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A Study on the applications of Silver nanoparticles synthesized usingaqueous extract and purified secondary metabolites of seaweed *Hypneacervicornis*

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***Abstract**:Seaweeds are commonly referred to as macroalgae and are plant like organisms that live attached to rock or any other hard substrata. They are one among the commercially important sources which possess the ability to synthesize numerous secondary metabolites that have wide applications in the field of medicine as anti-inflammatory or anticancer agents. They are also widely utilized in the production of polysaccharides such as carrageenan, fucoidan etc., and have both therapeutic as well as industrial applications. The Present study was carried out on the synthesis, characterization and applications of silver nanoparticle using the aqueous extract and the individual purified compounds of Seaweed Hypneacervicornis. The Silver nanoparticles were synthesized and 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. Not much work has been reported before on the nanoparticle synthesis using the individual secondary compounds from seaweed Hypneacervicornis so this study was one such attempt to identify the stabilizing and reducing ability of the secondary compounds in the synthesis of silver nanoparticle that could prove to be useful to the mankind for different applications.*

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

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I. INTRODUCTION

Seaweeds are marine non flowering plantsfound to grow abundantly in shallow waters of seas and estuaries.They are considered to be potentially beneficial sources of highly bioactive secondary metabolites that could be used widely for different applications. The compounds extracted from seaweeds are found to possess antimicrobial, antioxidant, antitumor, larvicidal and antiviral properties **[1].** Compounds such as laminaran, porphyran, ulvanderived from seaweeds are found to reduce cholesterol and high blood pressure levels so they are used as drugs in pharmaceutical industries in the form of nutraceuticals**[2].**

Hypneacervicornis is a red macroalgae that belongs to the family Hypneaceae and also referred to as hooked red weed. It remains attached to the upper branches of other reef algae in the subtidal region. These are generally yellowish green in colour but appear to be red in shaded conditions**[3].**They produce numerous secondary compounds that play an important role in food and pharmaceutical industries. They are the chief source in production of polysaccharides such as carrageenan which is used as a binding and smoothening agent in ice creams, tooth paste and also acts as a principal component in bacterial culture media.

Nanotechnology is an emerging field of scientific study that incorporates biotechnology at a nanoscale. Nanoparticle is a microscopic particle with dimension less than 100nm and can be of different types based on the metal used such as silver, gold, copper, zinc etc. Among them silver nanoparticles are mostly preferred due to their specialized magnetic, electrical and optical properties. Silver nanoparticles are widely utilized for different applications such as in textile industries, in cosmetics, in water treatment and as antimicrobial drugs etc. where they are found to possess effective antimicrobial activity**[4](Fig 1).**They are also been extensively studied in different fields such as materials science, electronics and in medicine **[5]**. Silver nanoparticles can be produced by different methods but biological synthesis is widely preferred due to low toxicity, eco-friendly and low cost.

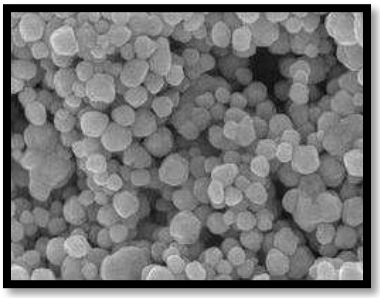

Figure 1: SEM Image of Silver nanoparticles

Secondary metabolites are organic compounds widely synthesized by various natural sources such as bacteria, fungi, algae, plants and animals and are not involved in the growth and development of an organism. These are generally produced during a transition state from growth to stationary phase and are widely utilized as colorant, aromatic agents and as medicinal drugs. In the present study Silver nanoparticles were synthesized using two different secondary metabolites they are Glycosides and Alkaloids. Glycosides are organic compounds in which a sugar molecule is bound to a functional group through a glycosidic bond while alkaloids are chemical compoundsthat contain basic nitrogen atoms both the compounds play a vital role and possess wide pharmaceutical and industrial applications.

Thus the present study was aimed at determining the antimicrobial, antioxidant and antidiabetic applications of Silver nanoparticles synthesized using the aqueous extract as well as the purified glycoside and alkaloid compounds of Seaweed*Hypneacervicornis*.

II. MATERIALS AND METHODS

2.1 Sample Preparation:

The Seaweed *Hypneacervicornis* samples were collected manually from Pazhaverkadu brackish water area at Thiruvallur district, Chennai and were identified based on their morphological characteristics **[6].** The samples were cleaned, washed under tap water and distilled water to remove the dirt and were dried for about 3 days and powdered. The powdered samples were stored in clean bottles for further analysis.

2.2 Extraction of Samples:

2.2.1 Methanolic Extraction: The crude extract from the lichen sample was obtained by means of cold extraction method using methanol as solvent. 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. The solution was filtered with the help of Whatman No.1 filter paper and the filtrate obtained was evaporated. The dried extracts were then stored for further experiments **[7].** The yield of respective crude extract was calculated as:

Percentage yield (%) = (dry weight of extract/dry weight of samples) × 100.

2.2.2 Aqueous Extraction: For aqueous extraction about 50g of the powdered lichen sample was added to 500ml of double distilled water separately in a conical flask and was kept in water bath at 65ºc for about 1 hour to enhance complete extraction. The aqueous solution thus obtained was filtered with the help of a Whatman no 1 filter paper and the filtrates thus obtained were collected in a beaker and was utilized for further analysis.

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, glass or an aluminium foil coated with an absorbent material. The adsorbent could be cellulose, silica or aluminium oxide (alumina). The layer of adsorbent material is called as stationary phase while the solvent or mixture of solvents acts as a mobile phase via capillary action. TLC plate is cut and using a pencil line is drawn about 1cm from one end of the edge. The samples were spotted on to the plate using a capillary tube for each spot. The spots should be air dried. The TLC chamber consists of a glass jar with a lid and the solvent mixture is added to chamber. The Mobile phase used for isolation of glycosides are **Toluene: methanol: glacial acetic acid: water = 7:4:3:1** and for alkaloids are **Butanol: acetic acid: water = 4:1:3**. The TLC plates were placed carefully in the TLC chamber. The solvent mixture should be below the spot and the chamber is kept closed. As the solvent moves up due to capillary movement, the compounds present in the extract are separated. The TLC plates are then removed from the chamber and kept for drying. The dried plates are observed for spots by using a suitable spraying reagent **[8].**For glycosides Iodine chamber is used while for alkaloids Dragendroff's reagent is used for identification **[9,**

