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THE PHYTOCHEMICAL SCREENING AND GREEN SYNTHESIS OF SILVER NANOPARTICLES, CHARACTERIZATION BY USING *PARMELIA PERLATA* (HUDS)ACH (LICHENS)

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ABSTRACT:

Parmelia perlata (Huds) Ach. (Lichen, parmeliaceae), Commonly known as black stone flower(pathar ke pool) in India, and is usually used as a spice to enhance the taste and flavor of the foods. It is also useful to treat sores, boils, inflammations Seminal weakness and amenorrhea.

OBJECTIVE:

This study was to make photochemical screening to determine the secondary metabolite present on *Parmelia perlata* and to test efficacy of P.perlata, which is used in traditional medicine for infection and wound healing against test bacteria and fungi.a

METHOD:

Preparation of plant extract by percolation process, that is macerated plant powders were soaked in solvents such as Ethanol, Acetone, Aqueous Double distilled water individually. The phytochemical screening is done on the dried powder different solvents extract to investigate the phytochemical groups as alkaloids, flavonoids, terpenoids, tannins, phenolic compounds, quinines, cardiac glycosides, Saponines and steroids etc. and also identified primary metabolites like amino acids, proteins and polysaccharides. and also in this present study aims to green synthesis of silver nano-particles using parmelia perlate (Lichen) thalli extracts. And then characterization of the synthesized silver nano-particle with plant extract by UV-visible, FT-IR, XRD, SEM. The bio-reduction of aqueous silver ions by the plant extract of thalli parmelia perlata is a good source for green chemistry approach.

CONCLUSION:

This green method is simple, rapid, Eco-friendly, and reliable and itmay have a potential use in the bio-medical applications. In the future, selection of such plants may create a new plat-form for

realizing the potential of herbal medicines In Nano-science for drug delivery.

KEY WORDS:

Parmelia perlata (Lichen), Phyto chemical screening lichen extracts,Green synthesis of silver Nano-particles of Lichen extracts, UV-visible, FT-IR, XRD, SEM.

ABBREVIATIONS:

UV-visible - Spectroscopy

FT-IR – Fourier Transforms Infra-Red spectroscopy

XR D – X-Ray diffraction studies

SEM – Scanning Electron Microscopy

INTRODUCTION

The Indian subcontinent is rich in medicinal plants and is one of the richest Countries in terms of genetic diversity of medicinal plants. It exhibits a wide range in topography and elimate. Several plants have been used in folklore medicine.

Medicinal plants are of great importance to the health of individual and communities. The medicial value of these plants lies in some chemical active substances that produce a definite physiological action on human body.

The most important of these chemically active constituents of plants are alkaloids, tannins-flavonoid and phenolic compounds. Medicinal plants are expensive gift from nature to human. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the bio-chemical pathway.Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate.

Natural products have very important role in the drug discovery for the curing of human, animal and plant diseases.

New drug preparations of natural origin are in need due to the numerous side effects and resistance development through the continuos and uncontrolled use of synthetic drugs. Bio active natural compounds have positive influence on the whole organism and without side effects.

In search for those products, many of research teams have focused attention on lichens. Lichens are used since ancient times as one of the natural drug. Lichens represent a symbiotic association of a fungus with an algal partner and are important constituents ecosystem.

Lichens produce characteristic secondary metabolities such as Alkaloids,Quinones, Flavonoides, Tannins, Phenols, Cardiac glycosides which have considerable biological activities such as anti-viral, anti- bacterial, anti-fungal, anti-tumour, anti-oxidant etc.

Until now, about 700 biologically active components were structurally identified from lichens. The Lichen chosen for this study is parmelia perlata colloquially known as Rockflowers, Black stone flower, Sea lichen of Foliose lichen on rocks and tree trunks, Leafy thallus spicy.

This thallus is dirty white or grayish black nearby 5-10 cm long. It is usually used as a spice to enhance the taste and flavor of the foods. It has folkloric repute of cosmetics for skin bleach and has been prescribed to for the management of diarrhea, spermatorrhoea, amenorrhoea, dysentery and wood healing.

It has also been reported to possess the analgesic, antipyretic and astringent activity. Its smoke relives the headache and heal the wound.

The survey of the literature reveals that more than 71 species of parmelia genus have been identified so far and only few of them have been analysed chemically.

Lichens are complex plants living in symbiotic relationship with fungi and algae, and the pertinent partners are defined as mycobiont and phycobiont respectively. In all lichens, the fungus forms a thallus of

lichenized stroma that may contain characteristic secondary compounds. These secondary metabolites are unique with respect to those of higher plants. So lichens are used in folk medicines by many peoples.

