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# ANTI-MICROBIAL, OVICIDAL, LARVICIDAL, ANTI-DIABETIC AND CYTOXICITY ACTIVITY OF SILVER NANOPARTICLES FROM BROWN SEAWEED (*STOECHOSPERMUM MARGINATUM*)

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**Keywords:**

*Steochospermum marginatum*, UV-Vis, FTIR, SEM, Cytotoxicity, VERO cell lines

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**ABSTRACT:** The present study aimed to green synthesis the nanoparticles using bioactive compounds from unique seaweeds and testing its application for anti-microbial, anti-diabetic, ovicidal, larvicidal and cytotoxic activity. Seaweeds are marine non- flowering plants have much of the secondary metabolites and had not been explored widely. There are very limited amount of literature are there for seaweeds. *Steochospermum marginatumis* a species of brown algae in the family Phaeophyta, they are found on rocky shores. The green synthesis of silver nanoparticles (AgNPs) was synthesized from the seaweed *Steochospermum marginatum*. The AgNPs was formed at 60°C at 5000 rpm when the solution of silver nitrate was added to aqueous extract of *Steochospermum marginatum*. The UV-Visible Spectroscopy, Fourier transform-infrared spectroscopy (FT-IR), Scanning electron microscope (SEM) were used for characterization of AgNPs. The green synthesized silver nanoparticles were tested for various applications such as Anti-microbial, ovicidal, larvicidal and anti-diabetic and cytotoxicity using VERO cell lines.

**INTRODUCTION:** Seaweeds are multicellular organisms that are present in the intertidal and sub tidal zone. Most of the marine organisms and animals depend upon algae as their source of food. It ranges from single celled, microscopic flagellates to giant kelp which grows to hundred feet long. They do not have any seeds or flowers and their reproduction is accomplished by asexual spores. They do not have roots but are attached to substratum by the anchoring substance called as holdfast and absorb their minerals and nutrients directly from the sea water through their leaf like structures.

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The seaweeds possess chlorophyll and they utilize the sunlight and synthesize proteins, carbohydrates, fats and other inorganic chemicals.

**FIG. 1:** ***STOECHOSPERMUM MARGINATUM***

Marine seaweeds have nutritional value. The chemicals which have been derived from them have peculiar properties. Hence they are mainly

used in various fields such as medicine, food and other industries. Even have been cultivated in different places and harvested which is used up to their full potential. Seaweeds have a wide range of therapeutic properties. It has primary and secondary metabolites which have high medicinal value. The marine seaweeds are mainly needed for the production of agar and algin in large amount.

## MATERIALS AND METHODS:

**Collection of Seaweeds:** *Stoechospermum marginatum* were collected from the coast of Ramanathapuram district in Tamil Nadu, India **Fig. 1**. The seaweeds were manually collected, the debris were removed and it was washed with tap water and again washed with distilled water and shade dried for 3 days at room temperature and using electric blender it was finely powdered.

**Extraction of Seaweeds:** 50 gm of the dried Brown seaweed were extracted in 500 ml of Chloroform, Methanol, Hexane and Aqueous (1: 10 ratio) for 2 days in separate conical flasks. The solvents were filtered using a muslin cloth. The solvents were allowed to evaporate to obtain the concentrated residue. The dry weight of the extracts was measured separately. The residues were stored in the tight screw cap bottle and used for further studies.

**Phytochemical Screening:**

**Qualitative Method:**

**Test for Alkaloids - Dragendroff's Test:** 1 ml of filtrates was taken in clean separate test tubes. Few drops of Dragendroff reagent were added to each test tube. Appearance of red precipitate indicates the presence of alkaloids.

**Test for Carbohydrates - Benedict Test:** 1 ml of filtrates was taken in clean separate test tubes. Few drops of Benedict's reagent were added to each test tube**.** Formation of Orange red precipitate indicates the presence of reducing sugars.

**Test for Saponins - Froth Test:** 1 ml of filtrates was taken in a clean separate test tubes and each test tube was diluted with 20 ml of distilled water and shaken for 15 minutes. Formation of 1cm of foam indicates the presence of saponins.

**Test for Phytosterols - Salkowski's Test:** 1 ml of filtrates was taken in clean separate test tubes and treated with 5 ml of Chloroform and few drops of Concentrated Sulphuric acid along the sides of the test tube. Appearance of reddish brown layer indicates the presence of Phytosterols.

