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A Study on The Applications of Silver Nanoparticle Synthesized Using The Aqueous Extract And The Purified Secondary Metabolites of Lichen *Parmelia perlata*

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Abstract: *Lichens are generally a symbiotic association between a fungal and an algal partner. They produce an array of secondary metabolites that are common in plants or higher fungi but 80% of metabolites are produced specifically by lichens. The secondary compounds produced by lichens are commonly referred to as "Lichen acids'' and are produced primarily by the mycobiont partner and are deposited externally on hyphae. The lichen acids possess pharmaceutical applications such as antimicrobial, antiviral and anticancer properties. The Present study was carried out on the synthesis, characterization and application of silver nanoparticles using the aqueous extract and the individual purified compounds of Lichen Parmelia perlata. The Silver nanoparticles were synthesized using the aqueous extract and using the purified compounds obtained from the methanolic extract and the nanoparticles thus obtained were characterized by means of UV-Visible spectroscopy, Fourier transform infrared spectroscopy and Scanning electron microscopy. The applications of these nanoparticles were determined by studying their antimicrobial, antioxidant and antidiabetic activity. Many reports are available for the synthesis of nanoparticle using the aqueous extracts but the nanoparticle synthesis using the individual secondary compounds were not carried out before so this study was one such attempt to identify the stabilizing and reducing potential of the secondary compounds in the synthesis of silver nanoparticle that could prove to be useful to the mankind for different applications.*

Keywords: *Fourier transform infrared spectroscopy, Parmelia perlata, Scanning electron microscope, Secondary compounds, Silver nanoparticle, UV-Visible spectroscopy.*

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I. Introduction

Lichens are composite organisms that arise from a symbiotic association between the fungal and an algal partner. They exist in different growth forms such as fruticose that are shrubby forms with branches, foliose that are complex with leafy forms and crustose which spread over the surface of their habitat **[1]**. They produce numerous secondary metabolites which are utilized for different purposes such as in the treatment of diarrhea, jaundice, fever, epilepsy and also in treating conditions such as injuries, bone fractures and skin eruptions. They possess high sensitivity towards specific pollutants and thereby are used as bioindicators and also play an important role in the absorption and accumulation of heavy metals **[**2]. *Parmelia perlata* is a species of lichen that belongs to the family Parmeliaceae and is commonly referred to as "Stone flower". This lichen grows in rosettes and spreads irregularly over the substratum **[3].** They possess innumerable applications in the field of medical sciences with antiemetic, analgesic and antipyretic properties. They are also widely utilized in treating boils, inflammations and also for pain in renal and lumbar regions. The powdered drug is used for improving digestion, to maintain normal body temperature and is also used as an important ingredient in cosmetics. Nanotechnology is an emerging field of study that deals with the development of nanoparticles within the size range of about 10-100nm **[4]**. Different types of nanoparticles can be produced using various metals and have wide applications **[5]**. The nanoparticles can be synthesized either by Physical, chemical or biological methods but nanoparticle synthesis through biological method has received much attention as it is considered to be environmentally safe. Among the metal nanoparticles Silver nanoparticles are of special interest due to their unique properties **[6]**.

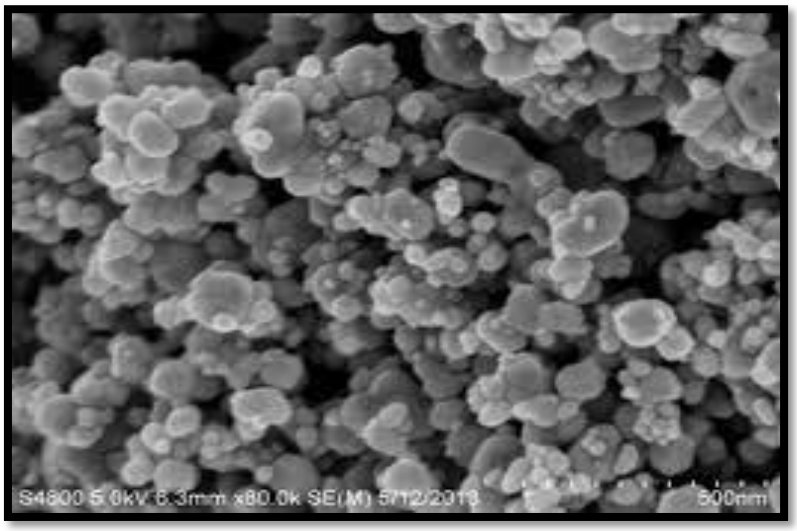

Figure 1: SEM image of Silver nanoparticle

Silver nanoparticles are widely employed in medical sciences for the diagnostic and therapeutic purposes such as in eye treatment, wound dressings, detecting pathogens, in diagnosis, in drug delivery and in detection of tumor **[7]**. They are commercially utilized in textile industries where the nanoparticles are incorporated or coated on to a fibre, in food industry because of their antibacterial activity and absence of preservatives also utilized in products like food, paste, plastic due to their germicidal activity **[8] (Fig 1).**Secondary metabolites are organic compounds synthesized by various natural sources and play an important role as colorant, aromatic agents and also in functional foods. In the current study Silver nanoparticles were synthesized using two different secondary metabolites they are Glycosides and Alkaloids. Glycosides are organic compounds which on hydrolysis yield sugar group along with the non sugar group while alkaloids are naturally occurring compounds that contain nitrogen atoms. These compounds are produced by bacteria, fungi, plants as well as animals and are widely utilized as medications, drugs and have numerous applications. Thus the present study was carried out to determine the antimicrobial, antioxidant and antidiabetic applications of Silver nanoparticles synthesized using the aqueous extract as well as the purified glycoside and alkaloid compounds of Lichen *Parmelia perlata*.

II. Materials And Methods

2.1 Sample Preparation

Dried samples of Lichen *Parmelia perlata* were obtained from the local wholesale market at Virugambakkam, Chennai and were identified based on the morphological and relevant keys given in the literature **[9]**. The sample was cleaned, washed under tap water and distilled water to remove the dirt, it was dried and powdered using a mixer. The powdered lichen sample was stored in clean bottle for further analysis.

2.2 Extraction of Sample

The crude extract from the lichen sample was obtained by means of cold extraction method using methanol and water. For methanolic extraction about 50g of the powdered lichen sample was added to 500ml of methanol in a conical flask, covered with aluminium foil and kept on a rotary shaker for 3 days at room temperature while for aqueous extraction about 50g of the powdered lichen sample was added to 500ml of double distilled water taken in a conical flask and was kept in water bath at 65ºc for about 1 hour to enhance complete extraction. The solution obtained from both methanolic and aqueous extraction were filtered using Whatman no 1 filter paper and the filtrates obtained were collected and utilized for further analysis **[10].** The yield of respective crude extract from methanolic extraction was calculated as: Percentage yield (%) = (dry weight of extract/dry weight of samples) × 100.

2.3 Identification, Isolation and Purification of Secondary metabolites

2.3.1 Thin Layer Chromatography:

Thin-layer chromatography (TLC) is a chromatographic technique used for separating different components from a mixture. It is generally carried out on a thin sheet of plastic or aluminium foil coated with an adsorbent material. The adsorbent material could either be cellulose, silica or alumina and acts as the stationary phase while the solvent or mixture of solvents acts as the mobile phase. A line is drawn about 1cm from one end of the cut TLC plate and using a capillary tube the samples were spotted on to the plate and allowed to air dry.

The solvent mixture utilized as mobile phase for isolation of glycoside compounds are **Toluene: methanol: glacial acetic acid: water = 7:4:3:1** and for alkaloids are **Butanol: acetic acid: water = 4:1:3 [11]**. The plates were placed carefully in the TLC chamber and kept closed. The compounds present in the extract get separated as the solvent rises up. The TLC plates were then removed from the chamber and kept for drying. The spots obtained on the plates were identified by using a suitable spraying reagent. Iodine chamber is utilized for the detection of glycoside compounds while dragendroff's reagent is used for the detection of alkaloids **[12] [13]**. The presence of glycoside and alkaloid compounds was confirmed by the development of brownish band and reddish orange band respectively. The R_f value for the developed spots were calculated using the formula: R_f = Distance travelled by the solute / Distance travelled by the solvent

2.3.2 Column Chromatography:

The Stationary phase utilized for column chromatography is silica gel (100-200 mesh). The glass column was packed with silica gel using methanol and was layered with the crude extract. The column was allowed to run by adding a suitable solvent mixture (**Toluene: methanol: glacial acetic acid: water** in the ratio of **35:20:15:10)** for glycosides and (**Butanol: acetic acid: water** in the ratio of **40:10:30)** for alkaloids. The collected fractions were monitored by means of thin layer chromatography. The fractions obtained were then subjected to Gas chromatography – Mass spectrometry (GC-MS) for the identification of individual compounds in the sample **[14].**

2.3.3 Confirmatory test: [15]

1. **Glycosides: Keller –Killani Test:** To 1ml of sample add 1ml of glacial acetic acid and 1ml of concentrated Sulphuric acid. Formation of reddish brown colour indicates the presence of glycosides.
2. **Alkaloids: Dragendroff's test:** About 2-3 drops of Dragendroff's reagent is added to 2ml of sample. Formation of orange red coloured complex indicates the presence of alkaloids

2.4 Identification of Secondary metabolites

2.4.1 Gas chromatography – Mass spectrometry (GC-MS):

It is an analytical technique used for identifying different components within a test sample. The Clarus 680 GC was used for the analysis and a fused silica column was employed and packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run.1μL of extract sample was injected into the instrument and the oven temperature was maintained as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The spectrums of the components were compared with the database spectrum of known components in the GC-MS NIST (2008) library **[16]**.