Aims of the Dissertation

Parmelia perlata L. Lichen. Family: parmeliaceae. a potent lichen. Commonly known as Black stone flower in India. This lichen Foliose lichen on rocks and tree trunks, and is usually used as a spice to enhance the taste and flavor of the foods. It is also useful to treat sores, boils, inflammations and amenorrhoea.

Objective:

This study is to make photochemical screening to determine the secondary metabolites present in parmelia perlata and also Green synthesis of silver nanoparticals and UV- Visible spectra, FTIR, XRD and SEM characterization of secondary metabolites ofparmelia perlata Lichen.

1.Method:

Ethanol, Acetone, Aqueous or double distilled water was used forextraction of parmelia perlata 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 parmelia perlata in this study reveal presence of different phytochemical groups as alkaloids, flavonoids, terpenoids, tannins, phenolic compounds, quinines, cardiacglycosides, saponines and steroids etc. and also identified primary metabolites like aminoacids, proteins and polysaccharides.

1. **Green Synthesis of Silvernanoparticles using parmelia perlata Plant extracts:**

The present study aims to synthesize Silver nanoparticles by a green biological route, using an extract derived from parmelia perlata (Lichens), And characterization of the synthesized silver nanoparticles with plant extract

characterized the bioreduced Silver nanoparticles are Utilizing UV-Visible spectroscopy, Fouriertransform Infrared spectroscopy (FT-IR) analysis, X- ray diffraction (XRD) and scanning electron microscope (SEM).

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:

1) Collection and Identification of Plant Meterials :

- The plant material parmelia perlata (Lichens) were parchased from the local more super market in Kurnool, A.P. This lichens identifiedby the botony department faculty K. Venkatarathnam, R.U, Kurnool.
- Preparation of Plant extract: Percolation Process :
- The dried lichen thalli parmelia perlata were powdered using mixer grinder. For the percolation process, the macerated plant powders were soaked in solvents such as Ethanol, Acetone, 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 whatmann No.1 filter paper, evaporated and concentrated under room temperature and used the deposited crude materials for phytochemical analysis

PARMELIA PERLATA (LICHENS) (BLACK STONE FLOWER)

PHYTOCHEMICAL SCREENING:

Phytochemical analysis of solvent extracts of the lichens samples was carried out using standard qualitative methods following the methodology of Harborne(1973) Trease and Evans(1989) and Sofowara(1993).

Tests For Alkaloids

a)Mayer's test:(Potassium Mercuric Iodide Solution)

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

a)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.

a)Hager's test:(Saturated solution of Picric acid)

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

Test for Phenolic Compounds

Ferric Chloride Test:

To the 1ml of solven**t extracts, 3ml of** double distilled water was added to this a few drops of neutral 5% of ferric chloride solution was added it gives dark green colour solution is formed. This indicates presence of phenolic compounds

Lead acetate test :

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

TEST FOR FLAVONOIDS

a)Shinoda test(Megnesium 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 after few minutes.

b)Zinc-HCl reduction test:

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

c)Alkaline reagent test:

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

d)Ammonia test:

A few drops of 1% NH_3 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 extracts were treated with 2 drops of alcoholic α- naphthol solution in a test tube and 2 ml conc.H_2SO_4 was addedcarefully along the sides of the test tube. Formation of dull violet/red ring at the interphase indicates the presence of carbohydrates.

TEST FOR REDUSING SUGARS:

Benedict's test:

The extracts were treated with Benedict's reagent and heated on a water bath. Formation of an orange red precipitate indicates the presence of reducing sugars

Test for Quinines:

To 1 ml of extract,1 ml of Conc.H_2SO_4 was added.Formation of red colour indicated the presence of quinines.

Test for Tannins:

To 1 ml of the solvent extract, few drops of 1% $FeCl_3$ solution were added. The appearance of a blue, black, green or blue green precipitate indicated the presence of tannins.

TEST FOR TERPENOIDS

Salkowski Test:

To 1 ml of the solvent extract, 2 ml of chloroform was added. Then 3 ml of conc.H_2SO_4 was added carefully to form a layer. A reddish brown coloration of the interfaces indicates the presence of terpenoids.

Test for Cardiac glycosides

Keller-Killani Test:

The extract was dissolved in glacial acetic acid containing traces of $FeCl_3$. The tube was then held at an angle of 45^o and 1 ml of Conc.H_2SO_4 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 H_2SO_4. Change in colour from violet to blue or green indicates the presence of steroids.

