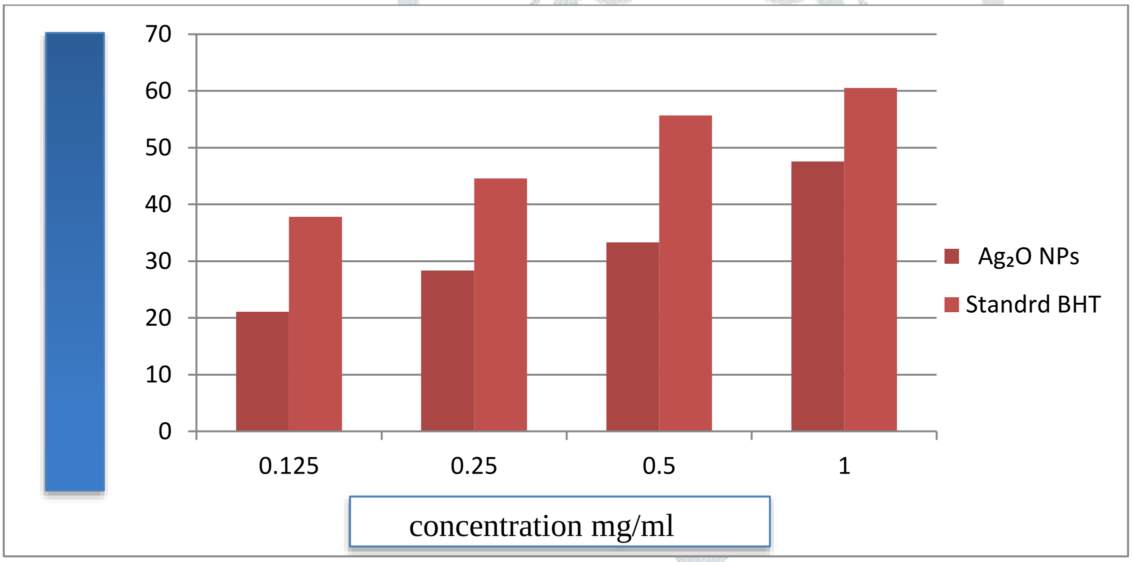

Fig: 3.3 DPPH radical scavenging activity (%) versus concentration (mg/mL) of Ag_2O Nps of *Carica papaya* root extract

β-Carotene Bleaching Assay

The *β*-carotene bleaching method is based on the loss of the yellow colour of β-carotene due to its reaction with radicals. The rate of *β*-carotene bleaching can be slowed down in the presence of antioxidants **.**

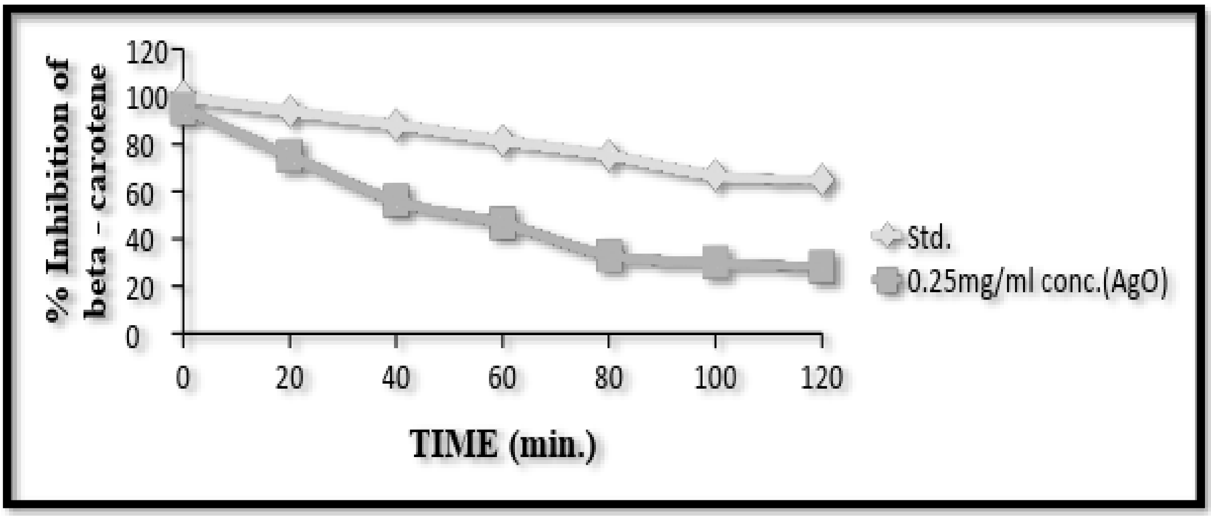

Fig: 3.4 % inhibition of β- carotene bleaching (%) versus time of Ag_2O Nps of *Carica papaya* root extract.