2.5 Synthesis of Silver nanoparticle

Using the aqueous extract and the purified fractions:

About 30ml of 1mM silver nitrate was prepared and taken in a beaker. The beaker containing magnets were placed on the magnetic stirrer with a rotation of 5,000 rpm at 60ºc. 10ml of Lichen **(*Parmelia perlata*)** aqueous extracts and purified glycoside and alkaloid fractions obtained through column chromatography were taken separately in a conical flask and was added drop wise into the beaker containing silver nitrate. The colour change from colourless to dark brown indicates the formation of silver nanoparticles. The reduction of the Ag+ ions was measured by UV-Vis spectrum **[17].**

2.6 Characterization of Silver nanoparticles

2.6.1 Ultraviolet - Visible Spectroscopy:

UV-Vis spectral analysis was done by using UV-Visible spectrophotometer – UV - 1800. It is a type of absorption spectroscopy in which light of ultraviolet region is absorbed by the molecule and results in the excitation of the electrons from the ground state to a higher energy state. The energy of UV radiation absorbed is equal to the energy difference between ground state and higher energy state. The absorbance was recorded at different nanometers (400-500nm) and the Optical density (OD) was calculated **[18].**

2.6.2 Fourier Transform Infrared Spectroscopy (FTIR):

FTIR analysis was carried out using Shimadzu IRTracer-100 by means of KBr Pellet Method. To prepare a liquid sample, a drop of liquid is placed on the face of a highly polished salt plate (such as NaCl, AgCl or KBr), then a second plate is placed on top of the first plate so as to spread the liquid in a thin layer

between the plates, and the plates were clamped together and excess liquid is wiped off and the sandwich plate is mounted onto the sample holder. After finishing the experiment, the plates are wiped with isopropanol and returned to the desiccators **[19].**

2.6.3 Scanning Electron Microscopy (SEM):

The Synthesized nanoparticles were centrifuged at 5,500 rpm for 15 minutes. The supernatant was discarded and the pellet was dissolved in double distilled water and centrifugation was repeated twice to remove the impurities. The pellet obtained finally was taken in petriplates and kept in hot air oven at 70ºc for about 30 minutes for drying. After it has completely dried the nanoparticles were scrapped off, stored in eppendorf and utilized for SEM analysis **[20].**

2.7 Applications

2.7.1 Antimicrobial activity:

The antibacterial activity of Lichen (***Parmelia perlata***) extract, Silver nanoparticle from aqueous extracts and from glycoside and alkaloid fractions were screened against both gram positive and gram negative bacteria such as *Staphylococcus aureus, Streptococcus spp, Escherichia coli, Klebsiella pneumoniae, Salmonella spp* and *Pseudomonas aeruginosa* while the antifungal activity was carried out against *Aspergillus spp* and *Candida albicans.*

2.7.1.1 Agar well diffusion method:

The stock cultures were maintained at 4°C on the nutrient agar slant slopes. Nutrient broth was prepared for about 50ml and inoculated with a loop-full of stock cultures and incubated at 37°C for 24 hours while Potato dextrose broth was prepared for the growth of fungal strains. Muller Hinton agar medium (MHA) was prepared for about 250ml and used to study the antibacterial activity while Potato Dextrose agar medium (PDA) was prepared and used for antifungal activity.The media was poured into the petriplates and allowed to solidify. Once solidified the bacterial and the fungal cultures were swabbed onto the agar medium using a sterile cotton swab .The wells were punctured using a sterile cork borer. Different concentrations of samples (20, 40, 60, 80mg/ml) were dispensed into each well using a micropipette. The petriplates were then incubated at 37°C for 24 hours for bacteria and 37°C for 3-4 days for fungi and observed for the zone of inhibition. The diameter of the zone of inhibition was measured in mm **[21].**

2.7.2 Antioxidant activity: The antioxidant potential of Lichen (***Parmelia perlata***) crude extract, Silver nanoparticles from aqueous extracts and from purified fractions was determined by 3 methods: Total antioxidant capacity assay, Hydrogen Peroxide scavenging assay and reducing power assay.

2.7.2.1 Total antioxidant capacity assay: About 1ml of the test sample is added to 3ml of freshly prepared phosphomolybdenum reagent and was incubated for about 90 minutes in water bath at 95ºC .It was then cooled to room temperature and absorbance was measured at 695nm in a spectrophotometer.1ml methanol without extract served as blank. Ascorbic acid was used as the standard **[22].** The Total antioxidant capacity can be calculated using the formula: Total antioxidant capacity (TAC) = (A of Control – A of test) / A of Control × 100

2.7.2.2 Hydrogen peroxide scavenging assay:

Solution of Hydrogen peroxide (40mM) was prepared in phosphate buffer **(pH 7.4).**1ml of the test sample was added to 3ml of Hydrogen peroxide solution and was incubated for about 10 minutes at room temperature and the absorbance was measured at 230nm in a spectrophotometer. Ascorbic acid was used as the standard. Phosphate buffer without hydrogen peroxide served as blank **[23].**It can be calculated using the formula: % scavenged (H_2O_2) = (A of Control – A of test / A of Control) × 100

2.7.2.3 Reducing power assay:

In this method 2.5ml of sample was mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5ml of 1% Potassium ferricyanide (10mg/ml).The mixture was incubated at 50ºc for 20 minutes then rapidly cooled and mixed with 2.5ml of 10% Trichloroacetic acid and centrifuged at 6500 rpm for 10 minutes. About 2.5ml of supernatant was diluted with 2.5ml of distilled water and then 0.5ml of 0.1% ferric chloride was added and allowed to stand for 10 minutes. The absorbance was read spectrophotometrically at 700nm **[24].**

2.7.3 Antidiabetic activity: The antidiabetic activity of Lichen crude extract, Silver nanoparticle from aqueous extract and from purified fractions were determined by means of Alpha amylase inhibition assay.

2.7.3.1 Alpha amylase inhibition assay:

About 1ml of the sample was added to 1ml starch solution and was incubated for 10 minutes at room temperature.0.5ml of the prepared enzyme solution was added to the mixture and incubated at 25ºc for about 10 minutes. The reaction was terminated by addition of 1ml of colorimetric reagent and was kept in water bath for 5 minutes and cooled to room temperature. This was further diluted by adding 10ml of distilled water and the absorbance of the mixture was measured at 540nm in colorimeter. Sample without extract served as blank **[25].** The % inhibition was calculated using the formula: % inhibition = (A of Control – A of test / A of Control) × 100

III. Results And Discussion

3.1 Extraction: The Lichen (***Parmelia perlata*****)** sample was extracted by means of cold extraction method using methanol. Methanol was found to be capable of dissolving polar compounds with a polarity index of 5.1. Therefore methanol was highly preferred and used for extraction purposes **[26]**. Water has a polarity index of about 10.2 and its ability is compared with that of methanol in extracting the compounds. The Percentage yield of Lichen crude extract was found to be 2.56 % and it has been reported to be the total yield for about 50 grams of the dry weight of the sample **[27] (Table 1).** A work has reported the extraction of Lichen *Parmelia perlata* using petroleum ether solvent and the total yield was found out to be 0.27 % **[28].**

Table 1: Estimation of Percentage yield of Lichen sample

S. no	Sample	Dry weight of sample (g)	Dry weight of extract (g)	Yield (%)
1	Lichen	50 Grams	1.28 Grams	2.56 %

3.2 Identification of Secondary metabolites – Thin layer Chromatography:

Thin layer chromatography is a technique used for the identification of secondary metabolites in a sample by using the solvents in different ratios. In this study TLC was employed to identify the presence of Glycoside and Alkaloid compounds in the Lichen sample using Toluene: methanol: glacial acetic acid: water = **7:4:3:1** for glycosides and the bands were visualized by placing it in the iodine chamber while Butanol: acetic acid: water = **4:1:3** was used for alkaloids and the bands were visualized by spraying it with Dragendroff's reagent respectively **(Fig 2) [11]**.