**Test for Phenols - Lead acetate Test:** 1 ml of filtrates was taken in clean separate test tubes and treated with 10% lead acetate solution. Appearance of white precipitate indicates the presence of phenols.

**Test for Tannins - Gelatin Test:** 1ml of filtrates were taken in clean separate test tubes and treated with 1% gelatin solution containing sodium chloride. Formation of white precipitate indicates the presence of tannins.

**Test for Flavanoids - Alkaline Reagent Test:** 1 ml of filtrates were taken in clean separate test tubes and treated with few drops of sodium hydroxide solution. Formation of intense yellow colour indicates the presence of flavonoids.

**Test for Proteins and Aminoacid:**

**Biuret Test:** 1 ml of filtrates was treated with 5% solution of sodium hydroxide and 1% copper sulphate. Appearance of pink or purple colour indicates the presence of proteins.

**Ninhydrin Test:** 1 ml of filtrates was treated with two drops of ninhydrin solution. Appearance of purple indicates the presence of amino acids.

**Test for Terpenoids - Salkowski's Test:** 1 ml of filtrates was taken in clean separate test tubes and treated with 5 ml of Chloroform and few drops of Concentrated Sulphuric acid along the sides of the test tube. Appearance of reddish brown layer indicates the presence of terpenoids.

**Test for Cardiac Glycosides - Keller-killani Test:** 1 ml of filtrates was taken in clean separate test tubes and treated with 2 ml of glacial acetic acid, one drop of 5% ferric chloride and few drops of Concentrated Sulphuric acid along the sides of the test tube. Formation of reddish brown colour at the junction of two layers indicates the presence of cardiac glycosides.

**Thin Layer Chromatography:** Thin layer chromatography (TLC) is a chromatographic

technique which is used to separate and identify various components from the plant source. The TLC chamber consists of a glass jar with a lid and the solvent mixture (Chloroform: Methanol - 9:1) is added to chamber. The TLC plates were placed carefully in the TLC chamber. The solvent mixture should be below the spot on the plate and the chamber is closed. As the solvent moves up the coloured pigment of the extract can be seen separating. The TLC plates are removed from the chamber when the solvent is approximately 1cm from the top of the TLC plates. After drying the plates the spraying reagent (Draggendroff reagent, Conc. sulphuric acid, Iodine vapour) which is used for different compounds are sprayed and left to dry. The development of the coloured spots indicates the presence of the secondary metabolites.

**Synthesis of Silver Nanoparticles:** 20 ml of Brown Seaweed extracts of (*Stoechospermum marginatum*) was taken in separate conical flasks. 1 mM silver nitrate was added drop by drop to the extract in a beaker at 5000 rpm at 60 °C. The colour change from light yellow to purple or brown colour indicates the formation of silver nanoparticles [1]. The reduction of the $Ag^+$ ions was measured by the UV-Vis spectrum [9].

**Characterization of Silver Nanoparticles:** Ultraviolet-visible Spectroscopy (UV-Vis) was performed in Shimadzu UV-VIS Spectro-photometer and at an ultraviolet range of (400 - 480 nm) the reading were noted. The studies of size, morphology and composition of the nanoparticles were performed by means of scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectral measurements were carried out using Shimadzu IR Tracer -100 to identify the potential biomolecules in *Steochospermum marginatum* extract which is responsible for reducing and capping the bio-reduced silver nanoparticles [2]. Samples for SEM studies were prepared by placing drops of the silver nanoparticles solutions on carbon-coated SEM grids and the morphology was studied.

**Applications of Synthesized Seaweed Nanoparticles:**

**Antibacterial Assay:** Nutrient broth and agar were used for the screening of the antibacterial activity of the seaweed extracts.

**Agar Well Diffusion Method (Jeyanthi *et al.*, 2013):** The authenticated organisms *Escherichia coli*, *Staphylococcus* sp, *Streptococcus* sp, *Pseudomonas* sp, *Proteus* sp, *Bacillus* sp, *Aspergillus* sp and *Candida* sp. used for testing were obtained from National Committee for Clinical Laboratories Standards (NCCLS), purchased from Institute of Microbial Technology, Chandigarh, India. Overnight cultures were prepared with nutrient broth.

