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The phytochemical screening and Green synthesis of silver nano-particles, Characterization by using *Opuntia dellini* (CLADODE)

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ABSTRACT:

Opuntia dellini (Cactaceae) family, Commonly known as pear bush, prickly pear. Is a succulent shrub growing under desert and dry conditions. *Opuntia dellini* is a wild Xerophytes is abundant in Himalayas, belived to be American origin and a native of India. Traditionally these plants used in the treatment of inflammation, hypoglycaemic, Stomach ulceration Neuro-protection through antioxidant action, Viral disease, diabetes, burns, bronchica, asthama and digestive problems throughout the world. Phytochemical screening was undertaken in Cladodes, and revealed the presence of phenols, Alkaloids, flavanoids, Saponins etc. and then Synthesize silver nano-particles by a green biological route, using an Cladode plant extract.

Thus, the synthesized silver nano-particles with plant extract. was confirmed by change of the colour of the solution from pale yellow into dark brown colour Solution, indicates to bio-reduction of silver ions in the plant extract. The Green synthesis of silver nano-particles was characterized by UV-visible spectroscopy, Fourier transforms Infrared spectroscopy (FT-IR), X-ray diffraction Studies (XRD) and Scanning electron microscopy (SEM). This study justifies the potential and applications of traditional medicine in health Care sector.

KEYWORDS:

Opuntia dellini Cladode, Phytochemical screening Cladode extract, Green synthesis of silver nano-particles with Cladode extract, characterization of UV-visible, FT-IR, XRD, SEM.

INTRODUCTION;

Medicinal Plants contain some organic compounds wich provide definite physiological action on the human body. Secondary metabolites are chemically and taxonomically extremely divers compoundes with obscure function. They are widely used in the human therapy. Secondary metabolites are the substances produced by plants as defens chemical s.They include alkaloids, flavaniods, essential oils ,phenols,saponins etc.Recently,many pharmaceutical compines have spent a lot of time and money in deveioping natural products extracted from plants, but the information available is quite meager.The present study was carried out to identify the active chemical principle compostioon in Opuntia dellini Opuntia dellini {Ker-Gawel Haw family cactaceae commonly known as pear bush,prickly pear,mal rachette or tuna, is a succulent shrub growing under desert and dry condisions. It is native to the American continent and the West Indies, but recently due to cultivation, it has become widely distributed throughout canaruy Island, Southern and Eastern Africas,Pakistan, India and Australlia.Opuntia dellini is a rich source of dietary fiberes, natural colorants and antioxidant vitamints and therefore, used as a food because of their edible fruit.Pharmacological evalution of Opuntia has shown its efficacy as antihypertlipidemic,antiviral anti-inflammatory antidiabetic antioxidantand antiulcerogenic activity.Ithas also been reported to protect nerve cells and used for the treatment of Alzheimers dieses,Parkinsons disease and stroke.Inrecent years, there haSs been a global trend to ward the use of natural resources As antioxidant and functional roods .Two characteristic historical exampies are the O. dellini plantationof srirangapatam{india}.Inthe first case,the Ruier of Mysore,Tippu sultan{1750-1799},reinforced the fortification around his residence with the cactus because of its formidable spins .secondly,in 1930 the Imam established the cactus near his castie in order to use the coloured fruit juice as ink. Opuntia dellini a wild xerophytes is abundant in Himalayas, belived to be of American origin and a native of India T raditionally,the plantis used in the treatment of inflammation, hypoglycaemic,stomach ulceration, Neuro-protection through antioxidant action,viral disease,diabetes,burns,bronchica,asthama and digestive problems throughout the world. Phytochemistry study was undertaken in cladodes and fruits and revealed the presence of phenols, Alkaloids,Flavanoids,saponins in cladode. Phenol content(6.8%)was found maximum followed by Alkaliods(5.4%),Flavaniods(3.5%),and

Saponins(1.05%)in cladose

Opuntia

Scientific classification

Kingdom: Plantae

(unranked): Angiosperms

(unranked): Eudicots

(unranked): Core eudicots

Order:* *Caryophyllales

Family:* *Cactaceae

Subfamily:* *Opuntioideae

Tribe:* *Opuntieae

Genus: Opuntia

Mill.

Aim:

Opuntia dellini cladode. Family cactaceae. Commonly known as green colour cladodes in India. Opuntia dellina a wild xerophytes is abundant in Himalayas, believed to be of American origin and a native of India. Traditionally, the plant is used in the treatment of inflammation, hypoglycaemic, stomach ulceration, Neuro-protection antioxidant action, viral disease, diabetes, burns, bronchial, asthma and digestive problems throughout the world.