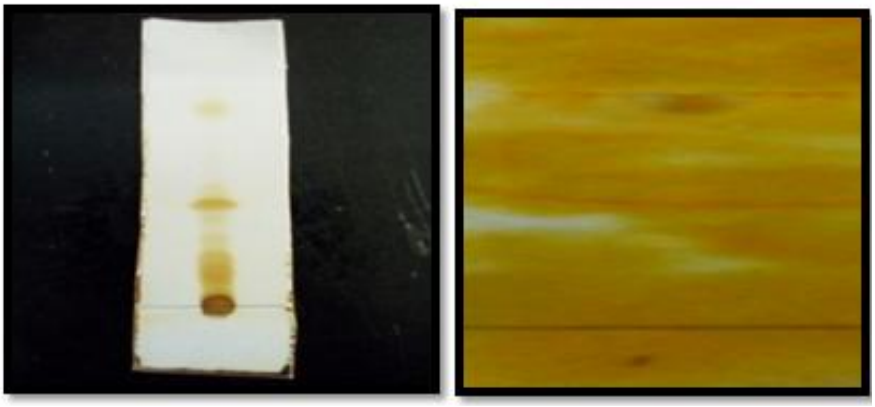

Figure 2: Thin Layer chromatography for detection of Glycoside and Alkaloid compounds

Table 2: R_f Values of the Glycoside and Alkaloid bands of Lichen

S. no	Compounds	R_f Values
1	Glycoside	0.325, 0.20, 0.15
2	Alkaloid	0.60, 0.526

The R_f values obtained for the TLC detection of glycoside compound was found to be 0.325, 0.20, 0.15 **[29]** and for alkaloid compound was found to be 0.60, 0.526 **[30] (Table 2).**

3.3 Isolation & Purification of Secondary Metabolites by Column chromatography:

Column chromatography is performed in order to purify the individual compounds present in the sample from a mixture of compounds. The Stationary phase used is Silica gel (100 – 200 mesh) while Mobile phase used for **Glycosides -** Toluene: methanol: glacial acetic acid: water = **35:20:15:10** and for **Alkaloids** – Butanol: acetic acid: water = **40:10:30 (Fig 3).** TLC was performed for the purified fractions and the corresponding R_f Values were recorded.

Figure 3a: Purified Glycoside fraction **Figure 3b:** Purified Alkaloid fraction

Figure 3: Purified Glycoside and Alkaloid fractions obtained through column chromatography

Table 3: R_f value of the Purified Glycoside and Alkaloid fractions of Lichen *Parmelia perlata*

S. no	Compounds	R_f Value
1	Glycoside	0.125
2	Alkaloid	0.526

The R_f values obtained for the TLC of individual purified fractions were found to be 0.125 and 0.526 **(Table 3).** The values were compared using various literatures and are considered to be glycoside and alkaloid compounds respectively **[29] [31]**. Both the purified fractions were then subjected to confirmation by performing Keller-Killani test and Dragendroff's test to detect the presence of glycoside and alkaloid compounds in that particular fraction.

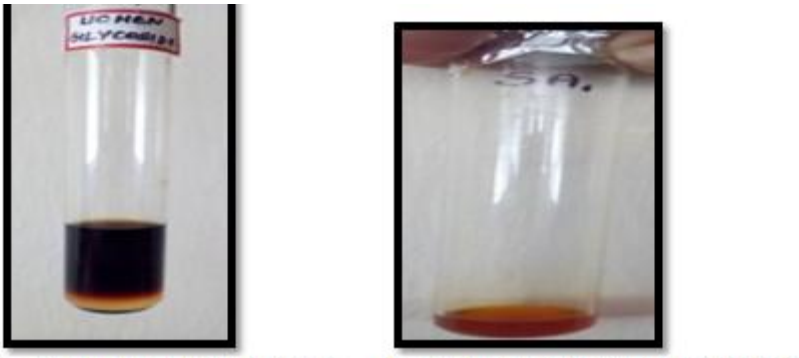

Figure 4a: Confirmatory test for Glycoside **Figure 4b:** Confirmatory test for Alkaloid

Figure 4: Confirmatory test performed for the purified Glycoside and Alkaloid fractions

The appearance of Reddish brown ring indicates the presence of Glycoside and the formation of orange red coloured complex indicates the presence of Alkaloid in the Lichen sample **(Fig 4).**

3.4 Identification by Gas Chromatography - Mass Spectrometry (GC-MS):

Gas Chromatography-Mass Spectrometry is an analytical method used for identifying different compounds within a test sample. In this study purified glycoside and alkaloid fractions obtained through column chromatography were subjected to GC-MS technique in order to identify the individual components present within the sample.

3.4.1 Lichen Glycoside:

The Glycoside compound obtained through column chromatography was subjected to Gas chromatography-Mass spectrometry and was identified to be Methyl 4-Methoxy Salicylate and the GC-MS spectrum for the corresponding compound is given in **Fig 5a.**

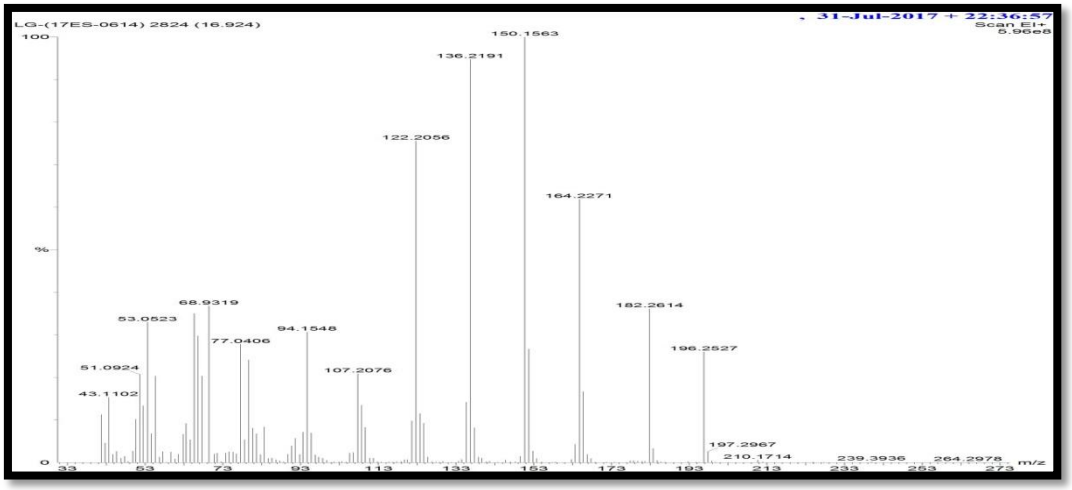

Figure 5a: GC-MS spectrum for Lichen Glycoside (Methyl 4-Methoxy Salicylate)

Methyl 4-Methoxy Salicylate compound is found to play an important role in pharmaceutical industry as an analgesic and as an antiseptic agent. It is used as a flavouring agent and also in anti herbivore defense. It is also widely employed in immunohistochemistry **[32]**.

3.4.2 Lichen Alkaloid: The Alkaloid compound obtained through column chromatography was subjected to Gas chromatography-Mass spectrometry (GC-MS) and was identified to be N-(1-Cyclohexen-1-yl) Piperidine and the GC-MS spectrum for the corresponding compound is given in the **fig 5b.**

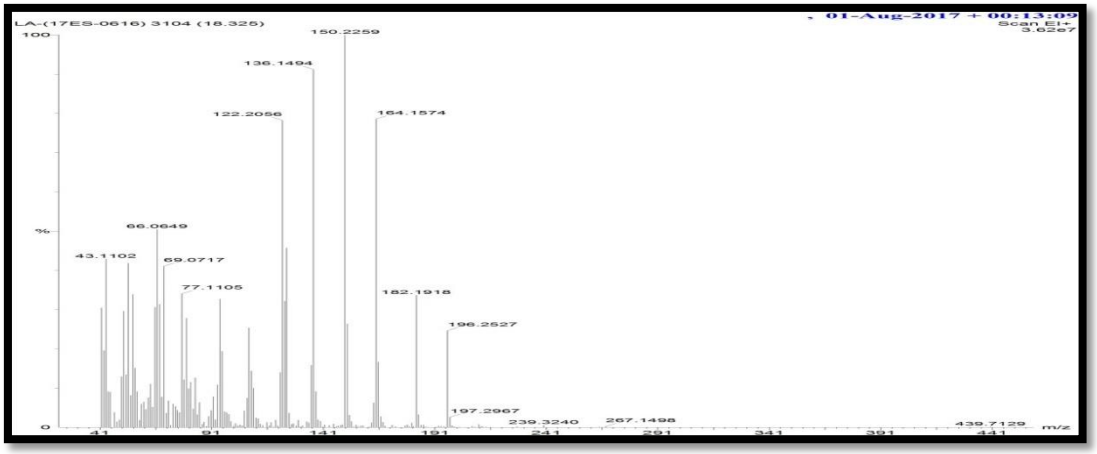

Figure 5b: GC-MS Spectrum for Lichen Alkaloid (N-(1-Cyclohexen-1-yl) Piperidine)

