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CHARACTERISATION AND FORMULATION OF COSMETIC PRODUCT FROM THE SEAWEED *Eucheuma Spinosum*

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Abstract : Seaweed is the marine algae that grows in oceans, rivers and lakes. This plant consists of vitamins, minerals, amino acids and proteins. The present study characterized and analyzed the petroleum ether of *Eucheuma Spinosum.* The extract was subjected to various phytochemical, antimicrobial and anti inflammatory and to determine its cosmetic potential. Due to its vast variety of bioagents it possess antimicrobial and antioxidant properties which have found their way into cosmetic product formulation.

Keywords : Phytochemical, Antimicrobial, Cosmetic potential and bioagents.

1. INTRODUCTION

Algae, the photosynthetic organism that are multicellular which possess photosynthetic pigments known as chlorophyll. Algae are important source of vitamins, minerals and fatty acids. They consists of a large heterogeneous assemblage of plants which are diverse in their habitat, size and organization. They are different in their physiologic and reproductive characteristics. Most algae don't have lignin associated to the cellulose of the plant cell membrane therefore the cellulose extraction is simpler, less costly and produces microcrystalline cellulose with chemical properties that can optimize the use of traditional cellulose in some applications as paper production and membrane filtration. (Krauss and Schmidt, 1987)

The algae cultivation is very efficient at converting light, water and carbon dioxide into biomass. Many metabolites isolated from marine algae are shown to possess bioactive effects. The invention of metabolites with biological activities from macro algae has increased significantly within the last three decades. (sheath & wher.,2015) Many species of algae are useful in food, dairy, pharmaceutical and cosmetic industry. Edible algae are composed of proteins, carbohydrates, vitamins and minerals.

Seaweed refers to the large marine algae that grows in the shallow waters. Seaweeds are plants because they use the sun's energy to produce carbohydrates from carbon dioxide and water. They are simpler than the land plants mainly because they absorb the nutrients that they require from the encompassing water and haven't any need for roots or complex conducting tissues. The large seaweed like the kelps have root-like parts called holdfasts but these only serve to attach them to the rock. Most seaweed has got to be attached to something so as to survive, and only a couple of will grow while drifting loose within the sea. (Chapman, R.L 2013)

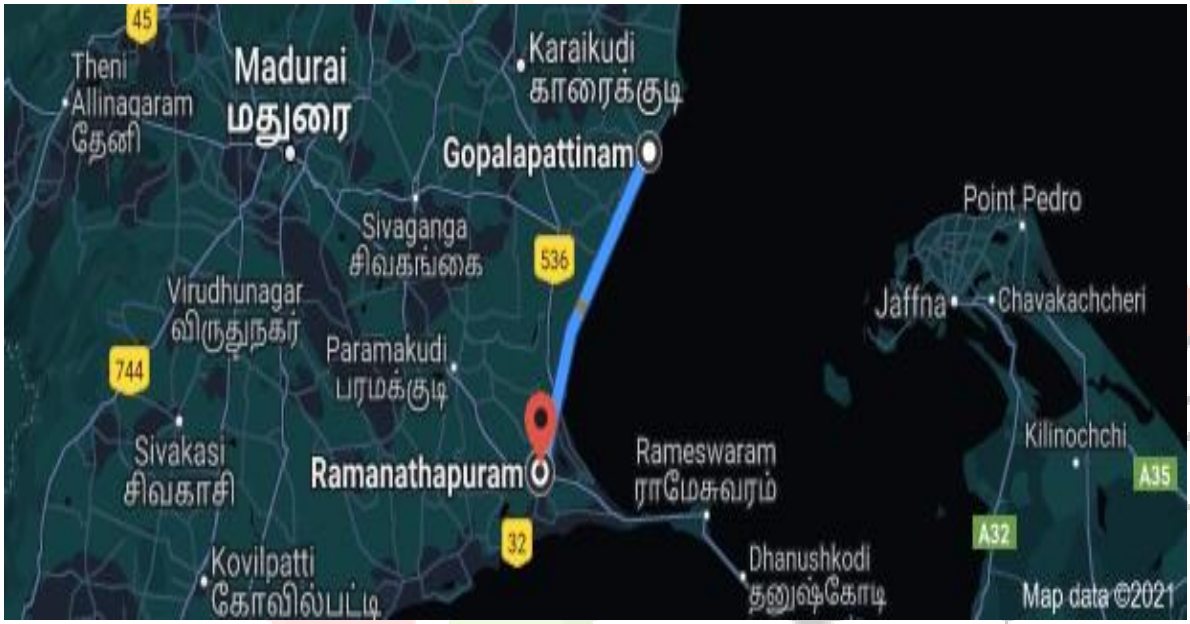
Seaweed provide home and food for many sea animals and also adds beauty to the underwater landscape. They are also useful to humans by serving as a raw material in food industries. They are the important source of bioactive materials. Seaweeds have recently received significant attention for their potential as natural antioxidants. In Asian countries, fresh seaweeds are used in the food diets and also been as a traditional remedy. Scientists are researching in the field of biologic drugs which are cheaper than the existing chemical drugs. (Graham et al., 2009)