10]. The formation of brown band indicates the presence of glycosides and the formation of reddish orange band indicates the presence of alkaloids. The R_f value is calculated using the formula:
R_f= Distance travelled by the solute / Distance travelled by the solvent

2.3.2 Column Chromatography:

Silica gel (100 - 200 mesh) was chosen as the stationary phase. The column was packed with silica gel using methanol once packed the crude residue from methanol extract was transferred onto the bed of silica gel. The column was run by using mobile phase (**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 fractions were collected at an interval of 5ml each and are monitored by means of thin layer chromatography. The fractions obtained were stored and utilized for the identification of individual compounds present in the sample by means of Gas chromatography – Mass spectrometry (GC-MS) **[11].**

2.3.3 Confirmatory test: [12]

1. **Glycosides: Keller –Killani Test:** To 1ml of sample add 1ml of glacial acetic acid and 1ml of concentrated Sulphuric acid. Appearance of reddish brown colour at the junction of 2 layers indicates the presence of glycosides.
2. **Alkaloids: Dragendroff's test:** To 2ml of sample 2-3 drops of Dragendroff's reagent is added. Appearance 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 method used for identifying different substances within a test sample. It also helps in identifying trace elements in a 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. The 1μL of extract sample was injected into the instrument and the oven temperature was as 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 fragments were obtained from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library **[13].**

2.5 Synthesis of Silver nanoparticles: [14]

The Silver nanoparticles were synthesized using the aqueous extracts and the purified glycoside and alkaloid compounds obtained through column chromatography from Seaweed *Hypneacervicornis*sample respectively.

2.5.1 Using the Aqueous extract:

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 Seaweed ***(Hypneacervicornis)*** aqueous extracts were taken 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 the UV-Vis spectrum.

2.5.2 Using 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 the purified glycoside and alkaloid fractions obtained through column chromatography were taken in a conical flask and was added drop wise into the beaker containing silver nitrate. The colour change from colourless to brown indicates the formation of silver nanoparticles. The reduction of the Ag+ ions was measured by the UV-Vis spectrum.

2.6 Characterization of Silver nanoparticles:

2.6.1 Ultraviolet - Visible Spectroscopy:

Instrument model: *UV*-Visible *Spectrophotometer* - *UV*-1800.

UV-Vis spectral analysis was done by using UV-Visible spectrophotometer. Ultraviolet-visible spectroscopy is a type of absorption spectroscopy in which light of ultraviolet region (400-500nm) is absorbed by the molecule. Absorption of the ultra violet radiations results in the excitation of the electrons from the ground state to the higher energy state. The energy of UV radiation absorbed is equal to the energy difference between the ground

state and the higher energy state. The absorbance was recorded at different nanometers from 400-500nm and the Optical density (OD) was calculated **[15].**

2.6.2 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.
Instrument Model: Shimadzu IRTracer-100
KBr Pellet Method: To prepare a liquid sample to IR analysis, firstly place a drop of the liquid on the face of a highly polished salt plate (such as NaCl, AgCl or KBr), then place a second plate on top of the first plate so as to spread the liquid in a thin layer between the plates, and clamp the plates together. Finally wipe off the liquid out of the edge of plate. Mount the sandwich plate onto the sample holder. After finishing the experiment, clean the plates with isopropanol and returned to the desiccators **[16].**

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**[17].**

2.7 Applications:
2.7.1 Antimicrobial activity:
The antibacterial activity of Seaweed(***Hypneacervicornis***) crude methanolic 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, Klebsiellapneumoniae, Salmonella spp*and *Pseudomonas aeruginosa*while the antifungal activity was carried out against *Aspergillusspp*and*Candida albicans.*

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 a loop-full of stock cultures were transferred to 50ml of nutrient broth and were incubated at 37°C for 24 hours. The strains were inoculated in nutrient broth for 24 hours. Potato dextrose broth was prepared for the growth of fungal strains and incubated for 48 hours. About 250ml of Muller Hinton agar medium (MHA) was prepared for antibacterial activity while Potato Dextrose agar medium (PDA) was prepared for antifungal activity and poured into the petriplates and was allowed to solidify. Once solidified the bacterial cultures 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 **[18]**.

2.7.2 Antioxidant activity:
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 at room temperature for 10 minutes and the absorbance was determined at 230nm in a spectrophotometer. Ascorbic acid was used as the standard. Phosphate buffer without hydrogen peroxide served as blank **[19].** It can be calculated using the formula:
% scavenged (H_2O_2) = (A of Control – A of test / A of Control) × 100

2.7.3 Antidiabetic activity:
The antidiabetic activity of Seaweed (***Hypneacervicornis***) crude methanolic extract, Silver nanoparticle from aqueous extracts, Silver nanoparticle from glycoside and alkaloid fractions were determined by means of Alpha amylase inhibition assay.
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 was incubated at 25ºc for about 10 minutes. The reaction was then terminated by the 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 **[20].**The % inhibition was calculated using the formula:

% inhibition = (A of Control – A of test / A of Control) × 100

III. RESULTS & DISCUSSION

3.1 Extraction of Sample:

The Seaweed (***Hypneacervicornis)*** sample was extracted by means of cold extraction method using methanol and water (aqueous). The Percentage yield of Seaweed crude extract was found to be 3.16 % and it is the total yieldobtained for about 50 grams of the dry weight of the sample**(Table 1).**The Percentage yield was calculated according to the paper **[21].**

S.NO	SAMPLE	DRY WEIGHT OF SAMPLE (g)	DRY WEIGHT OF EXTRACT (g)	YIELD (%)
1	Seaweed	50 Grams	1.58 Grams	3.16 %

Table 1: Estimation of Percentage yield of Seaweed sample

Methanol was highly preferred for extraction purposes because it has a polarity index of 5.1 and was found to be capable of dissolving polar compounds and the solvent was also reported to have been used by other authors for their extraction purposes**[22]**. Water has a polarity index of about 10.2 and is also used for extraction in order to compare its ability with that of methanol in extracting the compounds. A Study has reported the extraction of Seaweed *Sargassumwightii* using 4 different solvents such as hexane, diethyl ether, acetone and methanol wherein the percentage yield was found out to be maximum in acetone extract (0.969%) and minimum in hexane extract (0.225%) respectively **[23]**.