**Preparation of Plates and Antimicrobial Testing:** Muller - Hinton Agar (MHA) for antibacterial testing was prepared, autoclaved and the media was poured onto the petriplates and allowed to solidify, each organism was swabbed onto the plates under sterile conditions.The algal extracts of (20 µl, 40 µl, 60 µl, 80 µl) were dispensed into each well. The petriplates were incubated at 37 °C for 24 hours and observed for the zone of inhibition. The diameter of the zone of inhibition was measured in mm.

**Antidiabetic Activity:**

**Inhibition of α-amylase Enzyme Activity:** Starch solution was prepared by adding 1 g of starch in 100 ml of sodium acetate buffer and added to the tubes. The algal extracts were added in various concentrations (0.1 ml, 0.2 ml, 0.3 ml, and 0.4 ml) of amylase was added to the test tubes and incubated for 10 - 15 minutes in room temperature. 2 ml of 3, 5-dintro salicylic acid was added to all the tubes. 0.5 ml of amylase was added to control tubes and incubated in boiling water for 10 - 15 minutes and cooled. 1 ml of sodium potassium tartarate was added to all the tubes and the absorbance was read at 540 nm [7, 8].

% inhibition = OD of control- OD of test / OD of control * 100

**Ovicidal and Larvicidal Activity:** *Aedes aegypti* eggs and larvae were placed in the distilled water and the silver nanoparticles algal extract and the seaweed extracts were added in different concentrations (2, 4, 6, 8 µl). Larval food (yeast) was added to all the mosquito eggs and larvae [4]. Control mosquito eggs and larvae were exposed for 24 hours. Percentage of mortality was calculated as follows:

Percentage of mortality = (number of dead individuals/ number of treated individuals)*100

***In-vitro* Assay for Cytotoxicity Activity:**

**MTT Assay:** Cell lines such Vero cell lines were obtained from King's institute Guindy Chennai. The cells were maintained in Minimal Essential Media in a humidified atmosphere of $CO_2$ at 37 °C. The cytotoxicity of the brown seaweed was determined by the MTT assay [5]. 1 mg of sample is dissolved in 1ml of serum free MEM/DMSO. The stock is prepared fresh and filtered through 0.45 µ filter before each assay. Working concentrations of sample ranging from 1000 µg to 7.8 µg were prepared. Cell ($1\times10^5$/ well) (Vero Cells) were plated in 24 well plates and incubated at 37 °C with 5% $CO_2$ condition. After the cell reaches the confluence, media was removed from the wells carefully without disturbing the cells. The various concentrations of the samples (500 µl) were added and incubated for 24 hours.

After incubation, the sample was removed from the well and washed with phosphate buffered saline (pH-7.4) or MEM without serum.100 µl / well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazoium bromide (MTT) was added and incubated for 4 hours. After incubation, 1 ml of DMSO was added in all the wells. The absorbance at 570 nm was measured with ELISA reader using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition ($IC_{50}$) was determined graphically [11]. The % cell viability was calculated using the following formula:

% Cell viability = A570 of treated cells / A570 of control cells × 100

## RESULTS AND DISCUSSION:

**Extraction of Seaweeds:** The seaweed (*Stoechospermum marginatum*) was washed with marine water, the calcareous stones and epiphytes were removed my manual sorting and intensively washed with tap water and then again with distilled water. The seaweeds are shade dried for 3 - 4 days. The bioactive compounds from seaweeds were obtained by using the solvent extraction **Fig. 2**. The solvents such as Chloroform, methanol, hexane were taken in a conical flask and the seaweeds were weighed using an electronic weighing balance and added to the solvents in the ratio of 1:10 and left it for 2 - 3 days and the extract were filtered using a muslin cloth. The extracts were allowed to dry and the residues were collected and the dry weights of the samples were obtained. The residues were stored in a cork screw bottle and were used for the further analysis.