2. MATERIALS AND METHODS

Fig 2.1 collection & preparation of seaweed extract:

The sample Eucheuma spinosum were collected from intertidal zone of Gopalapattinam, ramanathapuram district (Lat. 9.9248° N; Lon. 79.1506° "E) of Tamil Nadu, India. The collected sample was cleaned with seawater to remove the epiphytes and sand particles. The sample has been packed in a polythene bag and brought to laboratory. Then it was washed with fresh water and shade dried. The shade dried sample is stored and preserved for further use. Preparation of extract petroleum ether extraction the seaweed was collected and dried it for 1 day, after the completion of drying; 50 g of seaweed is measured accurately and pulverize it gently. After pulverizing, add the seaweed in a conical flask and add 150 ml of petroleum ether to the added seaweed and place it in the orbital shaker for 24 h at 32°C in room temperature. After squeezing, the solvent was taken out, and the extraction liquid is kept ready for the filtration process. The extracted liquid was filtered by Whatman filter paper. The extracted sample was condensed using Soxhlet extractor at 50°C and stored for further use Eucheuma spinosum were collected and washed thoroughly with distilled water. The seaweed were air dried and then powdered. The extracts was filtered and evaporated under reduced pressure using Rotavapor.

Figure 2.2: Location of the Sample.



2. Qualitative Phytochemical Screening:

2.2.1 Test for Alkaloids:

To 2 ml of extract and 2 ml of concentrated hydrochloric acid was added to it. Following them few drops of Mayer's reagent were added to it. When there is presence of green color or white precipitate then it indicates the presence of alkaloids.

2.2.2 Test for Flavonoids:

To 2 ml of extract and 1 ml of 2N sodium hydroxide was added. When there is presence of yellow color then it indicates the presence of flavonoids.

2.2.3 Test for Saponins:

2 ml of extract and 2 ml of distilled water were added and then its shaken in a graduated cylinder for 15 min. It resulted in the formation of foam which indicated the presence of saponins.

2.2.4 Test for Tannins:

1 ml of extract and 2 ml of 5% ferric chloride was added. When there is dark blue or greenish black color formation then it indicates the presence of tannins.

2.2.5 Test for Phenols:

2 ml of distilled water followed by few drops of 10% ferric chloride was added to 1ml of the extract. When there is formation of blue or green color then it indicates presence of phenols.

2.3 Antimicrobial Activity by Agar Well Diffusion

The agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

2.4 Antioxidant Test:

2.4.1 Hydrogen peroxide scavenging activity:

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4).

Different concentrations (100,200,300,400 and 500µg/mL) of the synthesized compounds (or ascorbic acid as the control) were added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The hydrogen peroxide percentage scavenging activity was then calculated using the following equation:

Inhibition (%) = [1-(Abs sample/Abs control)] x 100

The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against various concentration of the extract used.

2.4.2 DPPH

The percentage of antioxidant activity (AA%) of each substance was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams *et al.* The samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of adding 0.5 mL of sample, 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV-VIS spectrophotometer (DU 800; Beckman Coulter, Fullerton, CA, USA). The mixture of ethanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA%) was determined according to (Mensor et al.,2012)

$$AA\% = 100 - \left[\frac{(Abs_{sample} - Abs_{blank})x100}{Abs_{control}}\right]$$

2.5 Formulation of serum:

A face serum preparation was made using components such as vitamin E oil, rose water, glycerin. All these components are mixed making it a Soap Base and then Essential oil is added.