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 thin layer chromatography was employed to identify the presence of Glycosides and alkaloids in the Seaweed sample. For Glycosides the mobile phase used is Toluene: methanol: glacial acetic acid: water = **7:4:3:1** and the bands were visualized by placing it in the iodine chamber while for Alkaloids the mobile phase used is Butanol: acetic acid: water = **4:1:3** and the bands were visualized by spraying it with Dragendroff's reagent **(Fig 2).**The solvent systems were used based on the paper **[8]**. The R_fValues obtained for the TLC detection of glycoside compound was found to be 0.375, 0.125 **[24]**similarly the R_f Values obtained for the TLC detection of alkaloid compound was found to be 0.63, 0.526 **[25] (Table 2).**

S.NO	COMPOUNDS	R_fVALUES
1	Glycoside	0.375,0.125
2	Alkaloid	0.63,0.526

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

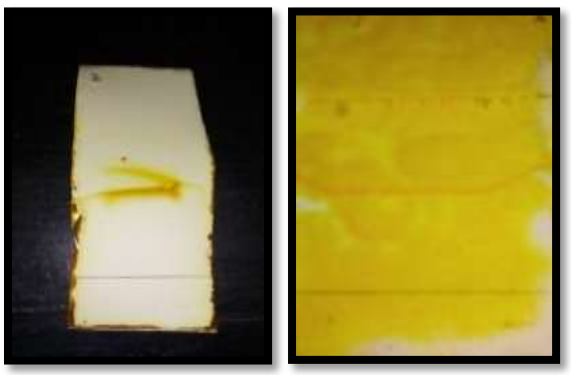

Figure 2a: TLC for Seaweed Glycoside**Figure 2b:** TLC for Seaweed Alkaloid
Figure 2: Thin Layer chromatography for detection of Glycoside and Alkaloid compounds

3.3 Isolation and Purification of Secondary metabolites – Column chromatography:
Column chromatography is performed in order to purify the individual compounds present in the sample from a mixture of different compounds. In column chromatography 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.**The purified fractions of glycoside & alkaloids are obtained **(Fig 3).**

Figure 3a: Purified Glycoside fraction **Figure 3b:** Purified Alkaloid fraction
Figure 3: Purified Glycoside and Alkaloid fractions obtained through column chromatography

3.4 Confirmatory test for Glycosides and Alkaloids:
The Glycoside and Alkaloid fractions **(Fig 3)** obtained through column chromatography was subjected to confirmation by performing Keller-Killani test and Dragendroff's test to detect the presence of glycoside and alkaloid compounds in the fractions.The appearance of Reddish brown ring at the junction of two layers indicates the presence of Glycoside and the formation of orange red coloured complex indicates the presence of Alkaloid in the Seaweed sample **(Fig 4).**

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

3.5 Identification of Secondary metabolites - Gas chromatography-Mass spectrometry:
The Purified fractions were then subjected to identification by Gas chromatography-Mass spectrometry. It is an analytical method used for identifying different compounds within a test sample. In this study Glycoside and alkaloid compounds isolated through column chromatography were identified by means of GC-MS technique. The Glycoside compound was identified to be Pregnane-20-one, 3-hydroxy- and the GC-MS spectrum for the corresponding compound is given in the **Fig 5a** while the alkaloid compound was identified to be Pyrrolidine, 1-(1-Cyclohexen-1-yl)- and the GC-MS spectrum for the corresponding alkaloid is also given in the **Fig 5b.**

The Glycoside compound obtained through gas chromatography-mass spectrometry was found to play an important role in pharmaceutical industries and also in food industry in the form of food additives. They are also widely used in the treatment of obesity, in decreasing the blood sugar and blood pressure levels and also in lowering the body mass index (BMI) **[26]**. The alkaloid compound is also found to exhibit wide medicinal

applications such as radical scavenging, anti-inflammatory, antitumor and metal chelator functions. They are also found to possess antibacterial and antifungal activities and are mainly found in many pharmaceutical drugs. It is also widely distributed in food stuffs in trace amounts and acts as a flavouring agent **[27]**.

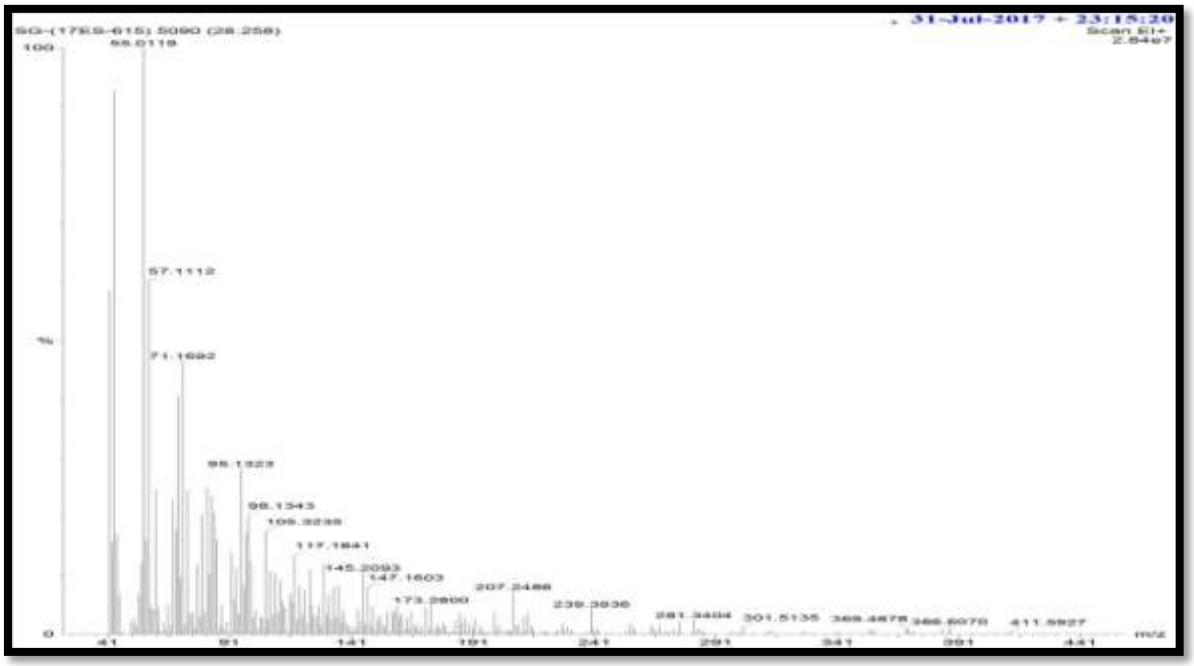

Figure 5a: GC-MS spectrum for Seaweed Glycoside (Pregnane-20-one, 3-hydroxy-)