Objective:-

This study is to make phytochemical screening to determine the secondary metabolites present in opuntia dellini and also green synthesis of silver nano particles and uv-visible spectra, FTIR,XRD and SEM characterisation of secondary metabolites of opuntiadellini cladode

Method:

Ethanol, ethyl acetate, Aqueous or double distilled water was used for extraction of opuntia dellini by percolation process. The phytochemical screening is done on the dried powder extract to investigate the chemical groups present in the extract. Phytochemical analysis of 90% Ethanol extract and Acetone extract and Aqueous extract f opuntia dellini in this study reveal presence of different phytochemical groups as alkaloids, flavonoids, terpenoids, tannins, phenolic compounds, quinines, cardiac glycosides, saponines and steroids etc. and also identified primary metabolites like aminoacids, proteins and polysaccharides.

1. Green Synthesis of Silvernanoparticles using opuntia dellini

Plant extracts:

a. The present study aims to synthesize Silver nanoparticles by a green biological route, using an extract derived from opuntia dellini, And characterization of the synthesized silver nanoparticles with plant extract characterized the bioreduced Silver nanoparticles are Utilizing UV-Visible spectroscopy, Fourier transform Infrared spectroscopy (FT-IR) analysis, X- ray diffraction (XRD) and scanning electron microscope (SEM).

b. The method provided a lot of advantages over other techniques such as being a simple method, low-cost, effective, eco-friendly and leading to point of care laboratory.

METHODOLOGY

METERIALS AND METHODS

Collection and Identification of Plant Meterials :

The plant material opuntia dellini(cladode). were collected by near rayalaseema university in Kurnool, A.P. This opuntia dellini(cladode) identified by the botony department facility,

R.U, Kurnool.

Preparation of Plant extract: Percolation Process :

The dried opuntia dellini (cladodes). Were powdered mixer grinder. For the percolation process, the macerated plant powders were soaked in solvents such as Ethanol,

ethylacetate, Aqueous individually. Extraction was done by soaking one part of plant powder to three parts of liquid solvent(1:3) and kept for percolation process for 3-5 days. Then the crude extracts were filtered using whatman No.1 filter paper, evaporated and concentrated under room temperature and used the deposited crude materials for phytochemical analysis.

PHYTOCHEMICAL SCREENING

Phytochemical analysis of solvent extracts of the lichens samples was carried out using standard qualitative methods following the methodology of sofowora A,(1993), Ross

JH Flora of southern Africa café.(1976).

Tests For Alkaloids

Mayer's test: Potassium Mercuri Iodide Solution)

The extracts were treated with Mayer's reagent. The formation of a yellow cream precipitate indicates the presence of alkaloids.

Wagner's test:Solution of iodine in Potassium iodide).

Few drops of Wagner's reagent were added by the side of the test tube to 1 ml of extract. A reddish-brown precipitate is produced

Hager's test:Saturated solution of Pinri acid

To the extract solution, add few dropes of Hager's reagent, Yellow precipitate is produced.

Test for Phenolic Compounds

Ferric Chloride Test :

To 1 ml of solvent extracts, 3 ml of distilled H2O was added. To this, a few drops of neutral 5% FeCl3 solution was added. Formation of a dark green colour indicated the presence of Phenolics.

Lead acetate test :

3 ml of 10% lead acetate solution was added to 1 ml of the extract. Appearance of bilky white precipitate confirms the presence of phenolic compounds.

Test for flavonoids: Shinoda test (magnesium hydrochloride reduction test)

To the extract solution add few fragments of magnesium ribbon and HCL drop wise, pink scarlet, crimson red or occasionally green to blue colour appears few minutes.

Zink- hydrochloride reduction test:

To the extract solution, add a mixture of zinc dust and concentrated HCl. It gives red colour after few minutes.

Alkaline reagent test:

To the extract solution, add few drops of sodium hydroxide solution, formation of an intense yellow colour that turns to colourless on addition of few drops of dilute acetic acid indicates the presence of flavonoids.

Ammonia test:

A few drops of 1% NH3 solution was added to 1 ml of the extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

Test for carbohydrates:

Molisch's test:

The extract were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml concentrated H2SO4 was added carefully along the sides of the test tube. Formation of dull violet and red ring at the inter phase indicates the presence of carbohydrates.

Test for terpenoids:

Salkowski test:

To 1 ml of the extract, 2 ml of chloroform was added. Then 3 ml of concentrated H2SO4 was added carefully to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids.

Test for cardiac glycosides:

Keller-killani test:

The extract was dissolved in glacial acetic acid containing traces of FeCl3 .The tube was then held at an angle of 450 and 1 ml of concentrated H2SO4 was added along the sides of the tube. Formation of a purple ring at the interface indicates the presence of cardiac glycosides.