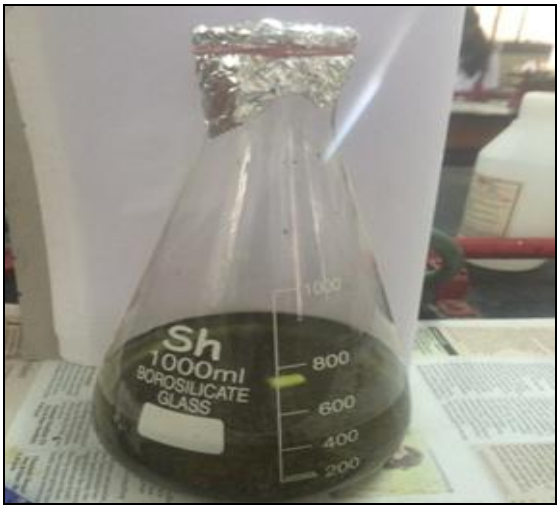



**FIG. 2: BROWN SEAWEED EXTRACT**

**Phytochemical Screening - Qualitative Analysis:**

**Phytochemical Analysis of *S. marginatum*:** In present study, brown seaweed (*Steochospermum marginatum*) was used to screen the presence of the secondary metabolites. The phytochemical such as the alkaloids, phenols, tannins, flavonoids, saponins, phytosterols, proteins, glycosides, fats and oils were the metabolites present in the seaweeds used as a protective mechanism against the microorganisms, insects and acts as a defence mechanism in protecting the body [3]. The brown seaweed samples indicate the presence of the bioactive compounds which has medicinal value and are being used for many applications [6] **Table 1**.

**TABLE 1: PHYTOCHEMICAL ANALYSIS OF *STOECHOSPERMUM MARGINATUM***

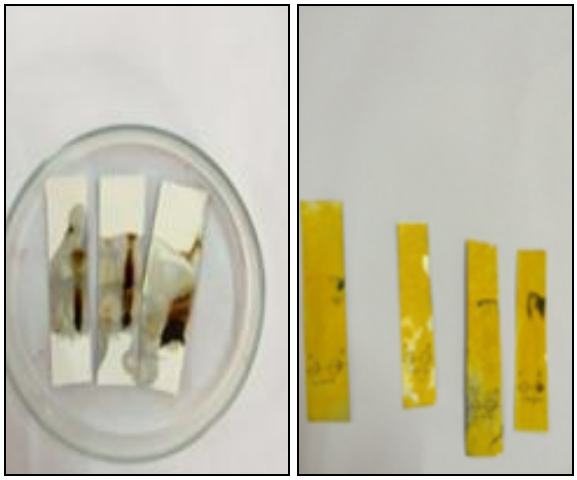
| Phytochemicals | Aqueous | Chloroform | Methanol | Hexane |
|---|---|---|---|---|
| Alkaloids | + | + | - | + |
| Carbohydrates | - | - | - | - |
| Saponins | - | - | - | - |
| Phytosterols | + | + | - | + |
| Phenols | + | + | + | + |

| | | | | |
|---|---|---|---|---|
| Tannins | + | - | + | - |
| Flavonoids | - | - | - | - |
| Proteins and aminoacids | - | - | - | - |
| Terpenoids | + | + | + | + |
| Cardiac glycosides | - | - | - | - |
| Fixed oils and Fats | + | - | - | - |

It was found that brown seaweed (*Stoechospermum marginatum*) have more secondary metabolites such as alkaloids, phytosterols, phenols, tannins, terpenoids, fixed oils and fats. Methanol and hexane extracts hasphytochemicals similar to aqueous and chloroform extracts of *Stoechospermum marginatum*.

**Thin Layer Chromatography:** Thin layer chromatography is a technique used for the separation of the secondary metabolites in the seaweed extract by using the solvents in different ratios. Thin layer chromatography was used to identify the presence of alkaloids, phenols, tannins, and Phytosterols.

The appearance of the orange bands in TLC plates indicated the presence of alkaloids in *S. marginatum* and the solvents used as a mobile phase was chloroform:acetone: methanol - 9: 1: 1 detected using Dragendroff reagent **Fig. 3B**. The appearance of the brown spots indicated the presence of Phytosterols used as a mobile phase was benzene: methanol - 9: 1 are detected using Vanillin reagent **Fig. 3A**.



**A** **B**

**FIG. 3: A) PHYTOSTEROLS B) ALKALOIDS**

The appearance of blue spots indicated the presence of phenols and the solvents used as a mobile phase is chloroform: methanol - 9: 1 are detected using Folin ciocalteau **Fig. 3C**.

**FIG. 3C: PHENOLS**

**Green Synthesis of Nanoparticles:** Silver nanoparticles were formed by the reduction of $Ag^+$ by the action of the 1 mM of $AgNO_3$. The solution was gradually heated and the colour change was observed which shows the presence of silver nanoparticles. The synthesis of silver nanoparticles was carried out in 30 min. The synthesized nanoparticles were stored in an air tight bottle at room temperature for further characterization studies **Fig. 4**. The silver nanoparticles were formed as compared with the algal mediated synthesis of seaweeds.