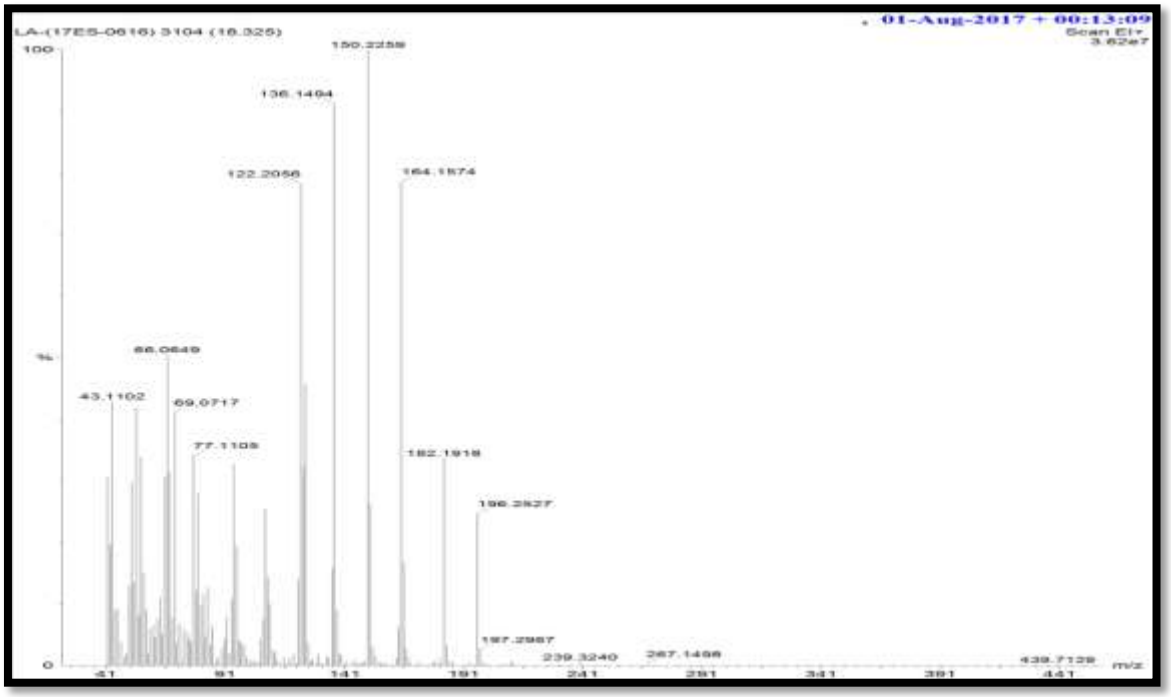

Figure 5b: GC-MS Spectrum for Seaweed Alkaloid (Pyrrolidine, 1-(1-Cyclohexen-1-yl)-)
Figure 5: GC-MS analysis of Glycoside and Alkaloid compounds from Seaweed

3.6 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 the Seaweed ***(Hypneacervicornis)***.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 from colourless to dark brown indicates the formation of silver nanoparticles. The synthesis was carried out in 30 minutes. The synthesized nanoparticles were then stored in an

air tight bottle at room temperature for further characterization studies**(Fig 6).**A study has reported the synthesis of silver nanoparticle from the aqueous extract of seaweed *Sargassumcinereum* and the nanoparticle formation was confirmed based on the colour change from yellowish green to orange**[28].**

Figure 6: Synthesis of silver nanoparticle using the aqueous extract and purified fractions

3.7 Characterization of Silver nanoparticles:

The Silver nanoparticles synthesized using the aqueous extract and purified fractions were further characterized by means of UV-Visible spectroscopy, Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscope (SEM).

3.7.1 UV-Visible Spectrophotometer analysis:

The Silver 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. The maximum absorption peak was obtained at 430nm for Seaweed aqueous extracts while showed peak at 440 nm for nanoparticle synthesized using Seaweed purified glycoside and alkaloid fractions **(Fig 7).**A study has reported the characterization of silver nanoparticles from *Murrayakoenigii* and *Zea mays* by UV-Visible spectroscopy wherein the absorption peak was found to be obtained at 420 - 440nm and thus confirmed the formation of nanoparticle **[29].**

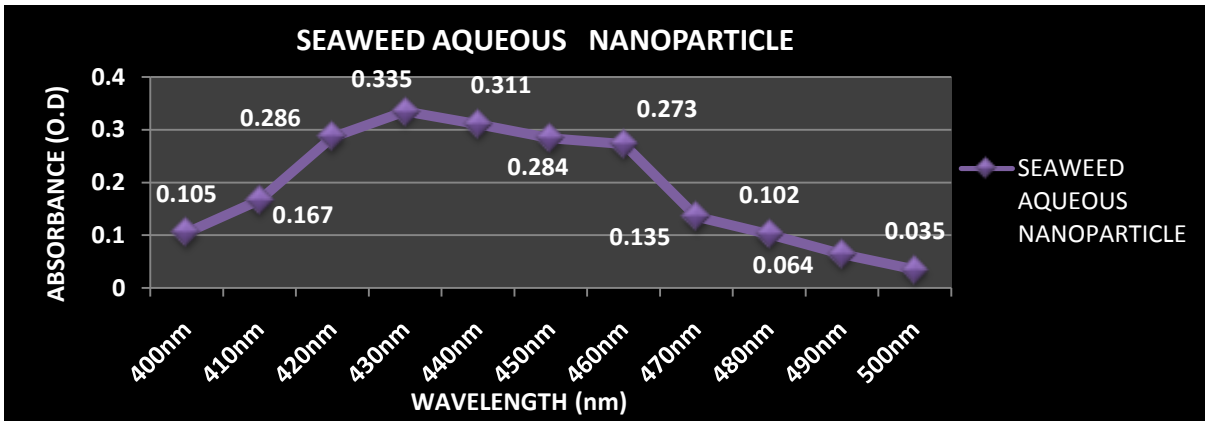

Figure 7a: UV-Visible spectra showing absorbance for Silver nanoparticle synthesized using Seaweed aqueous extract