Test for steroids:

2 ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H2SO4. Change in colour from violet to blue or green indicates the presence of steroids.

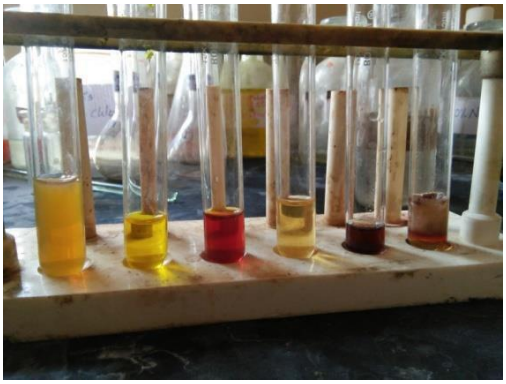
Phyto chemical result obtained from opuntiadellini cladodes(cactaceae)

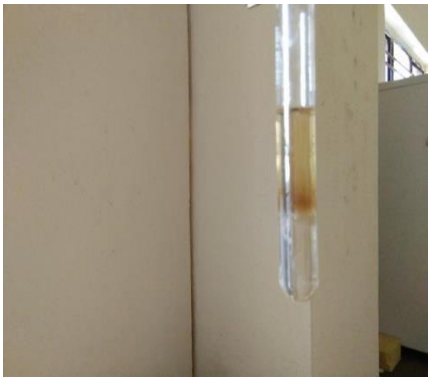
	Ethanol extract	Ethylacetate extract	Aqueous extract
Alkaloids			
Mayer's Test	+	+	+
Wagner's Test	+	+	+

Hager's Test	+	+	+
PHENOLS			
Ferric chloride test	+	+	-
Lead acetate test	+	+	-
FLAVONOIDS			
Shinoda test	+	+	+
Ferric chloride test	+	+	-
Zinc-HCireductiontest	+	+	-
Alkalinereagent(NaOH)	+	+	+
Ammonia test	+	-	-
Test for steroids	+	+	+
Test for Tannins	-	+	-
Test for Terpenoids Salkowski test	+	+	-
Test for Quinones	-	-	-
TEST FOR CARBOHYDRATES			
Molisch's	++	++	++
TEST FOR REDUCING SUGARS			
Benedict's test	-	-	-
TEST FOR CARDIAC GLYCOSIDES			
Keller-Killiani test	+	++	-

(+) = Indicate presence of the phyto constituents(-)= Indicate obsence of the phyto constituents

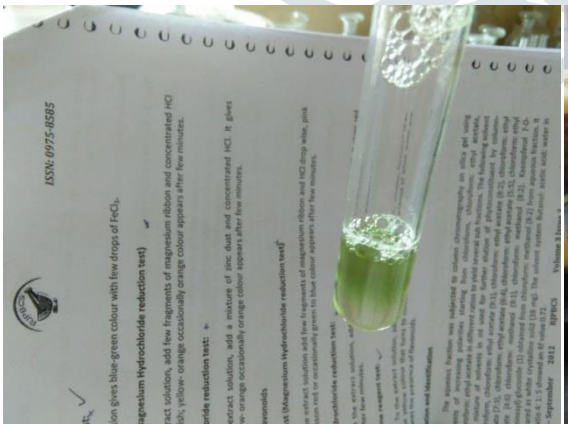
(++) = Indicate presencein more quantity of the phytoconstituent

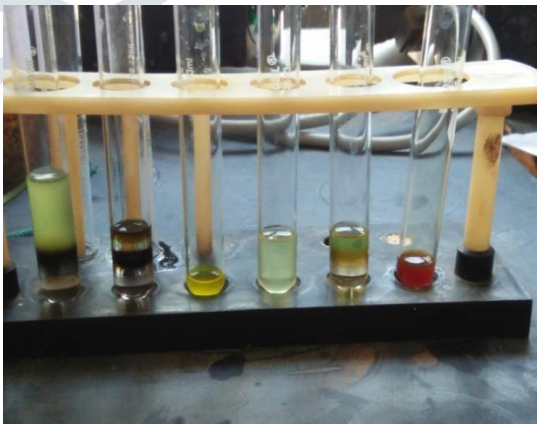




ETHYL ACETATE EXTRACT

ETHNOL EXTRACT





ACQEOUS EXTRACT

MARPHOLOGY

METERIALS AND METHODS

Preparation of plant extract:

Take 100 g of dried opuntia dellini (cladode) were powdered using mixer grinder. The macerated plant powder was done by soaking with 300 0ml double distilled water, one part of plant powder to three parts of solvent water(1:3) and then boiling the mixture and refluxed for 1h at 80oc and cooled that mixture. This cooled mixture was filter by Buchner flask 250 ml with Buchner funnel. Then collected filtrate is light orange in colour. The filtrate was stored in 4oc in the refrigerator, for further bio synthesis process.