Phyto chemical screening result obtained from Parmelia Perlata

(Lichens) (black stone flower)

Phytochemica lconstituents	**Ethano lextract**	**Acetone 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-HClreductiontest	++		
Alkalinereagent(NaOH)	+	-	++
Ammonia test	+	-	++
Test for steroids	+	-	+
Test for Tannins	++	+	+
Test for Terpenoids Salkowski test	++	-	+
Test for Quinines	++	+	+
TEST FOR CARBOHYDRATES			
Molisch's	++	++	++
TEST FOR REDUCING SUGARS			
Benedict's test	++	-	++

TEST FOR CARDIAC GLYCOSIDES			
Keller-Killani test	++	++	++

+ = Indicates presence of the phyto constituents

++= Indicates presence in more quantity of the phyto constituents

–= Indicates absence of the phyto constituent

Powdered Parmelia perlata (Black stone flower) soaked varies solvents

Acetone Extract

Aqueous Extract

Ethanol extract

METERIALS AND METHODS

Preparation of plant extract:

Taking 10 g of dried lichens thalli parmelia perlata were powdered using mixer grinder. The macerated plant powder wa done by soaking with 300 ml 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 80^{o}c 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 -4^{o}c in the refrigerator, for further bio synthesis process.

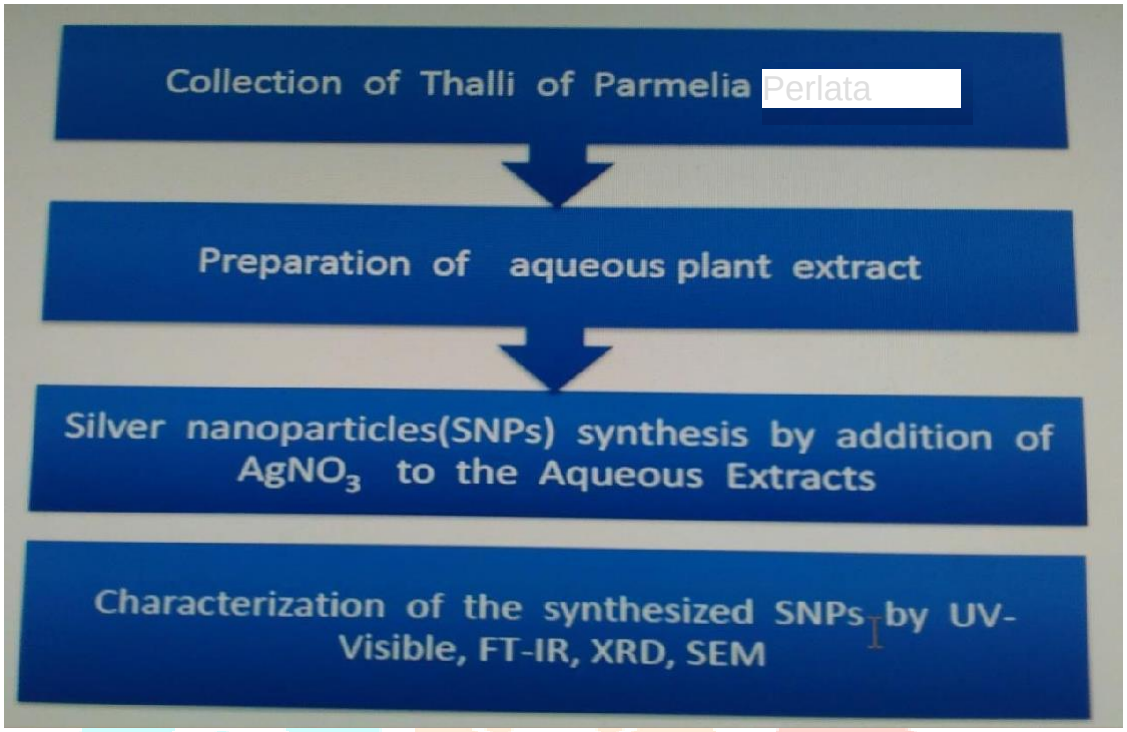
1)CHEMICALS:

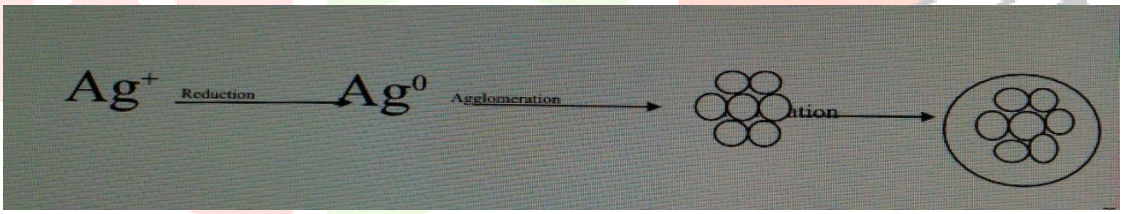
Silver Nitrate ($AgNO_3$) was purchased from Sigma Aldrich. Bangalore. India. Double distilled water was used throughout the experiment. All other chemicals were ofanalytical grade.