Figure 5: GC-MS analysis of Glycoside and Alkaloid compounds from Lichen *Parmelia perlata* N-(1-Cyclohexen-1-yl) Piperidine compound is known as the best representative structural element in alkaloids. It is widely used in the synthesis of organic compounds and also in degradation reactions. It is also employed as an antidepressant agent in pharmaceuticals **[33].**

3.5 Synthesis of Silver nanoparticles:

The Silver nanoparticles were synthesized using the aqueous extract and the purified glycoside and alkaloid fractions obtained through column chromatography from Lichen **(*Parmelia perlata*)**. Silver nanoparticles were formed by the reduction of Ag^+ by the action of the 1mM of $AgNO_3$. The solution was gradually heated and the colour change was observed which indicates the presence of silver nanoparticles. The synthesis of silver nanoparticles was carried out in 30 minutes. The synthesized nanoparticles were stored in an air tight bottle at room temperature for further characterization studies.

Figure 6: Synthesis of Silver nanoparticle using the aqueous extract and purified glycoside and alkaloid fractions of Lichen *Parmelia perlata*

The change in colour from colourless to brown indicates the formation of Silver nanoparticle **(Fig 6)** and the procedure was followed according to a paper wherein the synthesis of silver nanoparticles were carried out using the aqueous extract of lichen *Parmotrema praesorediosum* and nanoparticles were confirmed by colour change from colourless to yellowish brown **[34].**

3.6 Characterization of Silver nanoparticles:

3.6.1 UV-Visible Spectrophotometer analysis: The Nanoparticles synthesized using the aqueous extracts and purified fractions were characterized by means of UV-Visible spectrophotometer. The wavelength was selected from 400 to 500nm and the absorbance was recorded**.**

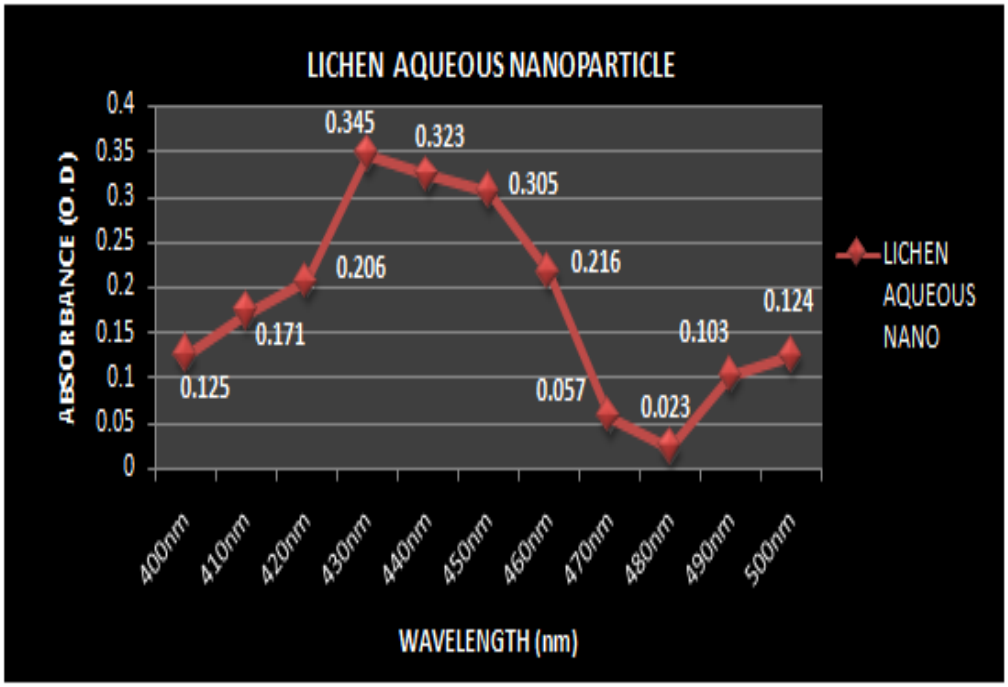

Figure 7a: UV-Visible spectra for Silver nanoparticle synthesized using Lichen aqueous extract

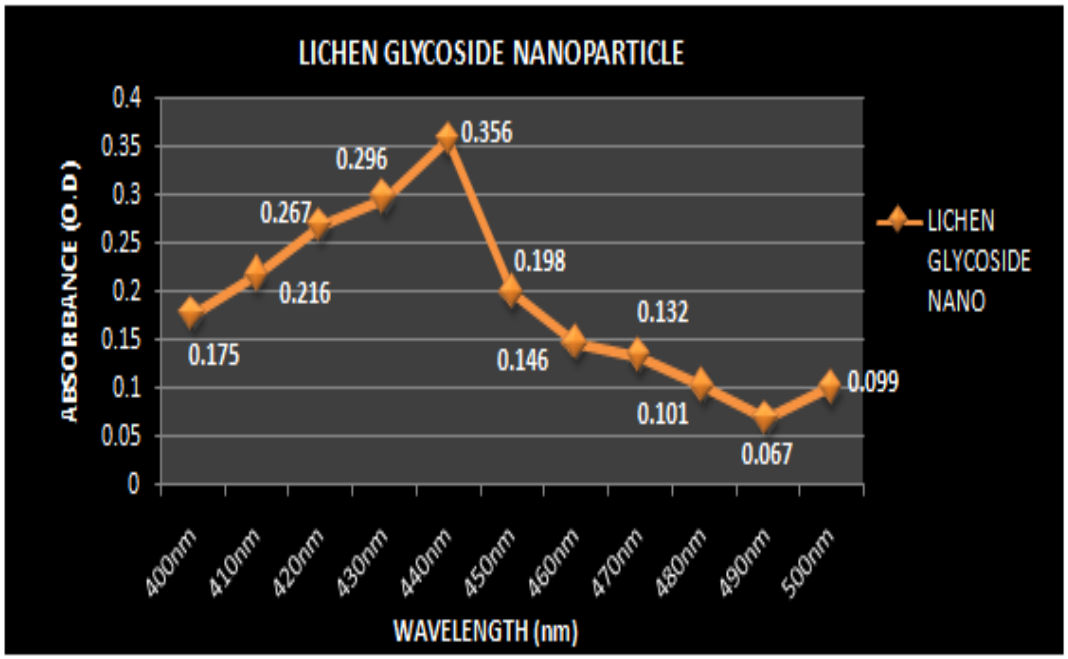

Figure 7b: UV-Visible spectra for Silver nanoparticle synthesized using Lichen purified glycoside fraction

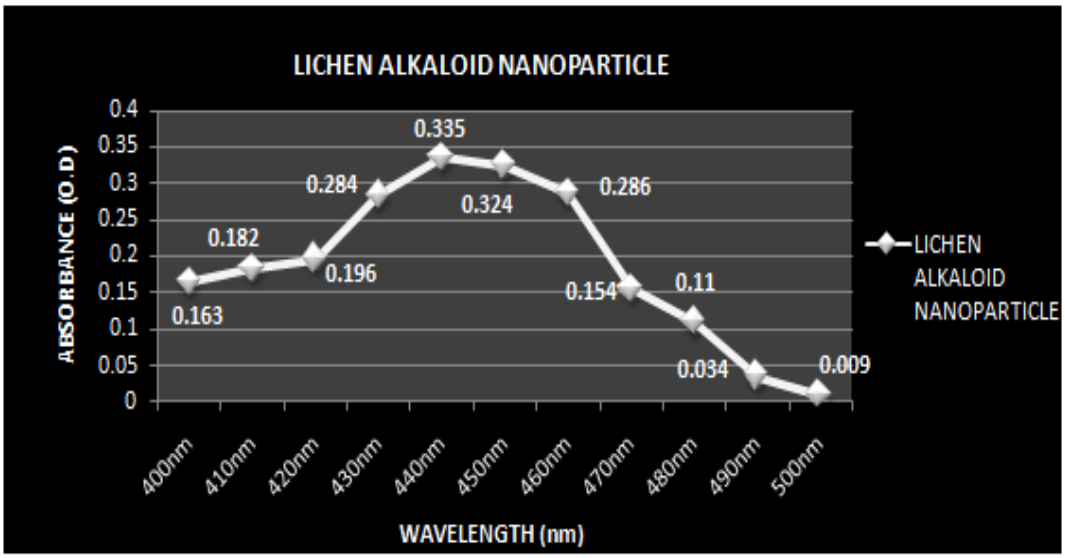

Figure 7c: UV-Visible spectra for Silver nanoparticle synthesized using Lichen purified alkaloid fraction

Figure 7: UV-Visible spectra for Silver nanoparticle synthesized using Lichen aqueous extract, purified glycoside and alkaloid fractions.