CHEMICALS:

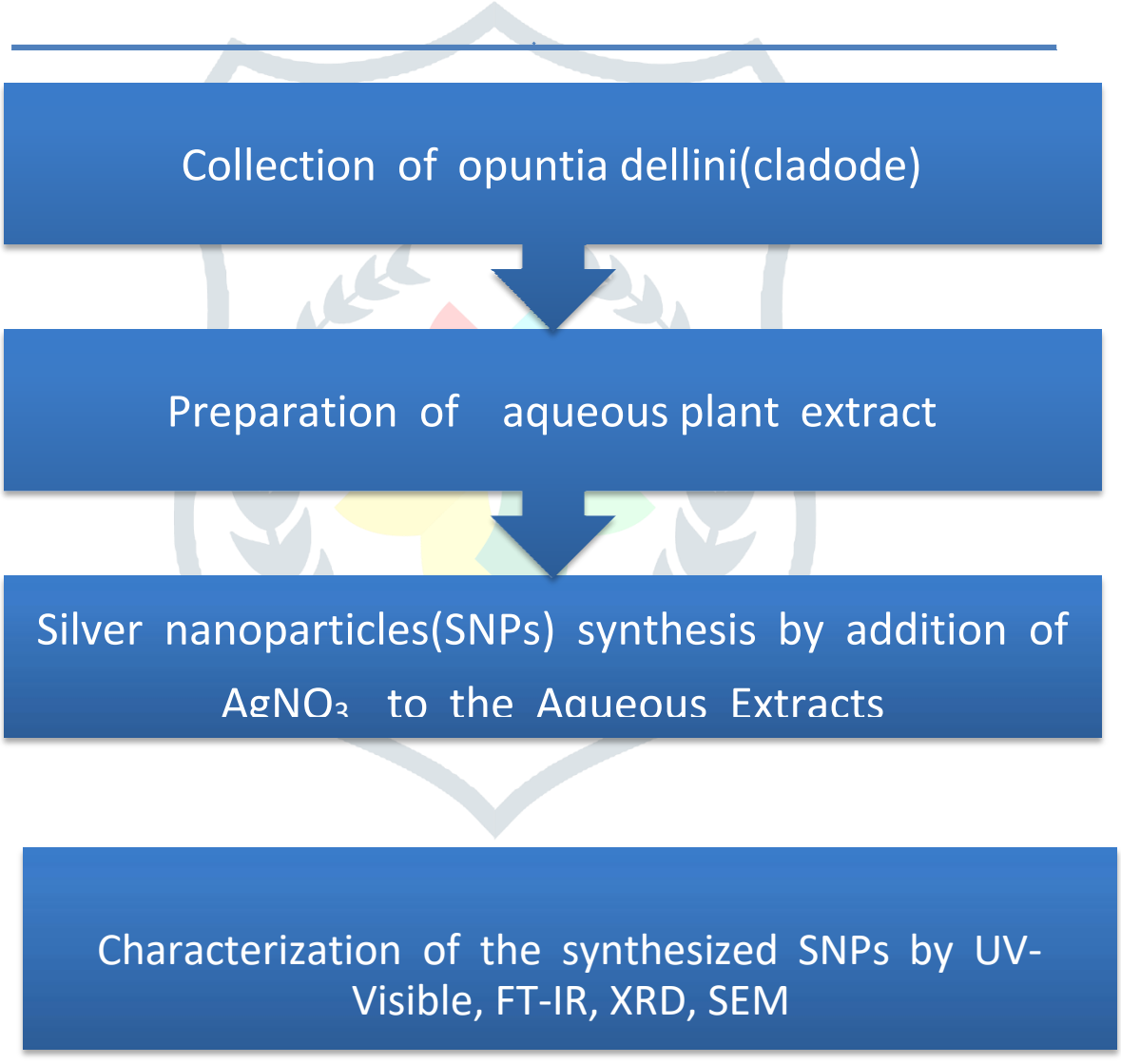
Silver Nitrate(AgNO3) was purchased from Sigma Aldrich. Bangalore. India. Double distilled water was usedthroughout the experiment. All other chemicals were of analytical grade.

BIO SYNTHESIS OF SILVER NANO PARTICLES

Bio synthesis of silver nanoparticles includes, 80ml supernatant of opuntia dellini(cladode) extract was added 200 ml of 1mM of aqueous solution of $AgNO_3$(1mM) at room temperature and stirred for 1h. The reaction mixture flask was kept in dark. Finally, the colour of solution changed from pale green coloured toblakish brown colour. That confirm the formation of silver nano particles. Further the colloidal solution was Ultra centrifuged at 8000 rpm for 20 min and the supernatant and solid was collected for further studies.