BIO SYNTHESIS OF SILVER NANO PARTICLES

Bio synthesis of silver nanoparticles includes, 80 ml supernatant of parmelia perlata 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 orange coloured to merune purple colour. That confirm the formation of silver nanoparticles. Further the colloidal solution was Ultra centrifuged at 8000 rpm for 20min and the supernatant and solid was collected for further studies.





RESULTS AND DISCUSSION

1) By color change

The sequential color change indicates the formation of AgNPS by plant materials. This is the primary test for the checking of formation of AgNPS. The color reduction of

$AgNO_3$ into nano particles was visibly evident from the colour change.

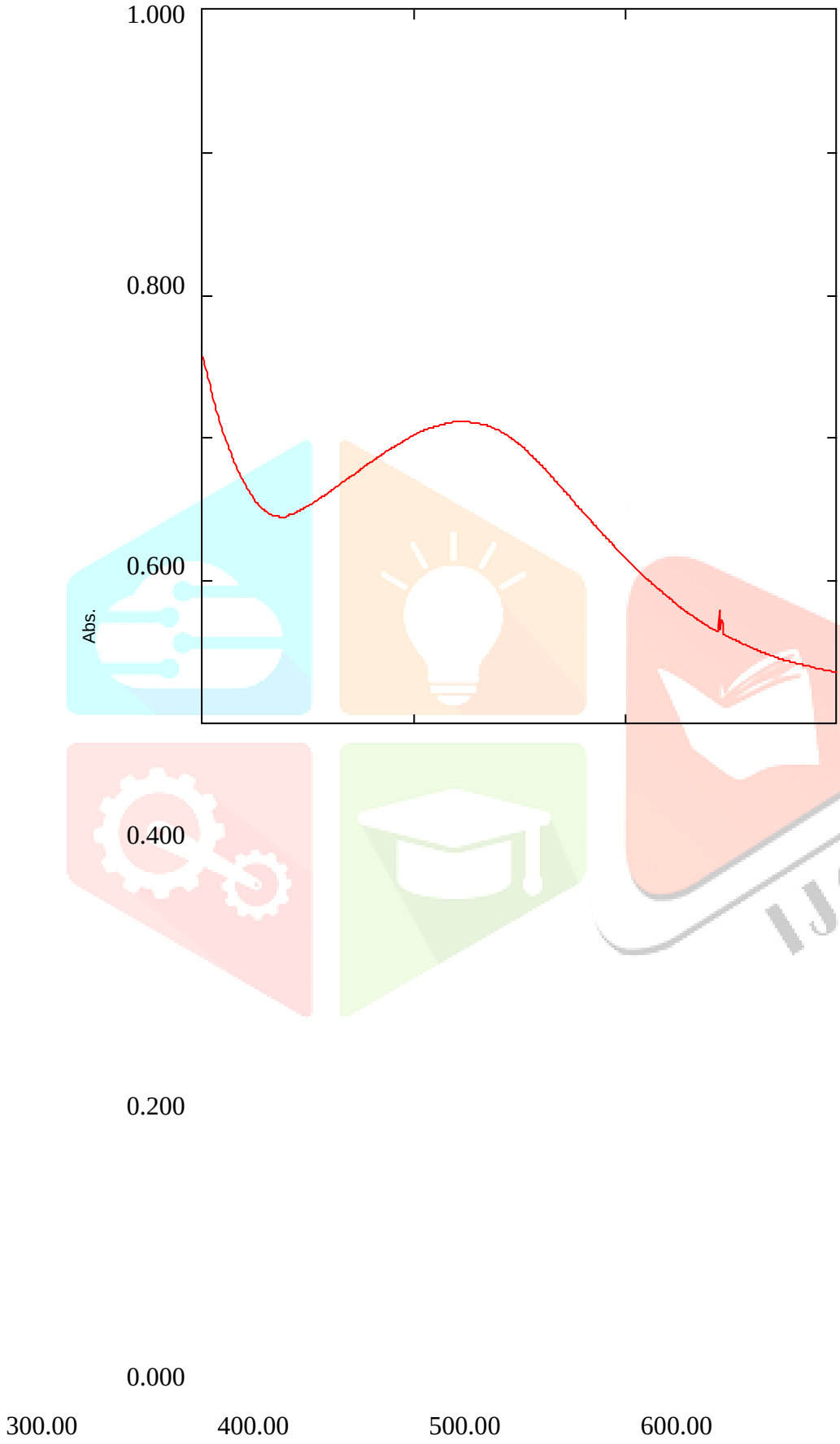
Pure filter Aqueous plant extract was added into a 1mM silver nitrate solution and boiled on water bath few minutes, the pale orange colour was changed from