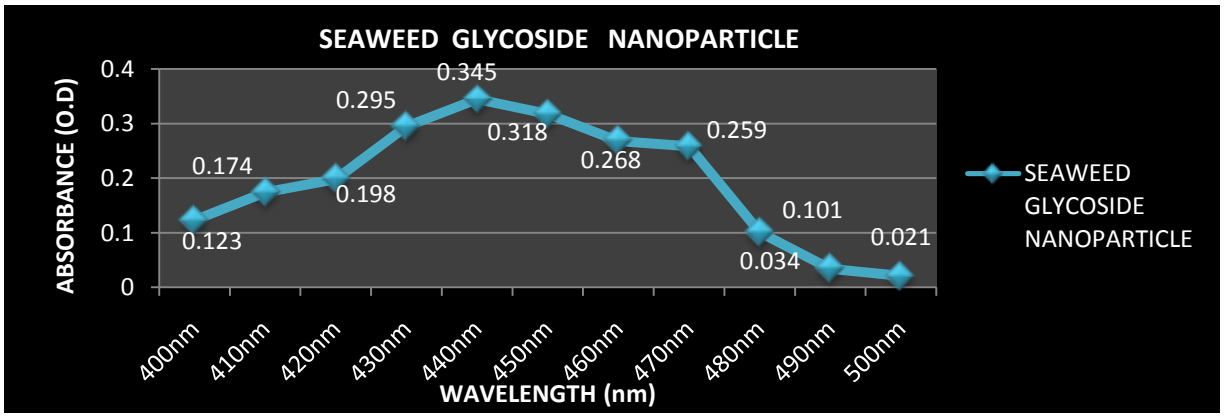

Figure 7b: UV-Visible spectra showing absorbance for Silver nanoparticle synthesized using purified glycoside fraction of Seaweed

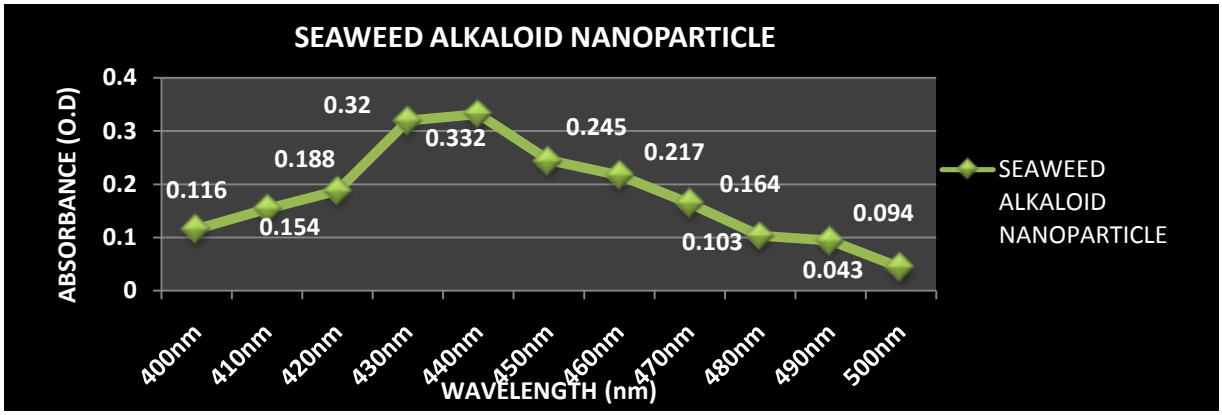

Figure 7c: UV-Visible spectra showing absorbance for Silver nanoparticle synthesized using purified alkaloid fraction of Seaweed

Figure 7: UV-Visible spectroscopic analysis of Silver nanoparticles from Seaweed

3.7.2 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. The FTIR results are interpreted according to a paper **[16].** The FTIR spectrum for the silver nanoparticles synthesized using the aqueous extract was analysed**(Fig 8a)** and absorption bands were observed at 3350.17cm^{-1}which corresponds to O-H group. The reduction of Ag^{+} to Ag^{0} was due to C=O stretch. The peak at 1234.89 cm^{-1},1386.90cm^{-1} corresponds with C-N stretching vibrations of aromatic and aliphatic amines, peak at 2922.97 cm^{-1}, indicates presence of C-H stretching of aromatic compounds and 1642.69cm^{-1} indicated presence of C=O group **(Table 3)**.The FTIR spectrum for the silver nanoparticles synthesized using the purified glycoside fraction was analysed**(Fig 8b)** and absorption bands were observed at 3431.77cm^{-1} which corresponds to C=O group. The peak at 1055.83cm^{-1} corresponds with C-N stretching vibrations of aromatic and aliphatic amines and the peak at 2850.81 cm^{-1}, indicates presence of C-H stretching of aromatic compounds , peak at 1643.46cm^{-1} indicated presence of C=O group and peak at 1730.58cm^{-1} indicates presence of C=C stretching **(Table 4).** The FTIR spectrum for the silver nanoparticles synthesized using the purified alkaloid fraction was analysed**(Fig 8c)** and absorption bands were observed at 3421.67cm^{-1} and 2919.56cm^{-1}which corresponds to O-H and C-H group. The peak at 1733.77 cm^{-1} corresponds with C-C stretching, peak at 2850.54cm^{-1}, indicates presence of C-H stretching of aromatic compounds and 1634.00cm^{-1} indicated presence of C=O group **(Table 5).**