Characterization of the synthesized SNPs by UV-Visible, FT-IR, XRD, SEM

- Characterization of the synthesized SNPs by UV-Visible, FT-IR, XRD, SEM



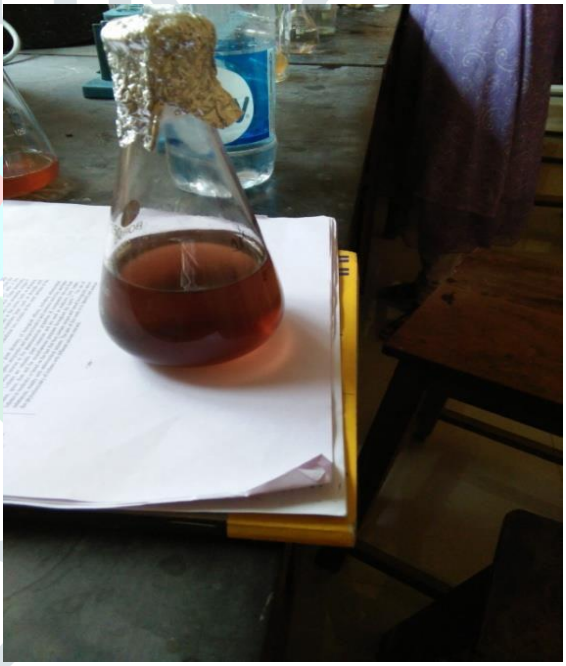
$$Ag^{+} \xrightarrow{\text{Reduction}} Ag^{0} \xrightarrow{\text{Agglomeration}} \text{Stabilization}$$

CHARACTERIZATION OF SILVER NANO PARTICLES BY USING Opuntia dellini(cladode) extract

RESULTS AND DISCUSSION

By color change

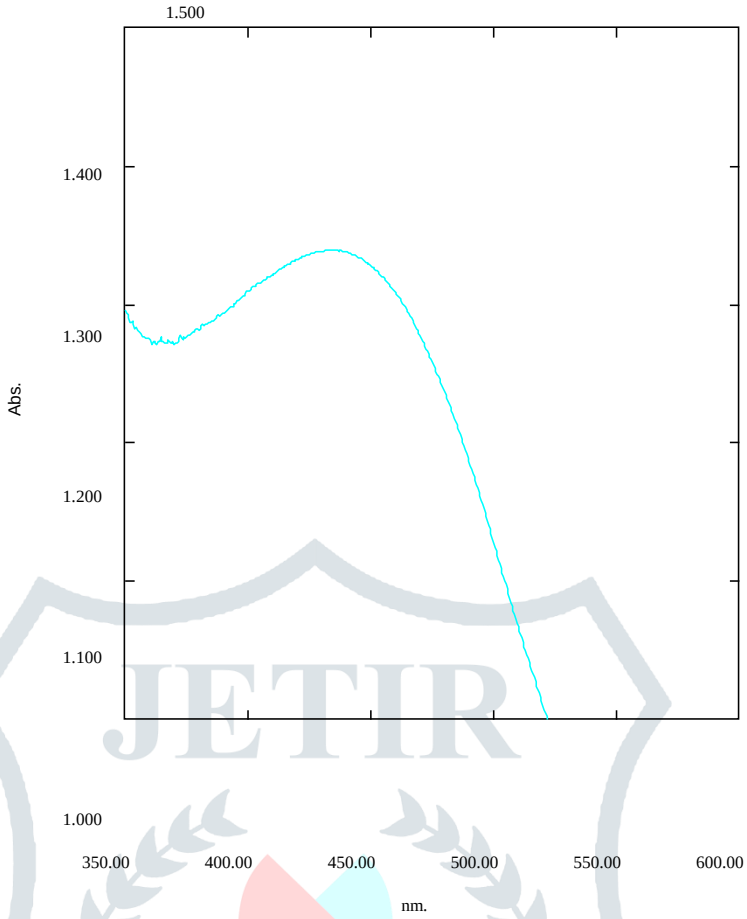
The sequential color change indicates the formation of AgNPS by plant materials. This is the primary test forthe checking of formation of AgNPS. The color reduction of $AgNO_3$ into nano particles was visibly evident from the colour change. Pure filter Aqueous plant extract was added into a 1mM silver nitrate solution and boiled on water bath few minutes, the pale green colour was changed from blakish brown colour after 30-35 minutes. After 18-24hrs colour was changed into dark brown. This colour change indicates the formation of AgNPS.