merune purple colour after 30-35 minutes. After 18-24 hrs colour was changed into dark brown. This colour change indicates the formation of AgNPS.

1) UV-Visible Spectroscopy Analysis of AgNPS:

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

Fig:1 shows the UV spectrum of AgNPS and at 422nm, 337nm, a peaks was observed that was identified to be AgNPS . Generally, it is well known that AgNPSexhibit reddish brown. The colour change ensued as of the active molecules present in the lichen 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


1.000
0.800
0.600
0.400
0.200
0.000
Abs.
300.00
400.00
500.00
600.00

nm.

No.	P/V	Wavelength nm.	Abs.
1	741.50	0.029	
2	267.00	4.000	
3	231.50	4.000	
4	224.00	4.000	
5	214.00	4.000	
6	707.00	0.022	
7	237.00	3.962	
8	227.50	3.979	
9	219.00	3.994	
10	209.00	3.997	

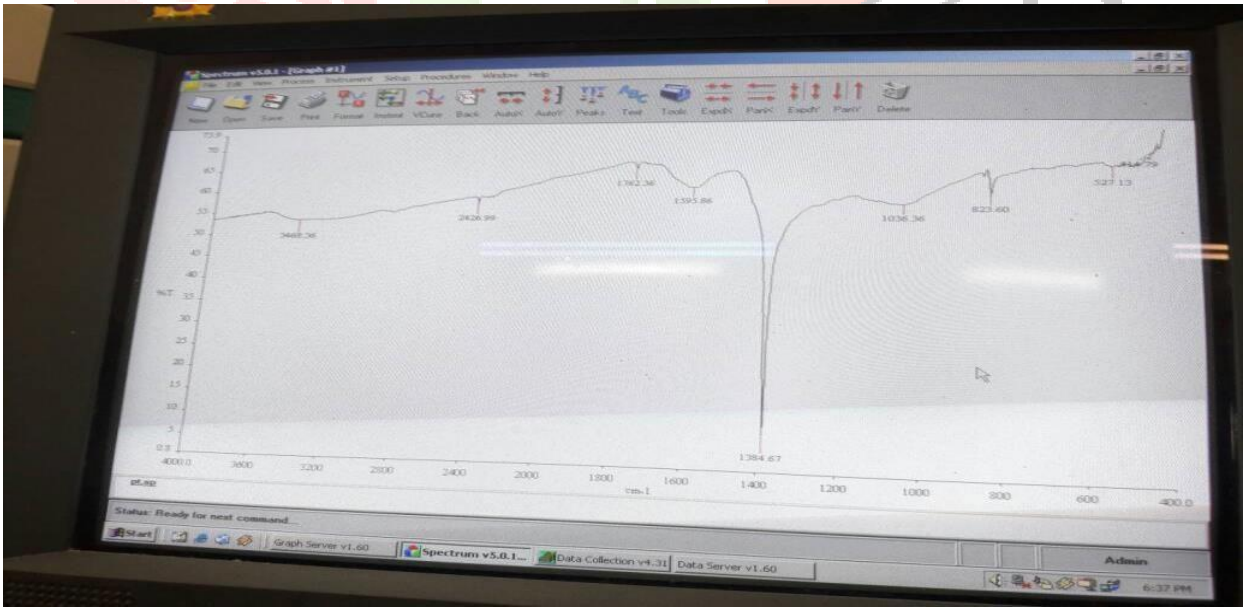
FT-IR Analysis:

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 silver ions into silver nanoparticles.

Figure(1a) show peaks at 3469cm^{-1} corresponds to the O- H stretching of -OH hydroxyl groups. 2426.99cm^{-1} was assigned NH^{+} asym stretching vibrations and also peak at 1782cm^{-1} represents C=O 1595.86cm^{-1} peak shows the C-NH Asym bending vibrations occurs. 1384.67cm^{-1} peaks shows NO_2 group present in the bio molecules.