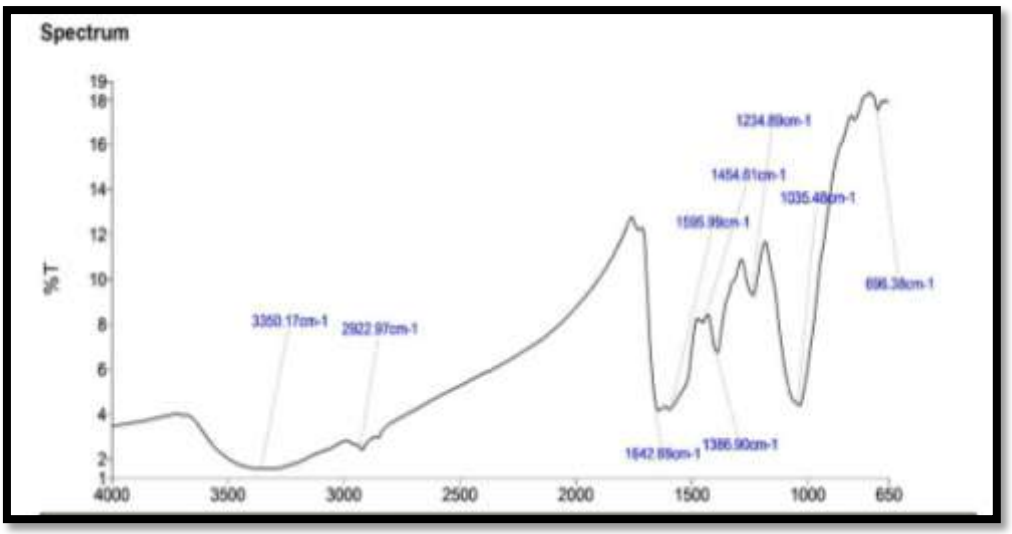

Figure 8a: FTIR Image of Seaweed aqueous nanoparticle

S.NO	BAND SPECTRA	BONDS
1	3350.17cm^{-1}	O-H group
2	2922.97cm^{-1}	C-H
3	1642.69cm^{-1}	C=O
4	1454.61cm^{-1}	C=C
5	1386.90cm^{-1}	C-N like amine
6	1234.89cm^{-1}	C-N stretching of amines
7	1035.48cm^{-1}	C-N stretching

Table 3:FTIR Spectra of Seaweed aqueous nanoparticle

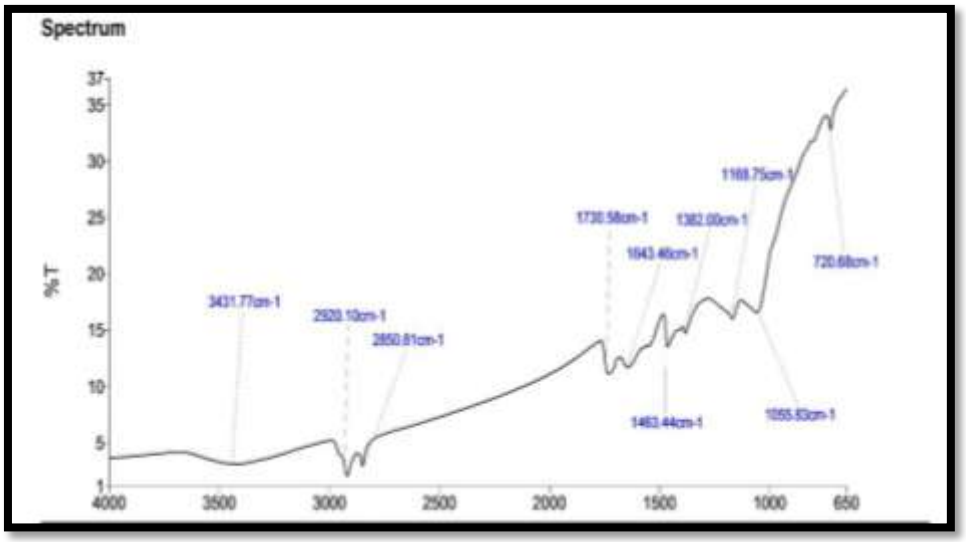

Figure 8b: FTIR Image of Seaweed Glycoside nanoparticle

S.NO	BAND SPECTRA	BONDS
1	3431.77cm^{-1}	C=O
2	2850.81cm^{-1}	C=H
1	1730.58cm^{-1}	C=C stretching
2	1643.46cm^{-1}	C=O
3	1382.00cm^{-1}	N=O
4	1055.83cm^{-1}	N-H and C-N

Table 4:FTIR Spectra of Seaweed Glycoside nanoparticle

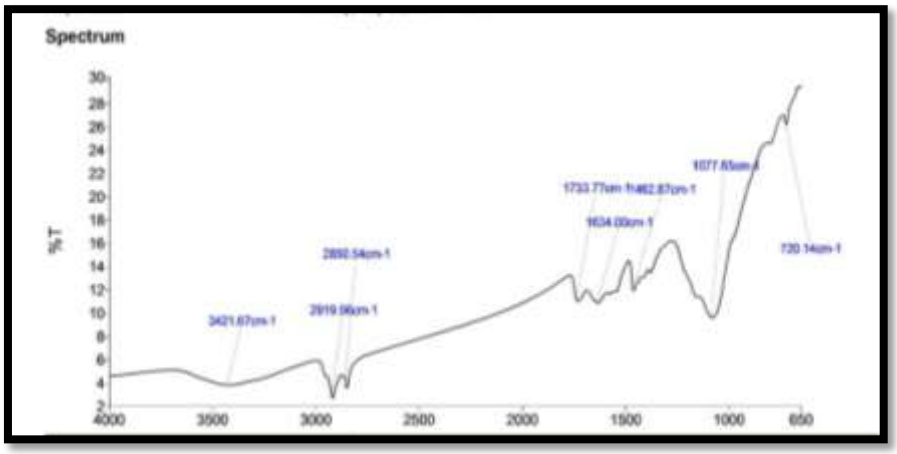

Figure 8c: FTIR Image of Seaweed Alkaloid nanoparticle

S.NO	BAND SPECTRA	BONDS
1	3421.67cm^{-1}	O-H group
2	2919.56cm^{-1}	C-H group
3	2850.54cm^{-1}	C-H stretching
4	1733.77cm^{-1}	C-C stretching
5	1634.00cm^{-1}	C=O

Table 5:FTIR Spectra of Seaweed Alkaloid nanoparticle

3.7.3 Scanning electron microscopy (SEM):

In the present study, SEM (Scanning electron microscope) analysis was performed to observe the morphology of the silver nanoparticles synthesized using the aqueous extract and purified glycoside and alkaloid fractions of Seaweed***Hypneacervicornis.***The shapes of the silver nanoparticles were reported to be spherical. The synthesized nanoparticles were found to be in aggregations which were distributed evenly. The particles formed aggregates on the surface of foil **(Fig 9).**