UV-Visible Spectroscopy Analysis of AgNPS:

The blakish sbrown coloured mixture turned into dark brown colour after 18-24h, indicating the bio transformation of ionic silver reduced to silver nano, asa result of the surface Plasmon resonance phenomenon(SPR).

Fig:1 shows the UV spectrum of AgNPS and at 435nm, 361nm, 227.50nm a peaks was observed that wasidentified to be AgNPS. Generally, it is well known that AgNPS exhibit blakish brown. The colour change ensued as of the active molecules present in the leaf extract that using to the excitation of SPR effect. The synthesized silver nano particles were there after analyzed at different time interval to find the stability of the particles. In this present work Gangs were analyzed in UV-Visible spectrometer



No.	P/V	Wavelength nm.	Abs.	Description
1		435.00 1.340	1.34	
2		227.50 4.000	4.00	
3		361.00 1.271	1.271	

FT-IR Analysis

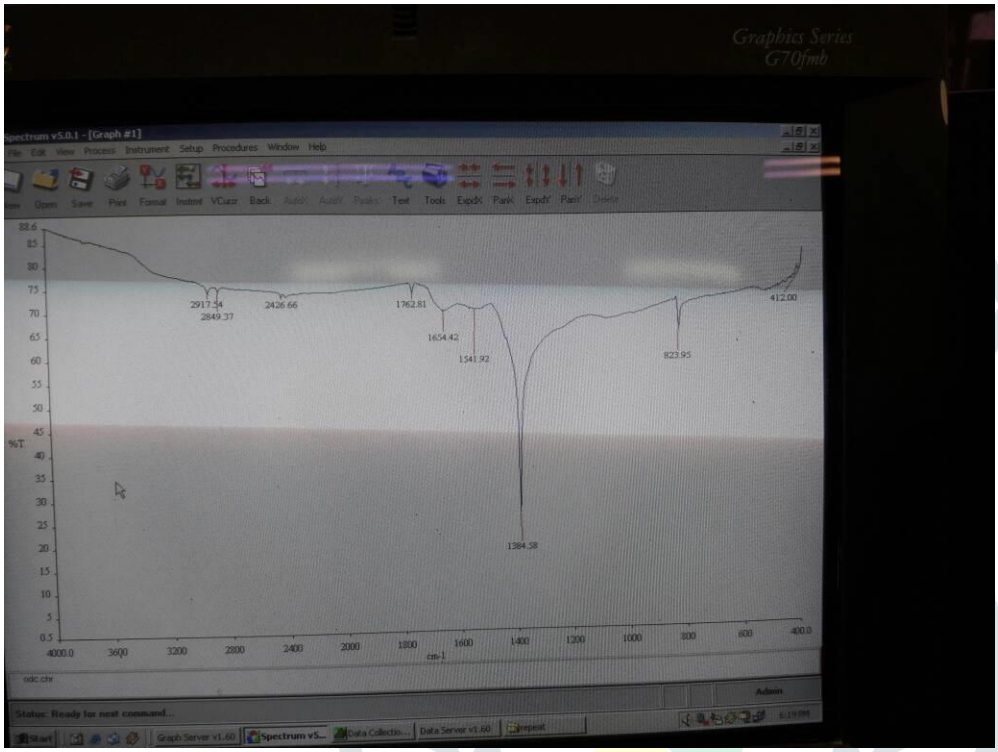
FT-IR Analysis was used to characterize the nature of capping ligands that stabilizes the silver nanoparticles formed by bio-reduction process. The FT-IR measurements were carried out to identify the possible bio-molecules responsible for the reduction of the silverions into silver nanoparticles.

Figure(1a) show peaks at 2917cm^{-1} corresponds to the O-H ,N-H stretching vibrations of -OH hydroxyl groups. 2849.37cm^{-1} C-Hstrtching(C-O-CH3)vibrations,2426.66nm was assignedmay be NH^{+}, NH_2^{+} asym stretching vibrations and also peak at 1762cm^{-1} represents C=O strtching 4ring saturated 1654.42cm^{-1} peak shows C=OstrtchingO-Hydrogenasymmetric intra

molecularhydrogen bonding,&1541.92cm^{-1}secondaryamidemonomer,and 1384.58cm^{-1}-O-CO-CH-assignedhigh intensityof these band dominate in region of the spectrum.Sothis group is present compounent,823.95cm^{-} $^{1}NO^{3-}$nitro compounds is present &412.00nm out