The synthesized silver nanoparticles were subjected to UV-Visible spectroscopy and maximum absorption peak was obtained at 430nm for Lichen aqueous nanoparticle and at 440 nm for nanoparticles synthesized from Lichen glycoside and alkaloid fractions **(Fig 7).** A study has reported the synthesis of silver nanoparticles using lichen *Parmotrema perlatum* and the nanoparticle was found to show an absorption peak at 420nm **[35].**

3.6.2 Fourier Transform Infrared Spectroscopy (FTIR):

Fourier transform infrared spectroscopy is a technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid, gas. It is generally an analytical technique to identify organic and inorganic materials **[19].**

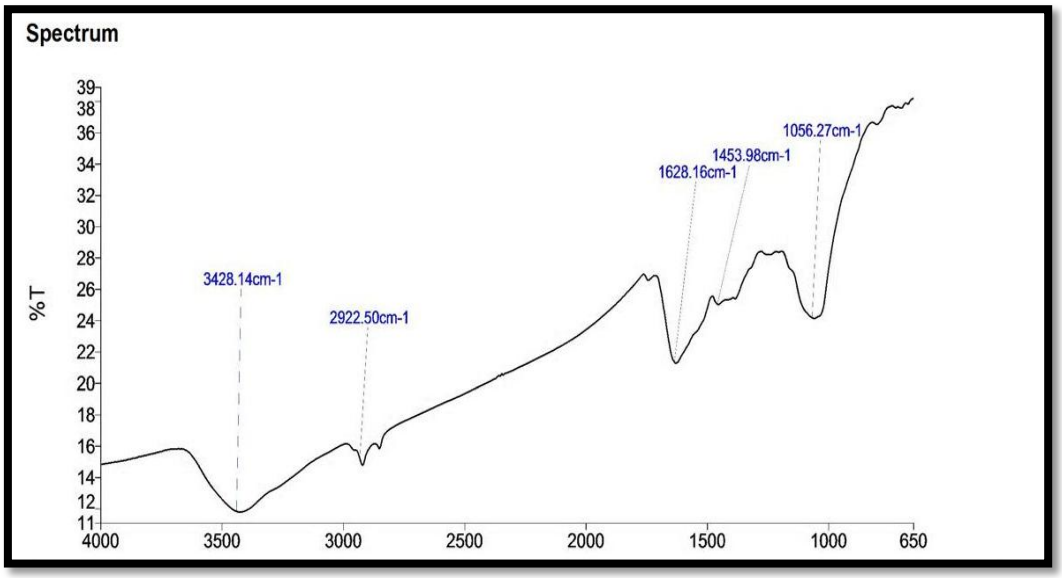

Figure 8a: FTIR Image of Lichen aqueous nanoparticle

Table 4: FTIR Spectra of Lichen aqueous nanoparticle

S. no	Band Spectra	Bonds
1	3428.14cm^{-1}	O-H stretching vibration(Indicates presence of alcohol and phenol)
2	2922.50cm^{-1}	C-H stretching of aromatic compounds
3	1628.16cm^{-1}	C=O and C=C stretching
4	1453.98cm^{-1}	N-H (amide linkages of protein)
5	1056.27cm^{-1}	C-N stretching vibration of aromatic and aliphatic amines

The FTIR spectrum for the silver nanoparticles were analysed and absorption bands were observed at 3428 cm^{-1} and 1628cm^{-1} which corresponds to O-H and C=O. The highest peak 1056.27cm^{-1} corresponds with C-N stretching vibration of aromatic and aliphatic amines and the peak at 2922.50cm^{-1}, indicate presence of C-H stretching of aromatic compounds and 1655.11cm^{-1} indicated presence of O-H group of alcohol and phenol **(Table 4).**

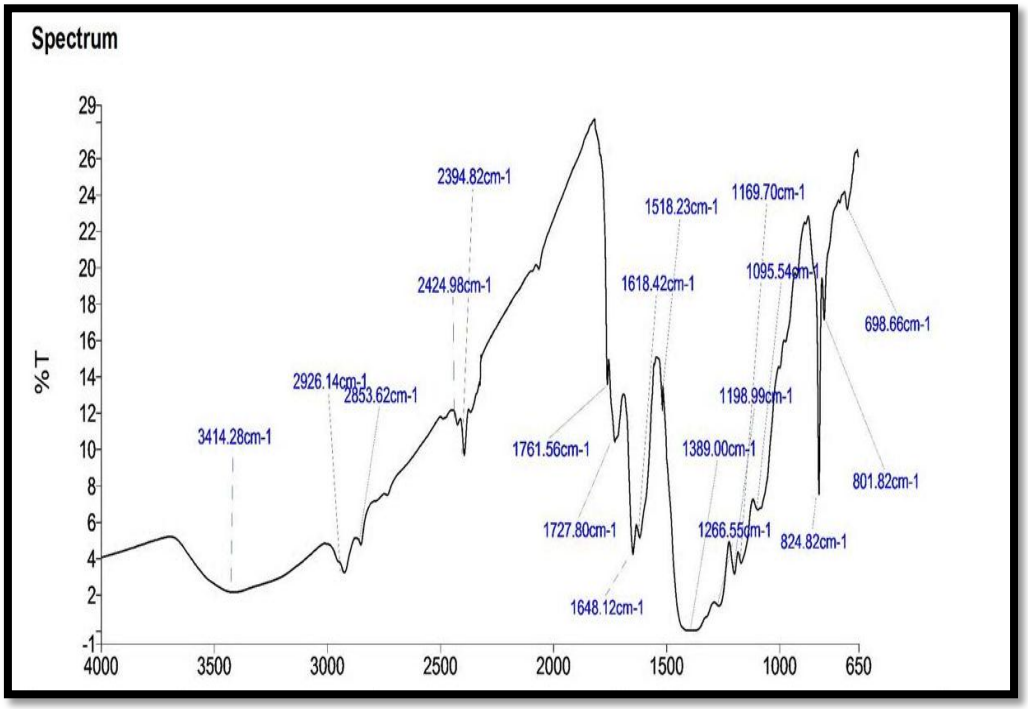

Figure 8b: FTIR Image of Lichen glycoside nanoparticle

Table 5: FTIR Spectra of Lichen glycoside nanoparticle

S. no	Band Spectra	Bonds
1	3414.28cm^{-1}	O-H stretching vibrations
2	2926.14cm^{-1}	C-H stretching of aromatic compound
3	2853.62cm^{-1}	C-H stretching of aromatic compound
4	1761.56cm^{-1}	C=C stretching
5	1648.12cm^{-1}	C=O
6	1618.42cm^{-1}	C=C
7	1389.00cm^{-1}	C-N-like amine/C-O- like phenol group
8	824.82cm^{-1}	Vibration of –O-H and C-H group

The FTIR spectrum for the silver nanoparticles was analysed and absorption bands were observed at 3414 cm^{-1} and 1648 cm^{-1} which corresponds to O-H and C=O. The peak at 1389.00 cm^{-1} corresponds with C-N stretching vibration of aromatic and aliphatic amines, peak at 2853.62 cm^{-1}, indicates presence of C-H stretching of aromatic compounds and 1648.12cm^{-1} indicates presence of C=O group **(Table 5).**

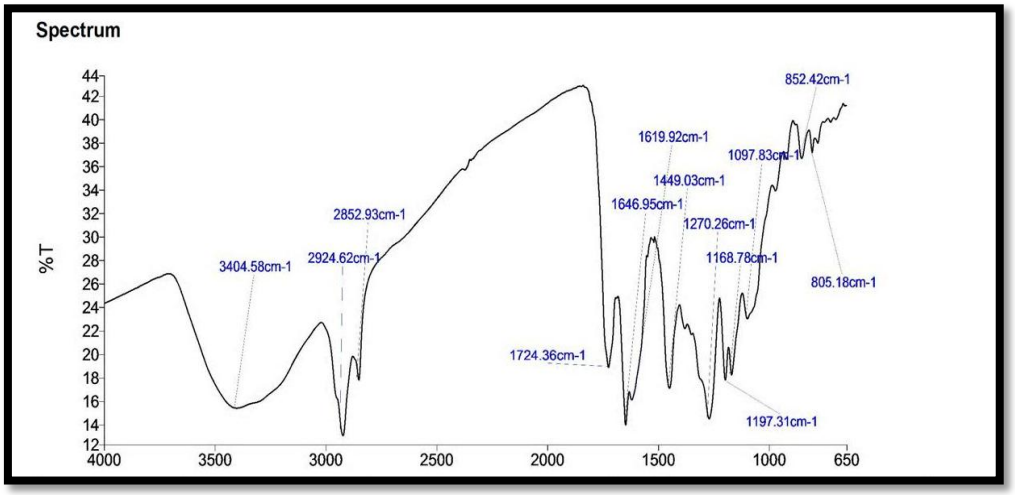

Figure 8c: FTIR Image of Lichen Alkaloid nanoparticle

Figure 8: FTIR Image for Silver nanoparticle from aqueous extract and purified fractions