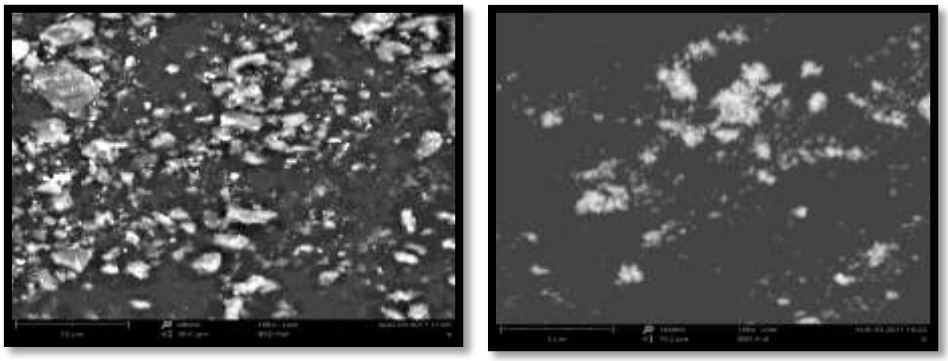

Figure 9a: Seaweed Aqueous nanoparticle**Figure 9b:** Seaweed Glycoside nanoparticle

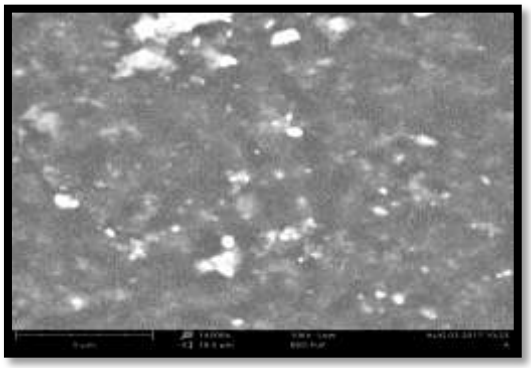

Figure 9c: Seaweed Alkaloid nanoparticle

Figure 9: SEM images of Silver nanoparticle synthesized using aqueous extract and purified fractions of Seaweed *Hypneacervicornis*

3.8 Applications:

3.8.1 Antibacterial activity:

The antibacterial activity of Seaweed (***Hypneacervicornis***) extract, Silver nanoparticle synthesized using aqueous extracts, Silver nanoparticle synthesized using glycoside and alkaloid fractions were screened against both gram positive and gram negative bacteria such as *Staphylococcus aureus, Streptococcus spp, Escherichia coli, Klebsiellapneumoniae, Salmonella spp*and *Pseudomonas aeruginosa***(Fig 10).** The Silver nanoparticle synthesized using the purified glycoside and alkaloid fractions showed good antibacterial activity compared to that synthesized using the aqueous extract. But among the purified fractions the nanoparticle synthesized using the glycoside fraction was found to have shown an increased zone of inhibition against all the organisms compared to that from the alkaloid fraction. The zone of inhibition obtained for the silver nanoparticle is given in the **Table 6.**

S.NO	ORGANISM	ZONE OF INHIBITION (in mm)				
		SEAWEED AQUEOUS EXTRACT	SEAWEED AQUEOUS NANO PARTICLE	SEAWEED GLYCOSIDE NANO PARTICLE	SEAWEED ALKALOID NANO PARTICLE	CONTROL
1	*E.coli*	0	4	12	8	0
2	*Streptococcus spp.*	0	3	8	6	0
3	*Klebsiella Pneumoniae*	0	3	11	6	0
4	*Staphylococcu s aureus*	0	5	9	6	0
5	*Salmonella typhimurium*	0	2	12	10	0
6	*Pseudomonas aeruginosa*	0	3	10	8	0

Table 6: Antibacterial activity of Silver nanoparticle synthesized usingthe seaweed aqueous extract and purified Glycoside and Alkaloid fractions were tabulated.

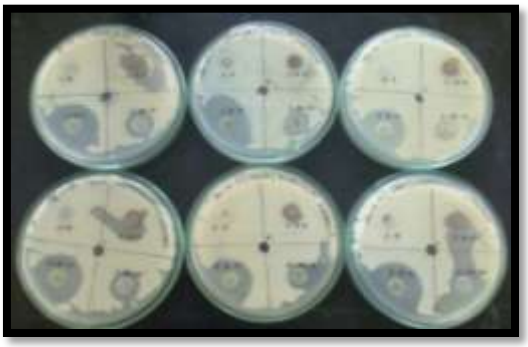

Figure 10: The antibacterial activity of silver nanoparticle synthesized using Seaweed extract and purified fractions were tabulated.

The antibacterial activity of silver nanoparticles synthesized from the seaweed *Gracilariabirdiae* has been reported in a study against 2 bacterial strains *Staphylococcus aureus* and *E.coli* wherein the antibacterial effect of silver nanoparticle against *E.coli* was found to be higher than that against *Staphylococcus aureus* respectively **[30]**.

3.8.2 Antifungal activity:

The antifungal activity of Seaweed (***Hypneacervicornis***) extract, Silver nanoparticle synthesized using aqueous extracts, Silver nanoparticle synthesized using glycoside and alkaloid fractions were screened against two fungal organisms namely *Aspergillusniger* and *Candida albicans***.**In case of antifungal activity the nanoparticle synthesized using the aqueous extract showed an increased zone of inhibition against both the fungal organisms when compared to that of the purified fractions. The zone of inhibition obtained for the silver nanoparticle is given in the **Table 7.**

S.NO	ORGANISM	ZONE OF INHIBITION (in mm)		
		SEAWEED AQUEOUS NANOPARTICLE	SEAWEED GLYCOSIDE NANOPARTICLE	SEAWEED ALKALOID NANOPARTICLE
1	*Aspergillusniger*	13	6	2
2	*Candida albicans*	11	3	2

Table 7: Antifungal activity ofSilver nanoparticle synthesized usingthe aqueous extract and purified Glycoside and Alkaloid fractions of Seaweed were tabulated.