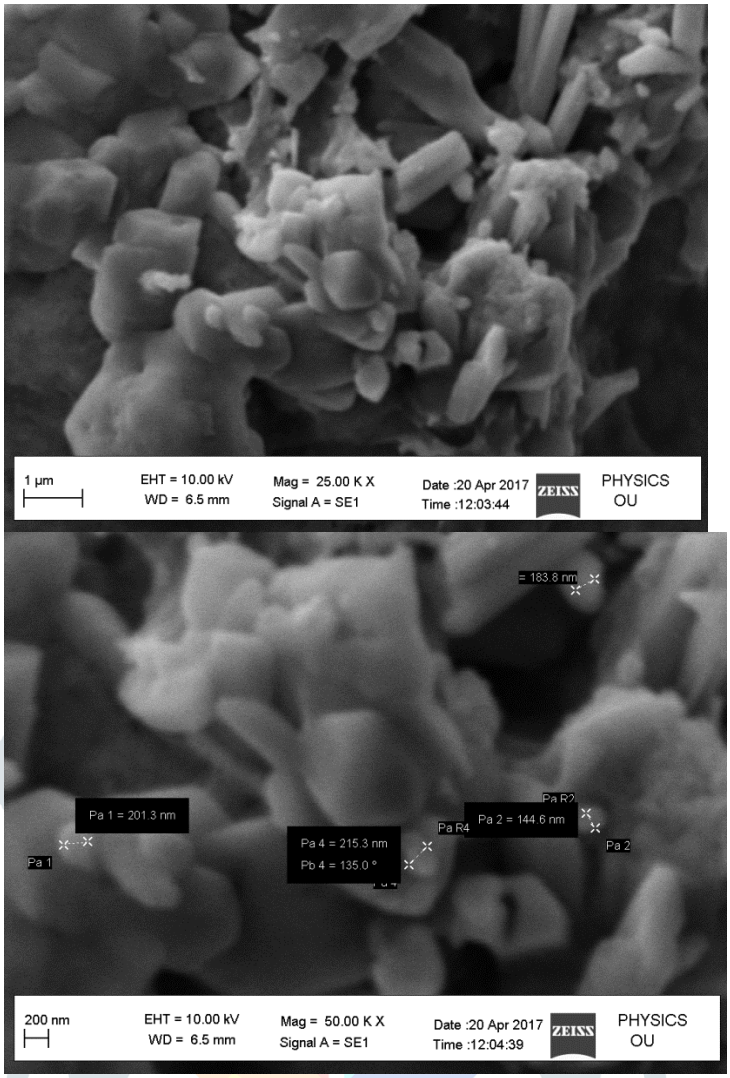
of –planering (N-H) bending, the Asym bending vibrations peak shows C-H ring vibrations are occurs. The hydroxyl groups and (C-N), (C=O) groups of these compounds have a stronger ability to bind silver ions and may beinvolve in the biosynthesis of AgNPs and act as reducing agent for the reduction of silver ions Ag^{+} to silver nanoparticles(Ag^{0}).

The biological molecules such as secondary metabolites may possible play a major role in the synthesis and stabilization of the metal nanoparticles was proved. The functional groups present in the figure are actively participates in the biosynthesis of silver nanoparticles.



Scanning Electron Microscope(SEM)