**FIG. 4: SILVER NANOPARTICLES *S. MARGINATUM***

**Characterization of Nanoparticles:**

**UV-Visible Spectrophotometer Analysis:** The newly synthesized nanoparticles were observed to show absorption peak at 400 - 480 nm under UV-Visible absorption spectroscopy. In this present study the *Stoechospermum marginatum* extract after the reduction of $Ag^{+}$ to Ag nanoparticles when subjected to UV-Visible spectroscopy showed the maximum absorption peak at 430 nm. The optical density of the brown seaweed was 0.33 OD which indicates the synthesized silver nanoparticles.

**Scanning Electron Microsope:** In this present study, SEM was performed to study the morphology of the synthesized silver nanoparticles. The shapes of the silver nanoparticles were reported to be spherical. The synthesized nanoparticles were found in aggregations which were distributed evenly varied in the size range of 100 µm - 200 µm. The particles form aggregation on the surface of the foil **Fig. 5**.

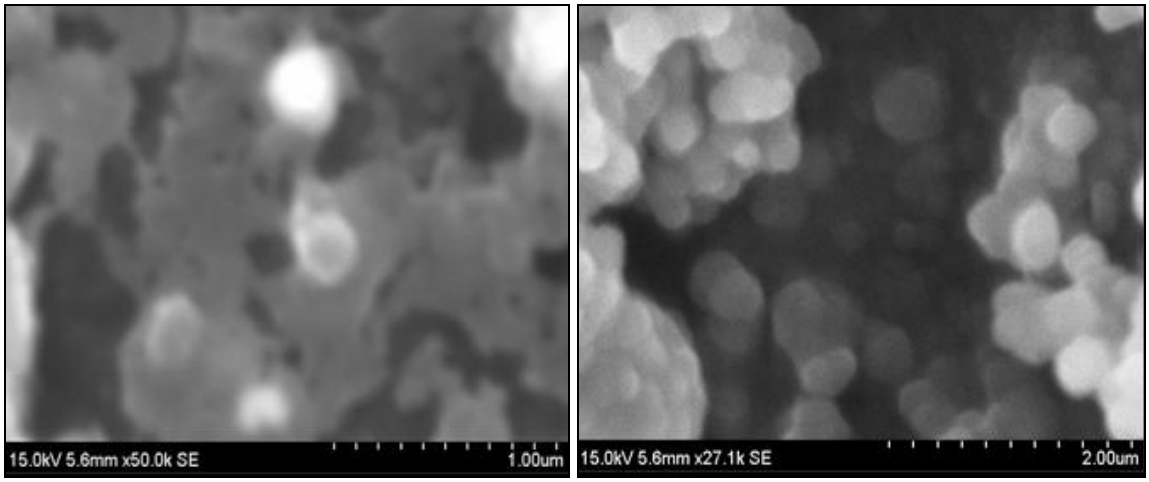



**FIG. 5: SEM IMAGES OF SYNTHESIZED BROWN SILVERNANOPARTICLES**

**FTIR Spectrum of Synthesized Nanoparticles:**

**FTIR Spectrum of Synthesized Nanoparticles from Brown Seaweed:**

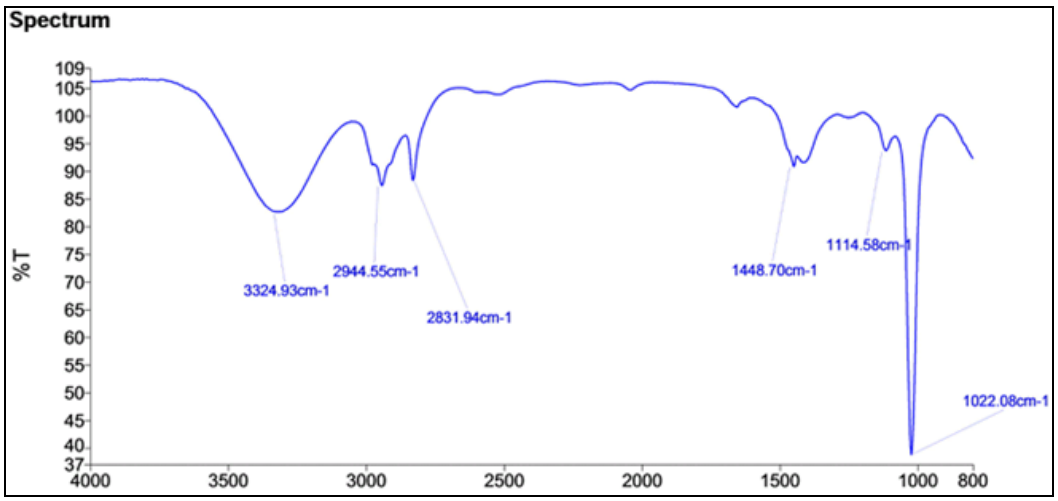



**FIG. 6: FTIR IMAGE OF BROWN NANOPARTICLES**

**TABLE 2: FTIR FOR BROWN SEAWEED SILVER NANOPARTICLES**

| S. no. | Band Spectra | Bonds |
|---|---|---|
| 1. | 3324.93$cm^{-1}$ | O-H |
| 2. | 2944.55$cm^{-1}$ | C-H |
| 3. | 2831.94$cm^{-1}$ | C-H |
| 4. | 1448.70$cm^{-1}$ | C=C |