Table 6: FTIR Spectra of Lichen Alkaloid nanoparticle

S. no	Band Spectra	Bonds
1	3404.58cm^{-1}	O-H stretching vibrations
2	2924.62cm^{-1}	C-H stretching of aromatic compound
3	2852.93cm^{-1}	C-H stretching of aromatic compound
4	1724.36cm^{-1}	C=C stretching
5	1646.95cm^{-1}	C=O
6	1619.92cm^{-1}	C=C
7	1449.03cm^{-1}	N-H amide linkages of protein
8	1270.26cm^{-1}	C-N stretching of amines

The FTIR spectrum for the silver nanoparticles were analysed and absorption bands were observed at 3404 cm^{-1} and 1646 cm^{-1} which corresponds to O-H and C=O. The peak at 1270.26 cm^{-1} corresponds with C-N stretching vibration of aromatic and aliphatic amines and the peak at 2852.93 cm^{-1}, indicates presence of C-H stretching of aromatic compounds and 1646.95cm^{-1} indicated presence of C=O group **(Table 6).** A study has reported the synthesis of silver nanoparticles using 4 lichens namely Parmeliopsis ambigua, Punctelia subrudecta, Evernia mesomorpha and Xanthoparmelia plitti and the FTIR spectrum for the corresponding lichens were found to be observed at 3331 cm^{-1}, 1639 cm^{-}1, 1252 cm^{-1} and 1656 cm^{-1} indicating the presence of polyphenolic, amide, carboxylic and carbonyl group respectively **[36].**

3.6.3 Scanning electron microscopy (SEM): In the present study, SEM analysis was performed to observe the morphology of the synthesized silver nanoparticles.

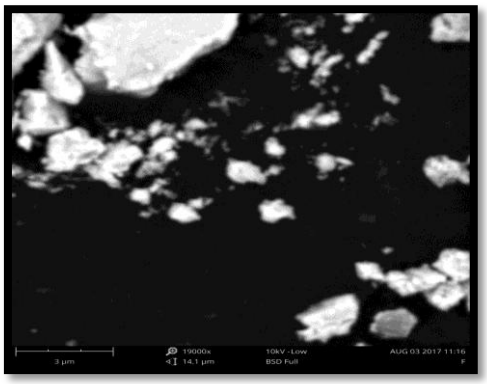

Figure 9a: Lichen Aqueous nanoparticle

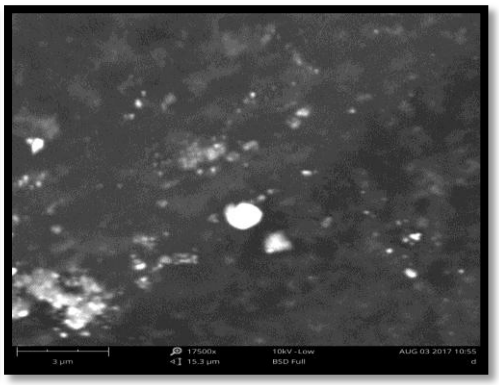

Figure 9b: Lichen Glycoside nanoparticle

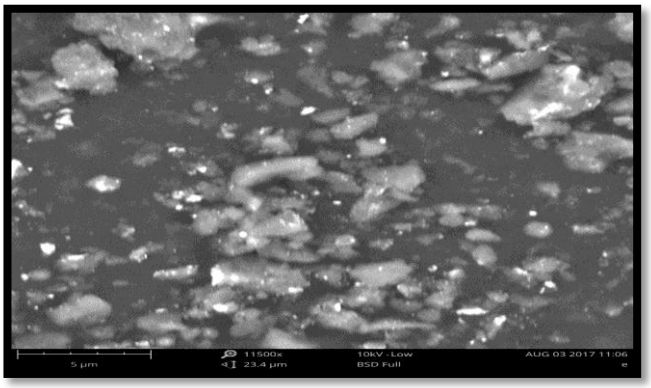

Figure 9c: Lichen Alkaloid nanoparticle

Figure 9: SEM images of Silver nanoparticle synthesized from aqueous extract and purified fractions of Lichen *Parmelia perlata*. The shapes of the silver nanoparticles were reported to be spherical. The synthesized nanoparticles were found in aggregations which were distributed evenly **(Fig 9).**The particles forms aggregate on the surface of the foil. A study has reported the Scanning Electron Microscopic analysis of silver nanoparticle synthesized using lichen Parmotrema perlatum where the nanoparticles are observed to be non-uniform in shape **[35].**

3.7 Applications:

3.7.1 Antimicrobial activity

The antibacterial activity of Lichen (***Parmelia perlata***) crude aqueous extract, Silver nanoparticle from aqueous extracts, Silver nanoparticle from glycoside and alkaloid fractions were screened against both gram positive and gram negative bacteria such as *Staphylococcus aureus, Streptococcus spp, Escherichia coli, Klebsiella pneumoniae, Salmonella spp* and *Pseudomonas aeruginosa.*

Table 7: The antibacterial activity of silver nanoparticle synthesized using lichen aqueous extract and purified fractions were tabulate

S. no	Organism	Zone of Inhibition (in mm)				
		Lichen Aqueous Extract	**Lichen Aqueous Nanoparticle**	**Lichen Glycoside Nanoparticle**	**Lichen Alkaloid Nanoparticle**	**Control**
1	*E.coli*	0	3	3	6	0
2	*Streptococcus spp.*	0	3	13	6	0
3	*Klebsiella Pneumoniae*	0	4	10	5	0
4	*Staphylococcus aureus*	0	3	13	5	0
5	*Salmonella typhimurium*	0	3	4	5	0

6	*Pseudomonas aeruginosa*	0	2	3	10	0

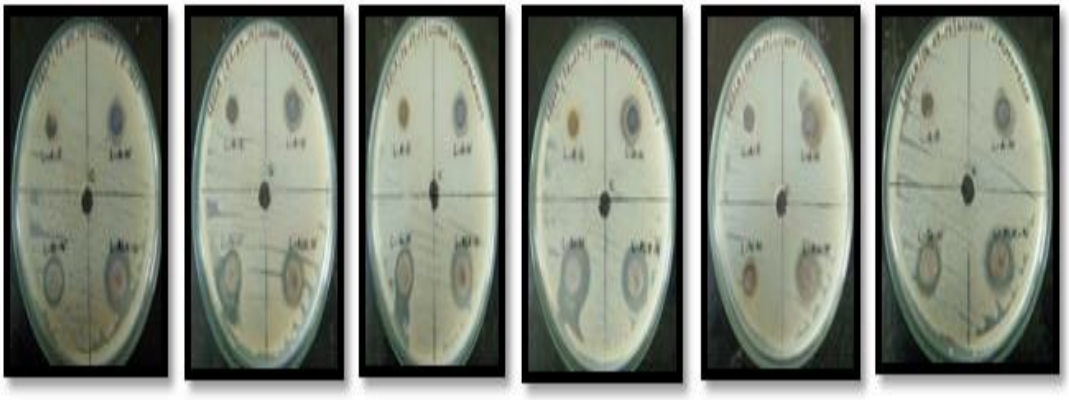

Figure 10: The antibacterial activity of the Silver nanoparticle synthesized using the Lichen aqueous extract and purified fractions

The antibacterial activity was carried out for the silver nanoparticles synthesized using the aqueous extracts and purified fractions of lichen against 6 bacterial organisms. Among them the nanoparticles synthesized using the purified fractions showed good antibacterial activity compared to that using the aqueous extract. But among the purified fractions the nanoparticle synthesized using the glycoside fraction showed an increased zone of inhibition against all the organisms compared to that using the alkaloid fraction **(Table 7).** A study has reported the antibacterial activity of silver nanoparticle synthesized using the lichen *Parmotrema perlatum* against 7 bacterial organisms at different concentrations as 25, 50, 75 and 100µl respectively wherein maximum inhibition was observed against *Streptococcus* (30mm) while minimum inhibition was observed against *Klebsiella planticola* (7mm) **[35].**The antifungal activity of Silver nanoparticles synthesized using aqueous extract and purified fractions of Lichen were determined against 2 fungi *Aspergillus niger* and *Candida albicans.*