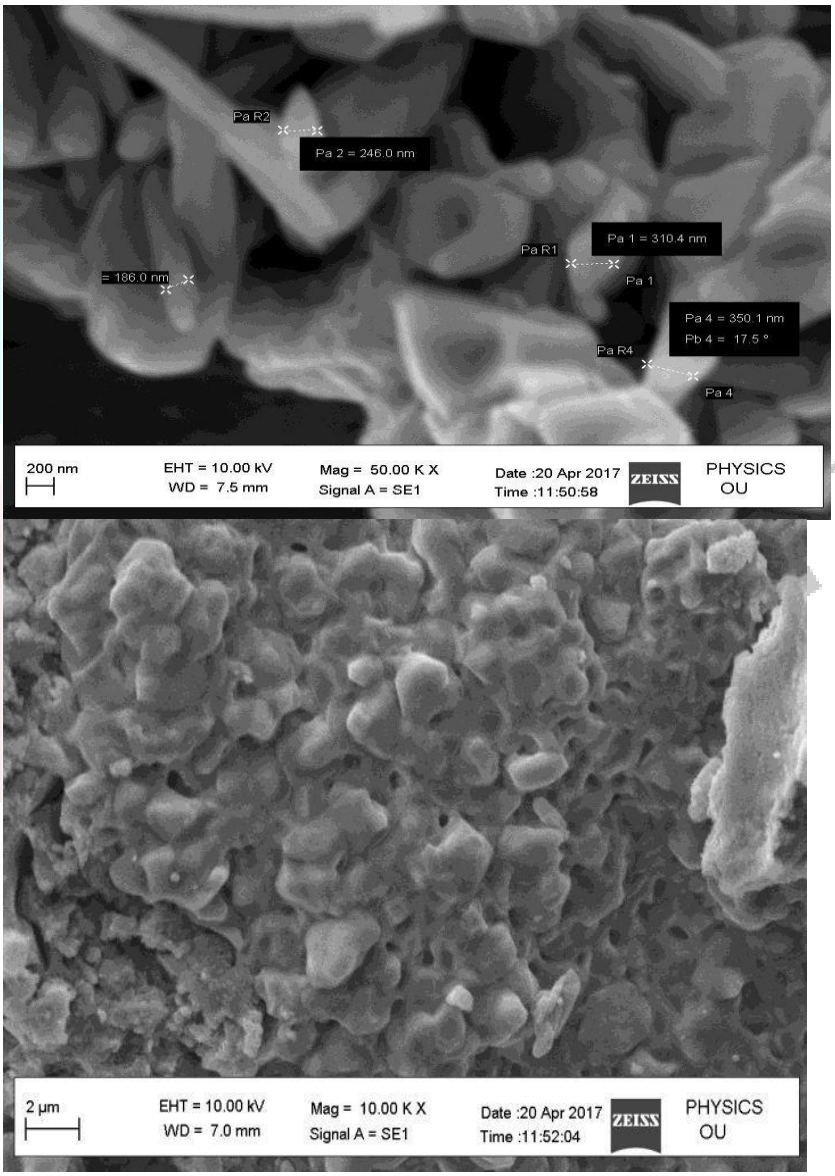
1036.36cm^{-1} peaks shows (C-N) vibrations. 823.60cm^{-1} peaks shows (C-H) vibrations of out of plane bending 527.13cm^{-1} peak shows C-H ring vibrations are occurs. The hydroxyl groups and (C-N), (C=O) groups of these compounds have a stronger ability to bind silver ions and may be involve in the 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.



1)Scanning Electron Microscope(SEM)

Figures shows representative SEM images of the Ag nanoparticles synthesized by treating $AgNO_3$ solution with plant extract the resulting AgNPS were predominantly spherical and the size range from 186.0 nm, 246.0 nm. The SEM analysis of Ag nanoparticles from parmelia perlata(Lichens) 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 Parmelia Perlata plant extract.



1) X-Ray Diffraction (XRD)

X-Ray diffraction is a very important method to characterize 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 parmelia perlata extract showed a diffraction peak 31.55 corresponding to nanosilver.

According to JCPDS standards of XRD of silver nanoparticles, the most intense peaks are related to '2θ' values of 27.51, 29.09, 34.81, 34.82, 63.57. The size of the nanoprticles was calculated by Debye Scherer's equation using FMWHS obtained from the diffraction peaks. The calculated average value for the size of the silver nanoparticles is about 113.73 nm.