Discussion

In the present study the antioxidant ability of the selected plant extracts was determined through DPPH free radical and Beta caroteen activity. In general, isolation of antioxidant compounds from plant is possible through extraction with different solvents and it depends on the nature of extracting solvent **(Sultana *et al.*, 2009).** Here results showed that, plant extracts exhibited promising free radical scavenging activity wherein, when the concentration increases the percentage inhibition of free radical also increased significantly ($P < 0.05$). Of the synthesis of *C.papaya* root extracts the silver oxide nanoparticle (47.56%) had higher DPPH radical scavenging activity. Studies pertained to antioxidant activity revealed that *C.papaya* are one among the best plants to quench free radicals.. Here the observed variation in antioxidant by crude ethanolic extracts of *C. papaya* may be due to significant differences in of nanoparticles of silver oxide nanoparticle i.e., the present study that, *C. papaya* recorded considerably lesser anti oxidant activity than silver nanoparticle synthesis by *C.papaya* root extract the metallic contant of nanoparticle than high anti oxidant activity of the chemical component of the palnt extract because the the chemical composition and chemical structures of active extract components are important factors governing the efficacy of natural antioxidants. The antioxidant activity of an extract could not be explained on the basis of their phenolic content which also needs their characterization of the nature of the metal and the oxidative property . For instance it has already been reported that nano particle parpered the plant extract depend with ortho- and para-dihydroxylations or a hydroxy and a methoxy group or both are more effective than simple phenolics Most often extracts from plants contain different classes of phenols, which have the different solubility's in different solvents.

The prepration and analysis of different methods of the formation of silver oxide nanoparticle by using *C. papaya* root extract had higher number of phytochemicals, followed by ortho para and meta position but the nanoparticle to show existence of anti oxidant activity of different Other plant extracts showed lesser number of phytochemicals. extracts showed dose dependent activity. Among the ethanolic extracts tested, at 1 mg ml- silver oxide nanoparticle showed higher DPPH radical scavenging activity; but this level increased significantly (P < 0.05) and it was high (46.20 ± 5.26at 1 mg/ ml. Similarly among the *C.papaya* root extract activity had showed maximum (47.50 ± 2.36% to 21.20 ± 6.48%) DPPH radical scavenging activity; but *C.papaya* displayed lesser activity (21.00 ± 3.38% with respect to increase in concentration.. A similar trend was noticed in Butylated hydroxyltoluene (standard); where in it recorded 99.30 ± 4.32% at 1 mg/ml . Based on the result of nanoparticle of different metal parepared by plant extract the antioxidant activity. It was found that ethanolic extracts of selected herbal plants had less activity than Silver nanoparticle parpered by root extract of *C. papaya*.

SUMMARY AND CONCLUSION

The green synthesized metal oxides nanoparticles were great interest due to their eco- friendlness , nontoxic , economic prospects , and feasibility and short time for synthesis may be wide range of application in nanomedicine, catalysis medicine mainly for the pharmaceutical industry for development of new formulations.

The present study was carried out to investigate the pharmaceutical potential of root parts of selected medicinal plants such as root of *C.papaya* using solvents of varying polarity and green synthesis of Silver nanoparticle. Results obtained from the present study were summarized below:

The prepration and analysis of different methods of the formation of silver oxide nanoparticle by using *C. papaya* root solvent extracts had higher number of phytochemicals, followed by ethyl acetate and chloroform extract had maximum occurrence of phyotchemicals. Next to this ethanol chloroform and ethyl acetate extract of *C. papaya* displayed almost all phytochemicals but failed to show existence of saponins, anthroquinones and terpenoids. Other plant extracts showed lesser number of phytochemicals extracts showed dose dependent activity.

In the present study the antioxidant ability of the selected plant extracts was determined through DPPH free radical and Beta caroteen activity. In general, isolation of antioxidant compounds from plant is possible through extraction with different solvents and it depends on the nature of extracting solvent **(Sultana *et al.*, 2009).** Here results showed that, plant extracts exhibited promising free radical scavenging activity wherein, when the concentration increases the percentage inhibition of free radical also increased significantly ($P < 0.05$). Of the two different metal nanoparticle synthesis of *C.papaya* root extracts the silver oxide nanoparticle (47.56%) had higher DPPH radical scavenging activity. Studies pertained to antioxidant activity revealed that *C.papaya* are one among the best plants to quench free radicals. Here the observed variation in antioxidant by crude ethanolic extracts of *C. papaya* may be due to significant differences in of nanoparticles of silver oxide nanoparticle i.e., the present study that, *C. papaya* recorded considerably lesser anti oxidant activity than silver nanoparticle synthesis by *C.papaya* root extract the metallic contant of nanoparticle than high anti oxidant activity of the chemical component of the palnt extract because the the chemical composition and chemical structures of active extract components are important factors governing the efficacy of natural antioxidants. The antioxidant activity of an extract could not be explained on the basis of their phenolic content which also needs their characterization of the nature of the metal and the oxidative property . For instance it has already been reported that nano particle parpered the plant extract depend with ortho- and para-dihydroxylations or a hydroxy and a methoxy group or both are more effective than simple phenolics Most often extracts from plants contain different classes of phenols, which have the different solubility's in different solvents.

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