Table 8: The antifungal activity of the Silver nanoparticle synthesized using the Lichen aqueous extract and purified fractions were tabulated

S. no	Organism	Zone of Inhibition (in mm)		
		Lichen Aqueous Nanoparticle	Lichen glycoside Nanoparticle	Lichen alkaloid Nanoparticle
1	*Aspergillus niger*	7	10	8
2	*Candida albicans*	7	5	4

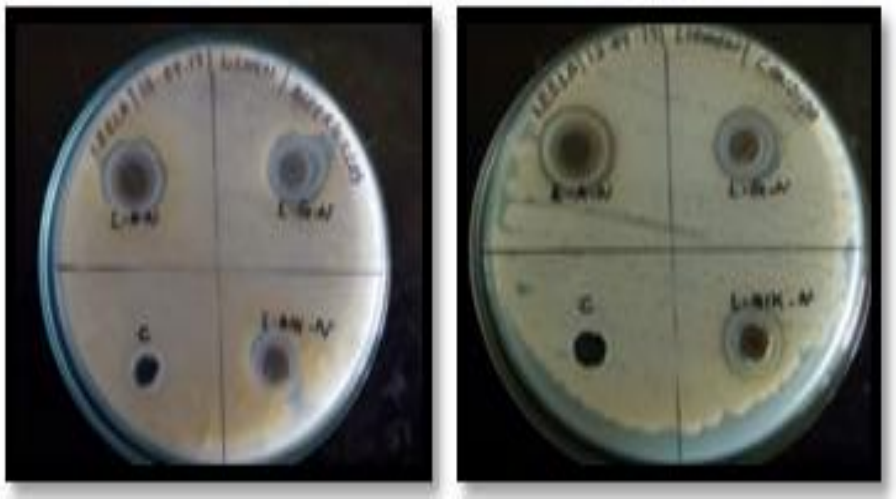

Figure 11: The antifungal activity of the Silver nanoparticle synthesized using the Lichen aqueous extract and purified fractions

The antifungal activity was determined for the silver nanoparticles synthesized using the aqueous extracts and purified fractions of lichen *Parmelia perlata* against 2 fungal organisms. The nanoparticle synthesized using the purified fractions as well as the aqueous extract showed good antifungal activity but among them the nanoparticle synthesized using the glycoside fraction showed an increased zone of inhibition against *Aspergillus niger* (10mm) while nanoparticle synthesized using the aqueous extract showed an increased zone of inhibition against *Candida albicans* (7mm) compared to that of the purified fractions **(Table 8).** A study has reported the antifungal activity of silver nanoparticle synthesized using the aqueous extract of *Parmelia perlata* against fungi *Aspergillus terreus* wherein the lichen was found to have shown a zone of inhibition at 14mm **[37].**

3.7.2 Antioxidant activity:

The antioxidant potential of Lichen (***Parmelia perlata***) crude extract, Silver nanoparticle from aqueous extracts, Silver nanoparticle from glycoside and alkaloid fractions were determined by 3 assays: Total antioxidant capacity (TAC) assay, Hydrogen peroxide scavenging (H_2O_2) assay and reducing power assay.

3.7.2.1 Total Antioxidant capacity (TAC) assay:

Total antioxidant capacity assay is a widely employed technique used for detecting the antioxidant potential of the biological samples. The amount of free radicals scavenged by the test solution or the sample can be measured. This assay is carried out by means of phosphomolybdenum method**.** This technique is based on the principle of reduction of Phosphate -Mo (VI) to Phosphate - Mo (V) by the test sample and the immediate formation of bluish green coloured Phosphate/Mo complex at an acidic pH **[38].**

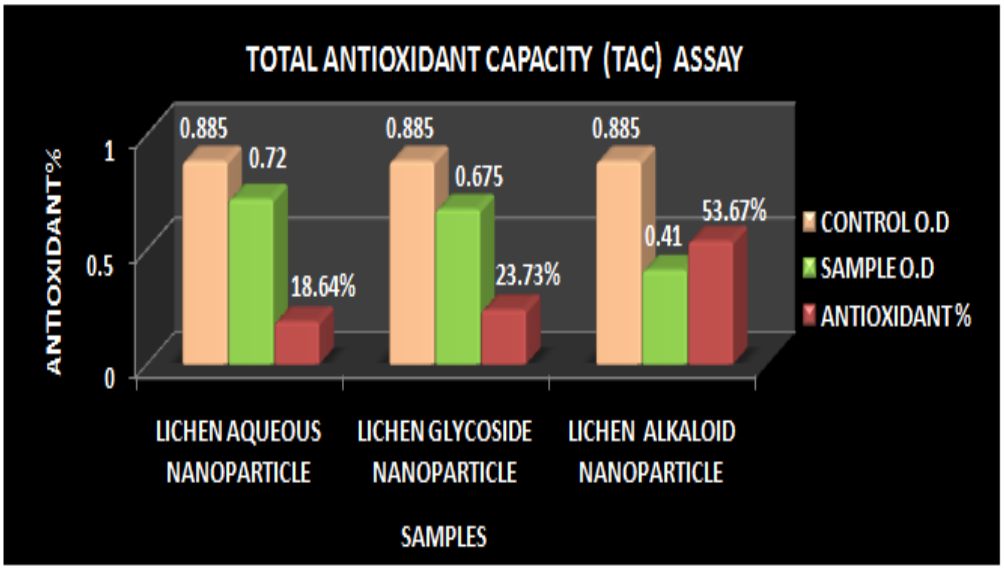

Figure 12: Total antioxidant capacity (TAC) assay for Silver nanoparticle synthesized using lichen aqueous extract and purified glycoside and alkaloid fractions.

The Total antioxidant capacity assay for the Silver nanoparticle synthesized using aqueous extract and purified glycoside and alkaloid fractions of lichen *Parmelia perlata* were determined. In TAC assay the silver nanoparticle synthesized using the purified fractions exhibited good antioxidant percentage when compared to that from the aqueous extract (18.64%) but when comparing nanoparticle from both the fractions the silver nanoparticle synthesized using the alkaloid fraction (53.67%) was found to have shown an increased antioxidant percentage than that of the glycoside fraction (23.73%) respectively **(Fig 12).** A study has reported the total antioxidant capacity of silver nanoparticles synthesized by four lichens namely *Parmeliopsis ambigua, Punctelia subrudecta, Evernia mesomorpha* and *Xanthoparmelia plitti* wherein the lichen *Punctelia subrudecta* was found to exhibit maximum activity compared to that of other lichen samples and is also attributed due to the presence of high polyphenol contents **[36].**

3.7.2.2 Hydrogen peroxide scavenging assay:

Hydrogen peroxide is a weak oxidizing agent which penetrates within the cell and reacts with Fe^{2+} or Cu^{2+} ion and gets converted into hydroxyl radical that could cause toxic effects thereby leading to cell damage. So it is important to prevent the formation of hydroxyl radical by preventing the accumulation of hydrogen

peroxide within the cell. This assay is based on the principle of decrease in absorbance of hydrogen peroxide upon oxidation of hydrogen peroxide. The ability of the extracts to scavenge the hydrogen peroxide is determined **[39].**

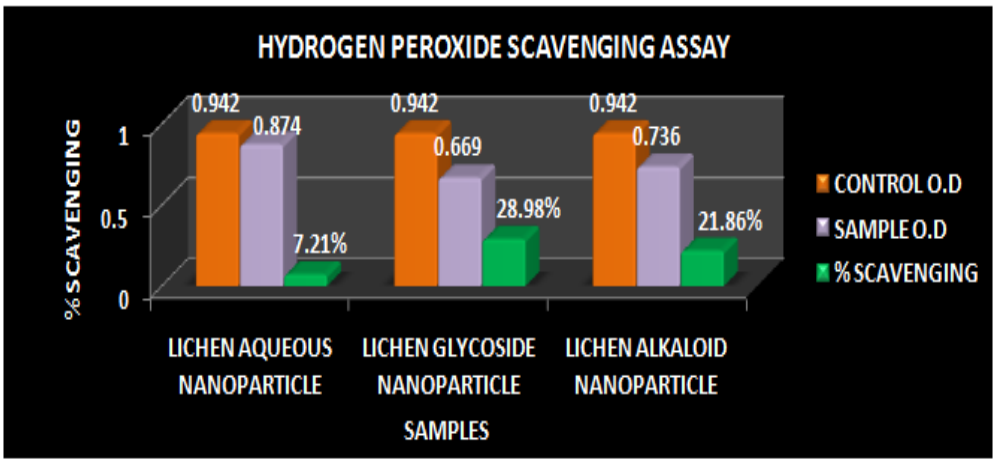

Figure 13: Hydrogen peroxide scavenging assay for Silver nanoparticle synthesized using lichen aqueous extract and purified glycoside and alkaloid fractions.