This is the simple example template containing only headers for each report item and the bookmarks.

Modify it according to your own needs and standards.

Measurement Conditions: (Bookmark 1)

Sample Identification 6

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Scan Type Continuous
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Generator Settings 40 kV, 30 mA Goniometer Radius

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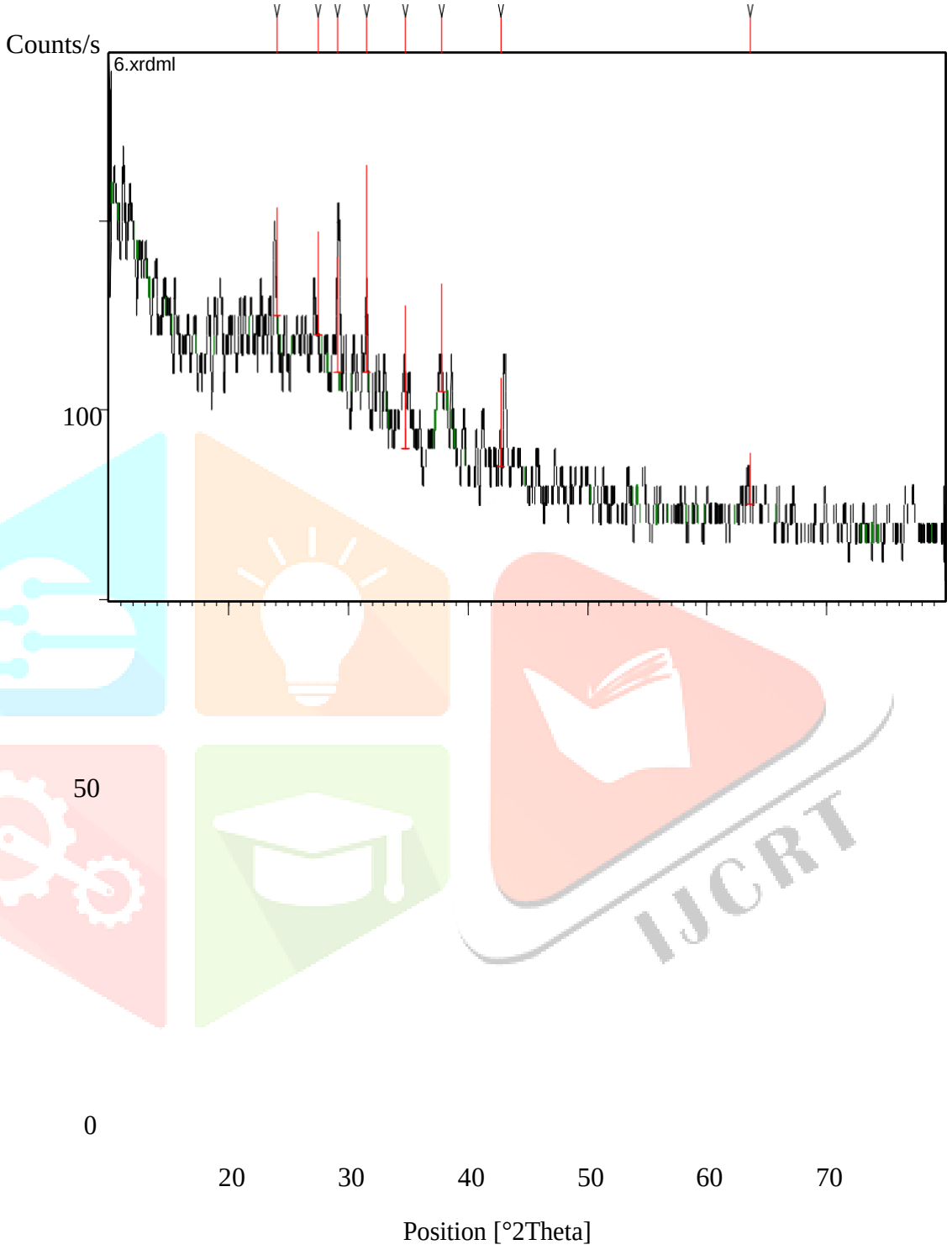

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27.5100	5.50	0.7866	3.24236	49.99
29.0980	6.19	0.6298	3.06892	56.25
31.5500	11.00	0.0200	2.83578	100.00
34.8100	7.60	0.8640	2.57732	69.07
34.8214	2.42	0.9446	2.57650	22.02
37.8100	5.77	0.0200	2.37944	52.46
42.7300	4.78	0.3835	2.11617	43.47
63.5700	2.80	0.7684	1.46362	25.45

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CONCLUSION

In the present investigation it was noted that silver nanoparticles synthesized using aqueous parmeli Perlata (Lichens) Black stone flower extract exhibited reddish brown colour in aqueous solution due to exeitation of surface Plasmon vibrations. The UV absorption spectra of biosynthesis – Nanoparticles in all methods gave absorption maximum at 422.0 nm-337 nm.