A study by has reported the antifungal activity of the silver nanoparticles synthesized using the brown seaweed *Padinaboergesenii*against 3 fungal pathogens namely *Aspergillusfumigatus*, *Aspergillusnidulans*and *Candida albicans* respectively wherein the maximum inhibition was found against *Aspergillusfumigatus* (21.2mm) followed by *Aspergillusnidulans* (17.3mm) and moderately against *Candida albicans* (17mm) **[31].**

3.9 Antioxidant activity:

3.9.1 Hydrogen peroxide scavenging assay:

Hydrogen peroxide is a weak oxidizing agent. It is capable of penetrating within the cell and thereby reacts with Fe^{2+} or Cu^{2+} ion and gets converted into hydroxyl radical that could cause harmful effects 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 **[32]**. The Hydrogen peroxide scavenging assay for the Silver nanoparticle synthesized using aqueous extract and purified glycoside and alkaloid fractions of Seaweed *Hypneacervicornis*were determined. In this assay the nanoparticle synthesized using the purified fractions and the nanoparticle from aqueous extract (19.95%) exhibited good scavenging percentage but among both the purified fractions the nanoparticle synthesized using glycoside fraction (32.48%) was found to show better scavenging percentage compared to that of the alkaloid fraction (18.36%) respectively **(Fig 11).**

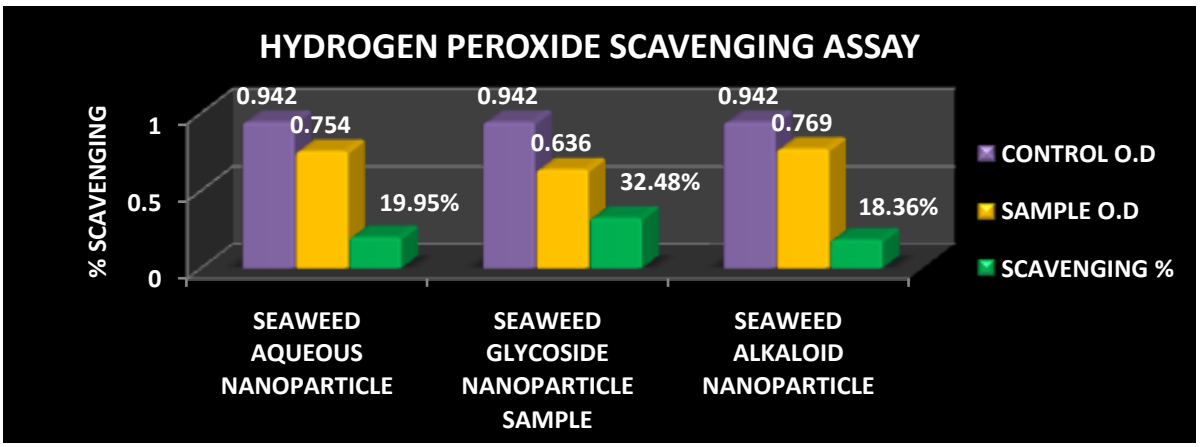

Figure 11: Hydrogen peroxide scavenging assay for Silver nanoparticle synthesized using seaweed aqueous extract and purified glycoside and alkaloid fractions.

The Hydrogen peroxide scavenging activity of the silver nanoparticles synthesized from the methanolic extract of brown seaweed *Himanthaliaelongata* have been reported in a study wherein the nanoparticles were found to have exhibited inhibition percentage of 39.1% to 77.4% at different concentrations from 200 to 800 ppm **[33]**.

3.10 Antidiabetic activity: The antidiabetic activity of the silver nanoparticle synthesized using the aqueous extract and the purified Glycoside as well as Alkaloid fractions of Seaweed *Hypneacervicornis* were determined

by means of alpha amylase inhibition assay. It is based on the principle of starch hydrolysis in the presence of α-amylase enzyme. This process is quantified using iodine which reacts with starch giving blue colour. The reduction in the intensity of blue colour indicates the breakdown of starch by the enzyme into monosaccharides. If the extracts show increased activity then the intensity of blue colour will be more or higher thus the intensity of blue colour developed is directly proportional to the alpha amylase inhibitory activity **[34]**. The Silver nanoparticle synthesized using the aqueous extract (27.77%) 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 (55.55%) showed an increased inhibition % than that of the alkaloid fraction (31.48%) respectively **(Fig 12).**The antidiabetic activity of silver nanoparticles synthesized using the seaweed *Halymeniapophyroides*has been studied at different concentrations as 0.2, 0.4, 0.6, 0.8 and 1ml respectively wherein the inhibition percentage was found to have shown an increase in dose dependent manner as 20% inhibition at a concentration of 0.2ml, 47% inhibition at 0.4ml, 61% inhibition at 0.6ml and 83% inhibition at 0.8ml **[35]**.

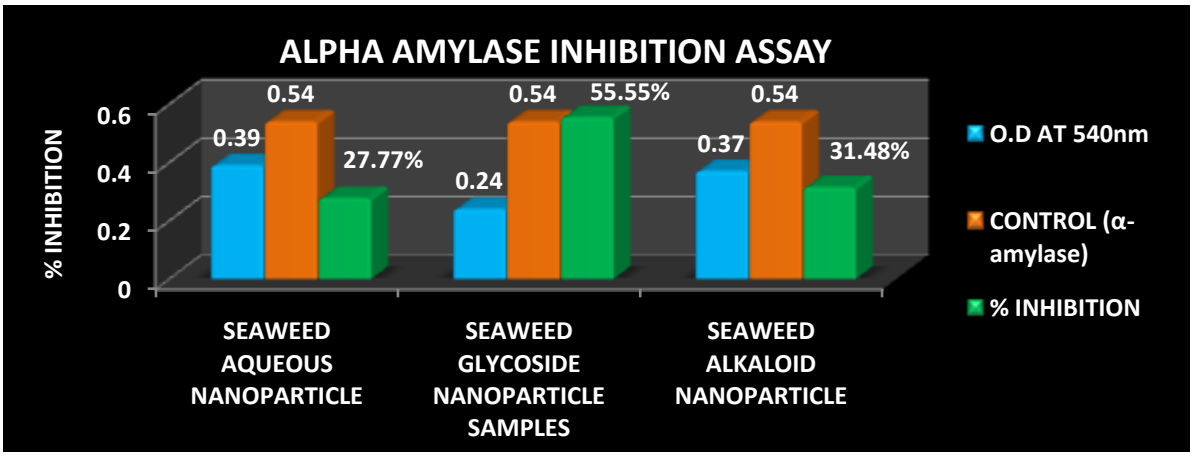

Figure 12: Alpha amylase inhibition assay for Silver nanoparticle synthesized using seaweed aqueous extract and purified glycoside and alkaloid fractions.

IV. CONCLUSION

The Current study was carried out in order to determine the therapeutic potential of Silver nanoparticles synthesized using the aqueous extract and purified glycoside and alkaloid fractions of Seaweed *Hypneacervicornis.*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 Seaweed*Hypneacervicornis*so that the combined efficacy of these nanoparticles with the secondary compounds could prove to be beneficial to the mankind in different aspects.

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