The Hydrogen peroxide scavenging assay for the Silver nanoparticle synthesized using aqueous extract and purified glycoside and alkaloid fractions of lichen *Parmelia perlata* were determined. In this assay the nanoparticle synthesized using the purified fractions exhibited good scavenging percentage than that of the nanoparticle from aqueous extract (7.21%) and among both the purified fractions the nanoparticle synthesized using glycoside fraction (28.98%) was found to have shown better scavenging percentage compared to that of the alkaloid fraction (21.86%) respectively **(Fig 13).** A study has reported the Hydrogen peroxide scavenging activity of the Petroleum ether, ethyl acetate, ethanol and aqueous extracts of lichen *Parmotrema grayanum* at different concentrations such as 20, 50, 100, 200µg/ml. Among all the extracts ethanolic extract of the lichen sample was found to have shown increasing scavenging percentage at all concentrations followed by petroleum ether extract respectively **[40].**

3.7.2.3 Reducing power assay: It is a widely employed technique and is based on the principle that the substances with reduction potential react with Potassium ferricyanide (Fe^{3+}) to form Potassium ferricyanide (Fe^{2+}) which again reacts with ferric chloride to form ferric-ferrous complex that have an absorption at 700nm.The presence of reducing compound in the extract will tend to cause the conversion of ferric form to ferrous form. By measuring the absorbance of blue colour formed at 700nm it is possible to determine the concentration of Fe^{3+} ion .Thus the reducing ability of the crude extract and individual purified fractions were determined **[41].**

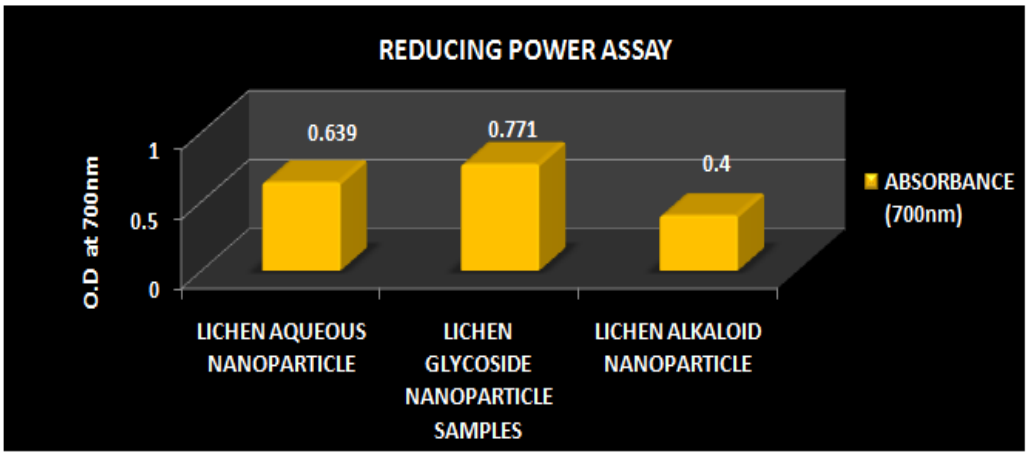

Figure 14: Reducing power assay for Silver nanoparticle synthesized using lichen aqueous extract and purified glycoside and alkaloid fractions.

The Reducing power assay for the Silver nanoparticle synthesized using aqueous extract and purified glycoside and alkaloid fractions of lichen *Parmelia perlata* were determined. In this assay silver nanoparticle synthesized using the aqueous extract as well as the purified fractions showed good absorbance but among these the nanoparticle synthesized using the glycoside fraction exhibited good absorbance (0.771) when compared to that of nanoparticle from alkaloid fraction (0.4) and aqueous extract (0.639) respectively **(Fig 14).** A study has reported the reducing power assay for the silver nanoparticles synthesized using 4 lichens namely *Parmeliopsis ambigua, Punctelia subrudecta, Evernia mesomorpha* and *Xanthoparmelia plitti* wherein among all the samples the nanoparticle synthesized using lichen *Punctelia subrudecta* showed highest reducing power **[36].**

3.7.3 Antidiabetic Activity:

The antidiabetic activity of the silver nanoparticle synthesized from the aqueous extract and from the purified Glycoside as well as Alkaloid fractions of Lichen *Parmelia perlata* were determined by means of alpha amylase inhibition assay. It is based on the principle of in vitro hydrolysis of starch in the presence of α-amylase enzyme. This process is quantified using iodine which gives blue colour on reaction with starch. The decrease in the intensity of blue colour indicates starch breakdown by the enzyme into monosaccharides. If the extracts possess a higher activity then the intensity of blue colour will be more thus the intensity of blue colour developed is directly proportional to the alpha amylase inhibitory activity **[42]**.

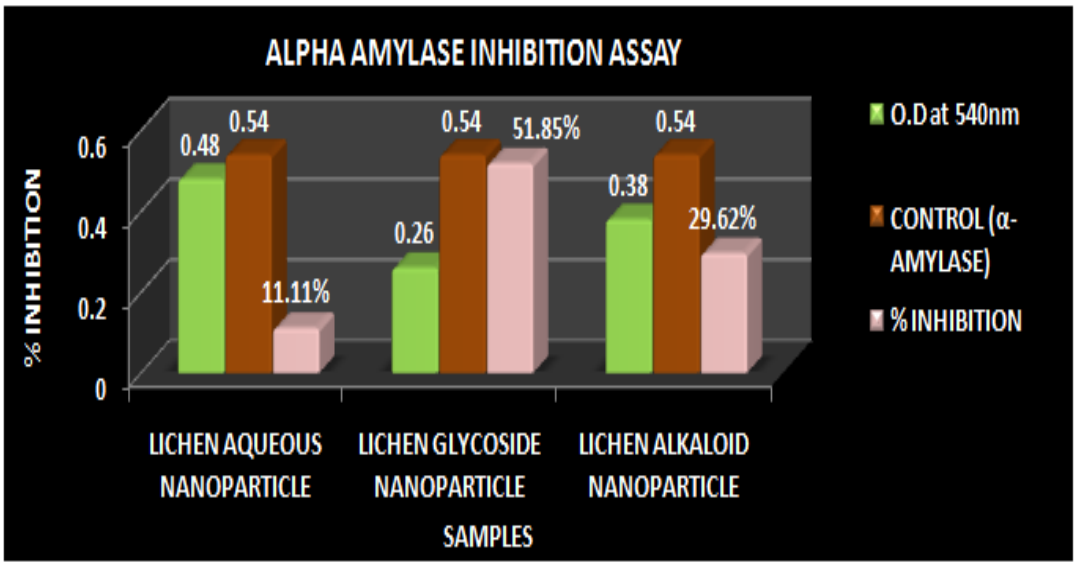

Figure 15: Alpha amylase inhibition assay for Silver nanoparticle synthesized using lichen aqueous extract and purified glycoside and alkaloid fractions.

The antidiabetic activity was carried out for the silver nanoparticles synthesized using the aqueous extract and purified fractions from lichen *Parmelia perlata* by means of alpha amylase inhibition assay. The nanoparticle synthesized using the aqueous extract (11.11%) as well as the purified fractions showed good inhibition percentage but on a comparative basis the nanoparticle synthesized using the purified fractions showed good inhibition percentage than that from the aqueous extract. Among the 2 purified fractions the nanoparticle synthesized using the glycoside fraction (51.85%) showed an increased inhibition % than that of the alkaloid fraction (29.62%) respectively **(Fig 15).** Many lichen extracts have been evaluated and are examined for their α-amylase inhibitory activity and were found to have shown beneficial effects in reducing the pace of digestion and sugar assimilation thereby leading to an improved management of type 2diabetes by lowering the postprandial hyperglycemia **[43]**

IV. Conclusion

The Present study was carried out in order to evaluate the therapeutic potential of Silver nanoparticles synthesized using the aqueous extract and purified glycoside and alkaloid fractions of lichen *Parmelia perlata*. Nanotechnology remains as the major field of interest due to its application in various fields and among the metal nanoparticles silver nanoparticle remains as the most promising source due to its wide importance in various disciplines. Silver nanoparticles exhibit various medicinal as well as industrial applications. Many reports have been proposed on the synthesis of silver nanoparticles using the aqueous extract of natural sources such as plants, fungi, algae etc. Since the compounds are derived from natural sources they

have been found to possess numerous pharmacological properties thereby do not cause any health impacts or don't pose any threat to the environment. Not much work has been carried out on the nanoparticle synthesis using a specific secondary compound so this study was an attempt to synthesize silver nanoparticles using the purified secondary metabolites from lichen *Parmelia perlata* so that the combined efficacy of these nanoparticles with the secondary compounds could prove to be beneficial to mankind in different aspects.

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