| 5. | 1114.58$cm^{-1}$ | C-O |
| 6. | 1022.08$cm^{-1}$ | C=O |

The FTIR spectrum for the silver nanoparticles was analysed and absorption bands were observed at 3340 $cm^{-1}$ and 1638 $cm^{-1}$ which corresponds to O-H and C=O. The reduction of $Ag^{+}$ to $Ag^{0}$ was due to C=O stretch. The highest peak was interpreted at 1022.08 $cm^{-1}$ corresponds to the C=O ketone group. The lowest peak was 1448.70 $cm^{-1}$ and 1114.58 $cm^{-1}$ C=C and C-O corresponds to carbonyl group **Fig. 6**, **Table 2**.

**Antimicrobial Activity:** Antibacterial activity of Brown Silver Nanoparticles:

**TABLE 3: ANTIBACTERIAL ACTIVITY OF BROWN SILVER NANOPARTICLES**

| Organisms | Samples | | | |
|---|---|---|---|---|
| | 20µl | 40µl | 60µl | 80µl |
| *E. coli* | - | - | - | 9mm |
| *Staphylococcus aureus* | 4mm | 9mm | 14mm | 18mm |
| *Klebsiella* spp. | - | - | 9mm | 12mm |
| *Proteus* spp. | - | 4mm | 7mm | 10mm |
| *Pseudomonas* spp. | 5mm | 8mm | 10mm | 13mm |
| Control | ---------- No Growth ---------- | | | |

The activity of the test was done using different sample concentrations such as 20 µl, 40 µl, 60 µl, 80µlof the brown silver nanoparticles. In 80 µl concentration 18 mm to 19 mm of zone shows the maximum activity against *Staphylococcus aureus*. The least activity of 9 mm was shown by the *E. coli*. The *Proteus* spp. and *Klebsiella* spp. Shows the zone of inhibition of 12 mm and 10 mm. When compared with antibacterial activity of the seaweed *S. marginatum*, *S. aureus* shows the maximum zone of inhibition **Table 3**.