The phytochemical screening of the aqueous extract of parmelia perlata(Lichens) revealed the presence of phytochemicals like Alkaloids, Flavonoids, Carbohydrates, Terpenoids, Sterols, Phenols, Tannins, Quinones are present in the thalli 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 parmelia perlata 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 average size of silver nanoparticles synthesized from plant extract was found as polynomial type=cubic shape and 113.73 nm in size and (SEM)-186.0 nm.

The bio reduction of aqueous silver ions by the plant extract of thalli Parmelia perlata 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, and reliable and it may have a potential use in the biomedical applications. In the future, selection of such plants may creat a new platform for realizing the potential of herbal medicines in nano science for drug delivery.

BIBLIOGRAPHY

References

1) A. Singh, R. Shukla, S. Hassan, R.R. Bhonde, M. Sastry. International Journal of Green Nanotechnology. 3(4), (2011),

2) D. Philip, C. Unni, S.A. Aromal, V.K. Vidhu. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. (2011),

3) A.K. Jha, K. Prasad. International Journal of Green Nanotechnology. (2011),

4) M.M.R. Mollick, B. Bhowmick, D. Maity, D. Mondal, M.K. Bain, K. Bankura,

J. Sarkar, D. Rana, K. Acharya, D. Chattopadhyay. International Journal of Green Nanotechnology. (2012)

5) S.S. Shankar, A. Rai, A. Ahmad, M. Sastry. Journal of Colloid and Interface Science. (2004),

6) A.M. Awwad, N.M. Salem, A.O. Abdeen. International Journal of Industrial Chemistry. (2013),

7) G. Rajakumar, A.A. Rahuman. Research in Veterinary Science. (2012),

8) A.A. Zahir, A.A. Rahuman. Veterinary Parasitology. (2012)

9) C. Krishnaraj, E.G. Jagan, S. Rajasekar, P. Selvakumar, P.T. Kalaichelvan, N. Mohan. Colloids and Surfaces B: Biointerfaces. (2010),

10) J.Y. Song, H.K. Jang, B.S. Kim Process Biochemistry. (2009),

11) Y.L. Balachandran, P. Peranantham, R. Selvakumar, A.C. Gutleb, S. Girija. International Journal of Green Nanotechnology. (2012),

12) D. Cruz, P.L. Fale, A. Mourato, P.D. Vaz, M.L. Serralheiro, A.R.L. Lino. Colloids and Surfaces B: Biointerfaces. (2010),

13)D.Philip, C.Unni. Physica E: Low-dimensional Systems andNanostructures. (2011),

14)S.R. Bonde, D.P. Rathod, A.P. Ingle, R.B. Ade, A.K. Gade, M.K. Rai. Nanoscience Methods. (2012),

15)D. Philip. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. (2011),

16)D. Philip. Physica E: Low-dimensional Systems and Nanostructures.(2010), 4

17)A.D. Dwivedi, K. Gopal. Colloids and Surfaces A: Physicochemical and Engineering Aspects. (2010),

18)K.B. Narayanan, N. Sakthivel. Materials Characterization. (2010),

19)K.B. Narayanan, N. Sakthivel. Materials Research Bulletin. (2011),

20)M.R. Bindhu, M. Umadevi. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. (2013),

21)K.Raja, A. Saravanakumar, R. Vijayakumar. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. (2012),

22)M.F. Zayed, W.H. Eisa, A.A. Shabaka. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. (2012),

23)Y.S. Rao, V.S. Kotakadi, T.N.V.K.V. Prasad, A.V. Reddy, D.V.R.S. Gopal. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. (2013),

24)R.Vivek, R.Thangam, K. Muthuchelian, P. Gunasekaran, K. Kaveri, S. Kannan. Process Biochemistry. (2012),

25)D. Prabhu, C. Arulvasu, G. Babu, R. Manikandan, P. Srinivasan. Process Biochemistry. (2013),

26)K. Prabakar, P. Sivalingam, S.I.M. Rabeek, M. Muthuselvam, N. Devarajan, A. Arjunan, R. Karthick, M.M. Suresh, J.P. Wembonyama. Colloids and Surfaces B: Biointerfaces.