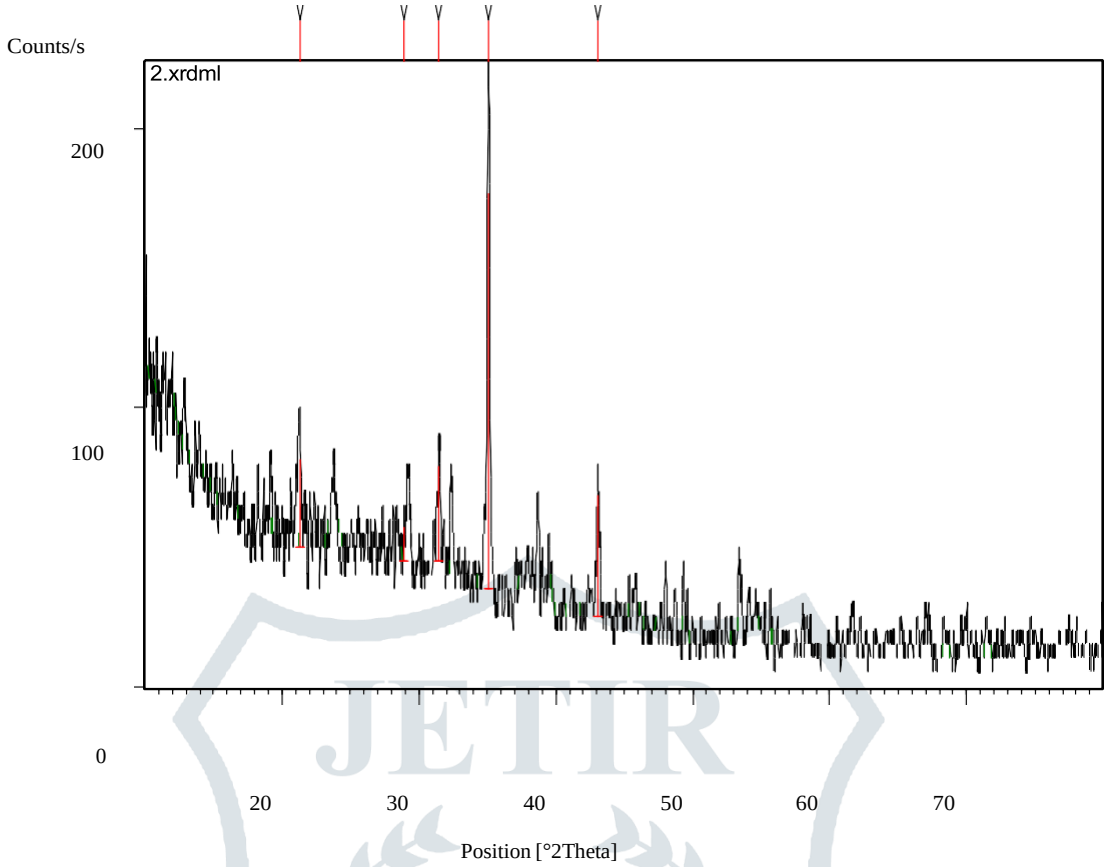
Figures shows representative SEM images of the Agnanoparticles synthesized by treating $AgNO_3$ solution with plant extract the resulting AgNPS were predominantly spherical and the size range from 186.0nm, 144.6 nm. The SEM analysis of Ag nanoparticles from opuntia dellini(cladode) supports the results. Also the rapid bio synthesis of silver nanoparticles of different shapes was observed and the sizes of nanoparticles were increased by high concentration of opuntia dellini (cladode) plant extract.



X-Ray Diffraction (XRD)

X-Ray diffraction is a very important method tocharacterize the structure of crystalline materials and used for the lattice parameters analysis of single crystals, or the phase, texture (or) even stress analysis of sample. X-Ray diffraction of the silver nanoparticles formed from aqueous opuntia dellini (cladode) extract showed a diffraction peak 31.96 corresponding tonanosilver.

According to JCPDS standards of XRD of silver nanoparticles, the most intense peaks are related to '2θ' values of 21.29,28.94,31.41,35.07,43.08. The size of the nanoprticles was calculated by Debye Scherer's equationusing FMWHS obtained from the diffraction peaks. The calculated average value for the size of the silver nanoparticles is about 160.36 nm.



Peak list;

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]
21.2959	6.38	0.4723	4.17232	22.41
28.9460	2.52	0.9446	3.08469	8.84
31.4181	6.90	0.4723	2.84739	24.23
35.0746	28.48	0.4723	2.55847	100.00
43.0841	8.82	0.5760	2.09786	30.97

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CONCLUSION

In the present investigation it was noted that silver nanoparticles synthesized using aqueous opuntia dellini (cladode) extract exhibited blakish brown colour in aqueous solution due to exeitation of surface Plasmon vibrations. The UV absorption spectra of biosynthesis – Nanoparticles in all methods gave absorption maximumat 435.00 nm-361nm.

The phytochemical screening of the aqueous extract of opuntia dellini (cladode) revealed the presence of phytochemicals like Alkaloids, Flavonoids, Carbohydrates, Terpenoids, Sterols, Phenols, Tannins, are present in theopuntia dellini (cladode) of plant extract which is responsible for reduction of silver bulk to silver nanoparticles. Which is revealed from FT-IR studies the presence of such metabolites are indicative of their role in the reduction of silver nitrate to silver nano particles synthesis of silver nanoparticles using aqueous opuntia dellini(cladode) extract at different conditions shows that sonication method formed nanosilver in 10 minutes. Where as 1:1 ratio of silver nitrate and plant extract formed silver nanoparticles in 25 minutes. XRD and SEM results reveals that averagesize of silver nanoparticles synthesized from plant extract was found as polynomial type=cubic shape and144.5 nm in size and (SEM)-183.8nm.

The bio reduction of aqueous silver ions by the plant extract of opuntia dellini (cladode) is a good source for green chemistry approach towards the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic feasility etc

This green method is simple, rapid, eco-friendly, andreliable and it may have a potential use in the bio medical applications. In the future, selection of such plants may creat a new platform for realizing the potential of herbal medicines in nano science for drugdeliver.

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