**Antidiabetic Activity:**

**Comparison between Aqueous Brown Seaweed and Brown Seaweed Nanoparticle:**

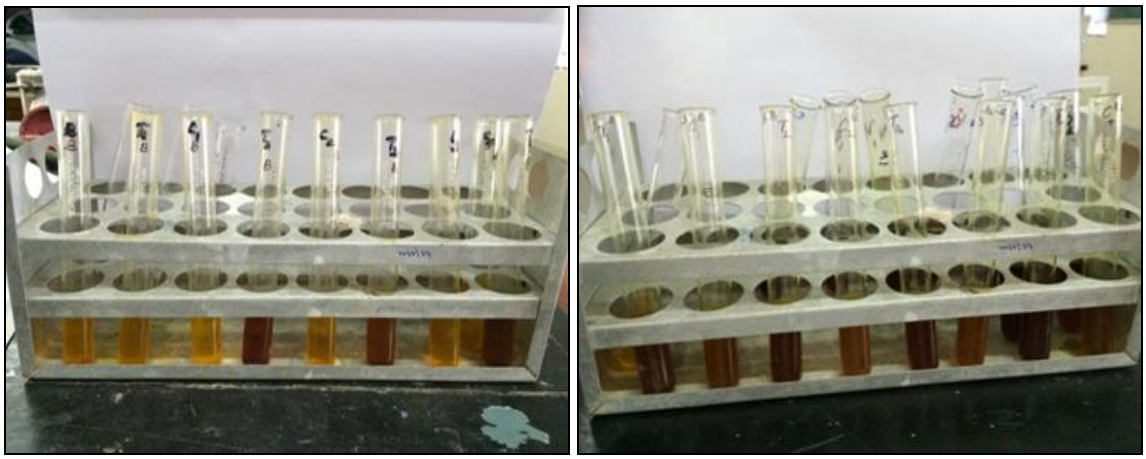


**FIG. 7: ANTIDIABETIC ACTIVITY OF AQUEOUS BROWN SEAWEED AND BROWN SEAWEED NANOPARTICLE**

**TABLE 4: COMPARISON BETWEEN AQUEOUS BROWN SEAWEED AND BROWN SEAWEED NANOPARTICLE**

| Sample Concentration | % Inhibition in Aqueous Brown Seaweed | % Inhibition in Brown Seaweed Nanoparticle |
|---|---|---|
| 0.1ml | 12.5 | 10 |
| 0.2ml | 20 | 8.1 |
| 0.3ml | 23 | 20 |
| 0.4ml | 25 | 21.0 |

The extract of the brown seaweed showed a good percentage of inhibition of diabetic activity. The aqueous extract was more effective when compared with the biosynthesized nanoparticles **Fig. 7**.

The reduction in the active component by silver nitrate reflects a poor anti-diabetic activity when compared to the aqueous extract with anti-diabetic activity of the seaweeds **Table 4**.

## Anti - Ovicidal Activity:

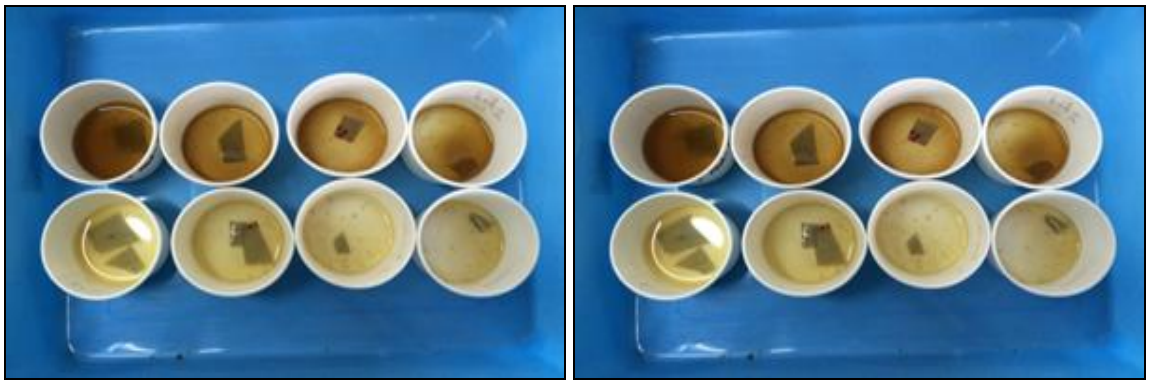


**FIG. 8: ANTIOVICIDAL ACTIVITY OF AQUEOUS BROWN SEAWEED AND BROWN NANOPARTICLE SEAWEED EXTRACT AGAINST MOSQUITO EGGS**

**TABLE 5: ANTIOVICIDAL ACTIVITY OF AQUEOUS BROWN SEAWEED AND BROWN NANOPARTICLE SEAWEED EXTRACT AGAINST MOSQUITO EGGS**

| Nanoparticle Dilution | % of Mortality of Mosquito Eggs in Brown Seaweed | % of Mortality of Mosquito Eggs in Brown Seaweed Nanoparticle |
|---|---|---|
| 1:2 | 100 | 100 |
| 1:4 | 100 | 100 |
| 1:6 | 100 | 100 |
| 1:8 | 100 | 100 |

The extract of both the biosynthesized nanoparticles and the aqueous extracts were found to be effective in killing the mosquito eggs and the percentage of mortality was found to be 100%. The extracts can be further purified and used as the mosquito repellent. The leaf *Steochospermum marginatum* extract nanoparticles showed maximum mortality rate against the *Aedes aegypti* when compared with seaweed extract **Fig. 8** (Kadarkari M *et al*., 2015) which correlated with the finding of the present study where, the seaweed *U. lactuca* nanoparticles exhibited maximum mortality rate when compared to the seaweed extract alone **Table 5**.

## Larvicidal Activity:

**FIG. 9: LARVICIDAL ACTIVITY OF AQUEOUS BROWN SEAWEED AND BROWN NANOPARTICLE SEAWEED EXTRACT AGAINST MOSQUITO EGGS**

**TABLE 6: LARVICIDAL ACTIVITY OF AQUEOUS BROWN SEAWEED AND BROWN NANOPARTICLE SEAWEED EXTRACT AGAINST MOSQUITO EGGS**

| Nanoparticle Dilution | % of Mortality of Mosquito Larvae in Brown Silver Nanoparticles | % of Mortality of Mosquito Larvae in Brown Seaweed |
|---|---|---|
| 1:2 | 57 | 20 |
| 1:4 | 66 | 50 |
| 1:6 | 75 | 62 |
| 1:8 | 85 | 88 |

The extract of the biosynthesized nanoparticles and the aqueous extract of the brown seaweed were found to have the maximum mortality rate in killing the larvae. The leaf *Steochospermum marginatum* extract showed maximum mortality rate against the *Aedes aegypti* when compared with seaweed nanoparticle extract **Fig. 9**. The ova and the larvae are treated and controlled. When correlated with the finding of the seaweed *U. lactuca* nanoparticles exhibited maximum mortality rate when compared to the seaweed extract alone seaweed extract and synthesized nanoparticles act as a best alternative than the other source of chemicals usedfor controlling and treating the mosquitoes **Table 6** Kadarkari M *et al*., 2015.

**Cytotoxic Assay (MTT Assay):**

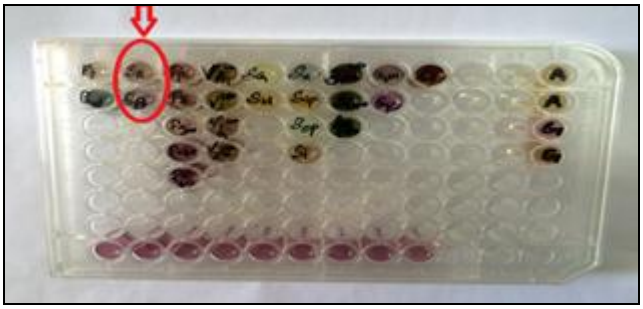


**FIG. 10: MTT ASSAY - VERO CELL LINES**

**TABLE 7: CYTOTOXICITY ASSAY**

| Samples | % Cell Viability |
|---|---|
| Brown silver nanoparticles | 84% |

The cytotoxicity assay was carried out using VERO cell lines the Brown seaweed silver nanoparticles were less cytotoxic **Fig. 10**. The percentage of cell viability was 84% in brown silver nanoparticles **Table 7**. The different concentrations of certain polysaccharides include sulfated galactans, sulfated rhamnans or mannans, carrageenans and agars which contribute to the different levels of toxic effects of the seaweeds which were found to be low in the brown seaweed.

**CONCLUSION:** The present study is to synthesis silver nanoparticles using brown seaweed *Steochospermum marginatum.* The Phytochemical analysis was carried out by the qualitative and quantitative analysis and showed the presence of alkaloids, phenols and tannins. The synthesized nanoparticles were tested for its activity for anti-microbial, anti-diabetic, ovicidal, larvicidal and less cytotoxic. The brown seaweed exhibited the antimicrobial activity which enhances a promising finding to utilize the bioactive compound in the field of medicine and pharmaceuticals which helps in enhancing the quality of the drugs.

About 50% of the anticancer drugs are been obtained from the natural sources such as medicinal plants, herbs and spices. The active compounds such as alkaloids, phenols and tannins have shown to posess this activity. Anti-cytotoxicity testing of the silver nanoparticles obtained from the brown seaweed extract showed 84% anti-cytotoxic activity. Hence the brown could be used in the treatment of the diseases as it is less cytotoxic.

This contributes to future pharmaceutical industry to obtain the active compound present in the brown seaweed and prepare silver nanoparticles which aids in targeted drug delivery of the active compound which can be produced in large scale and used to treat diseases.

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**CONFLICT OF INTEREST:** The authors declared no competing interests.

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