COMPONENTS	ROLE	QUANTITY
Vitamin E oil	Anti-aging property & Treat acne scarring	3ml
Glycerin	Relieve dryness & hydration	1ml
Seaweed extract	Antioxidant, anti-wrinkling, wound healing property &anti-aging.	6.5ml
Rose water	clean pores & tone the skin, helps to strengthen skin cells	4ml
Preservative	Either kill or prevent the micro-organism.	0.5ml

Components of the face serum.

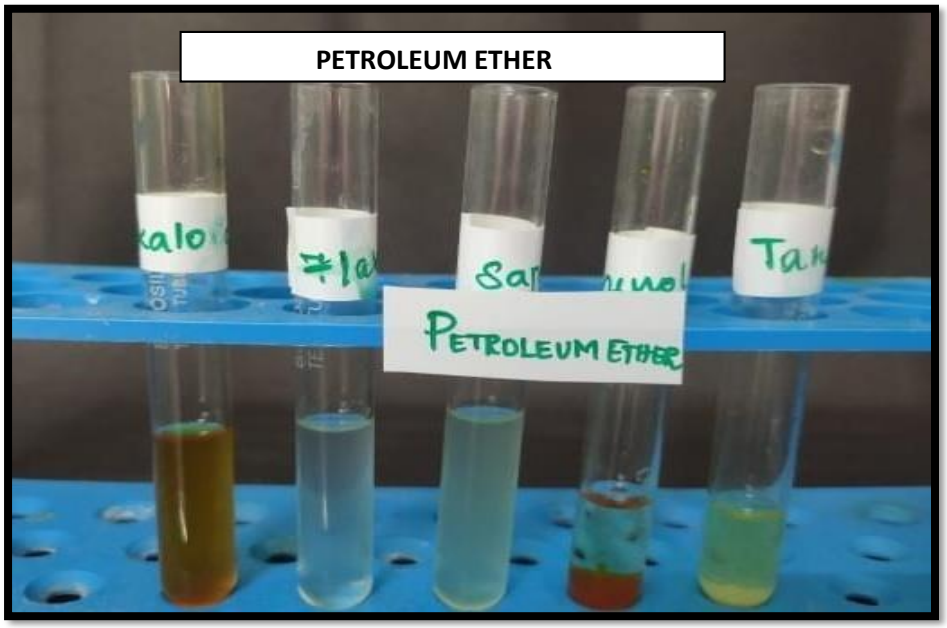
3.RESULT& DISCUSSION

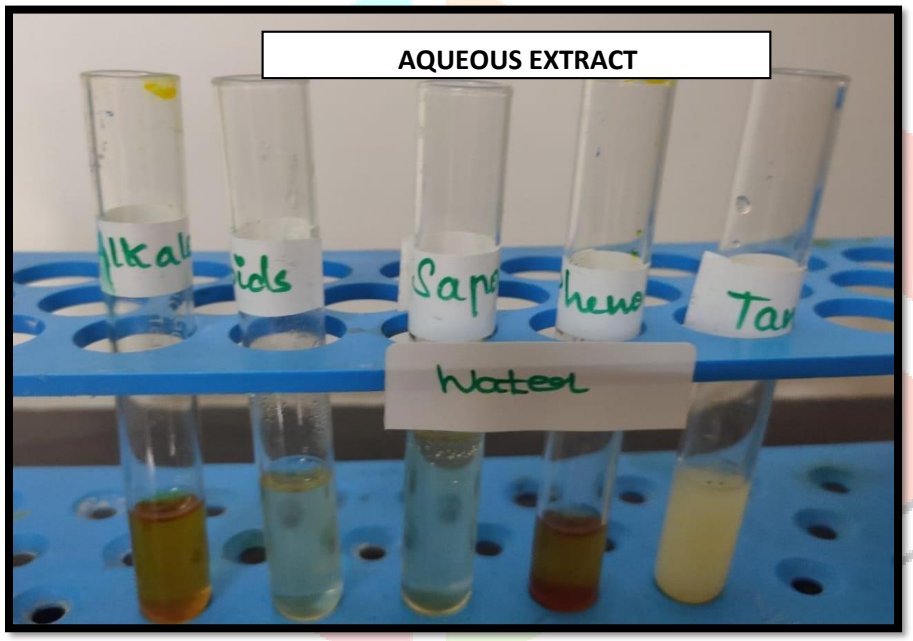
3.1 Phytochemical analysis of the petroleum ether Extract:

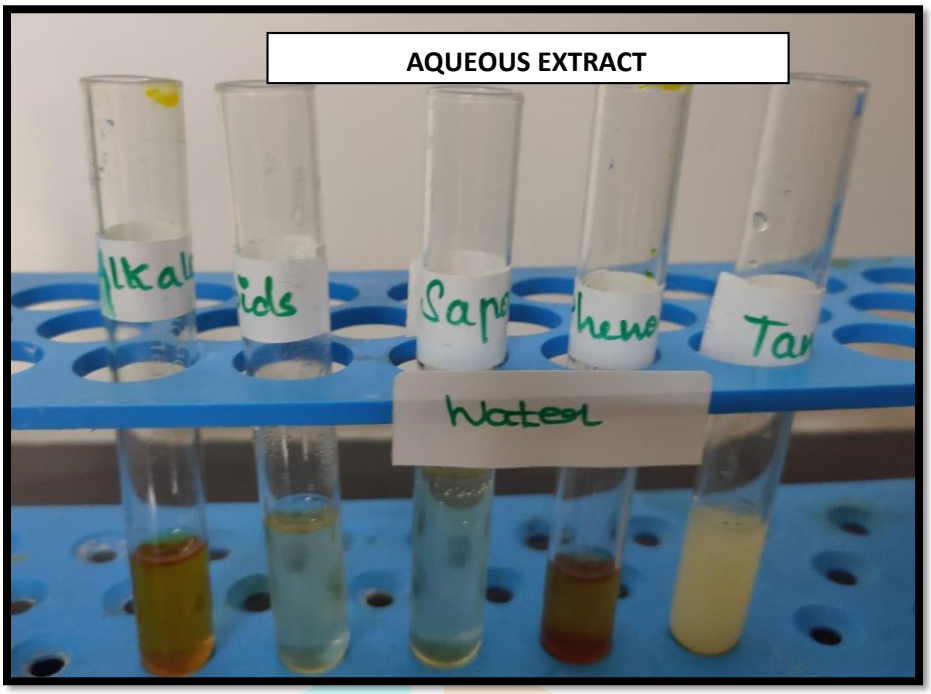
The results of phytochemical analysis of Petroleum ether, Aqueous, Methanolic extract of *E. spinosum*are in tabulated in Table (5.1).

PHYTOCHEMICAL COMPOUNDS	PETROLEUM ETHER EXTRACT	AQUEOUS EXTRACT	METHANOL EXTRACT
Alkaloids	+	+	-
Flavonoids	+	+	+
Saponins	+	-	-
Tannins	+	+	+
Phenols	+	-	-

Qualitative phytochemical screening of petroleum, aqueous & methanol extract of *E. spinosum.*

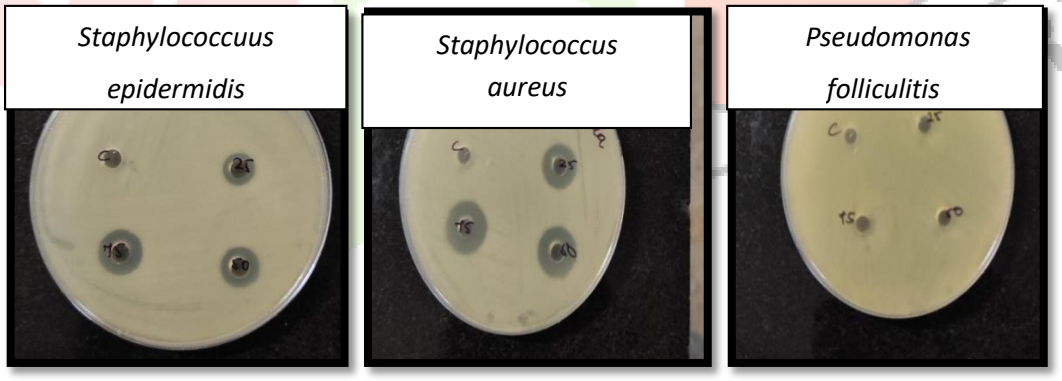

PETROLEUM ETHER
PETROLEUM ETHER

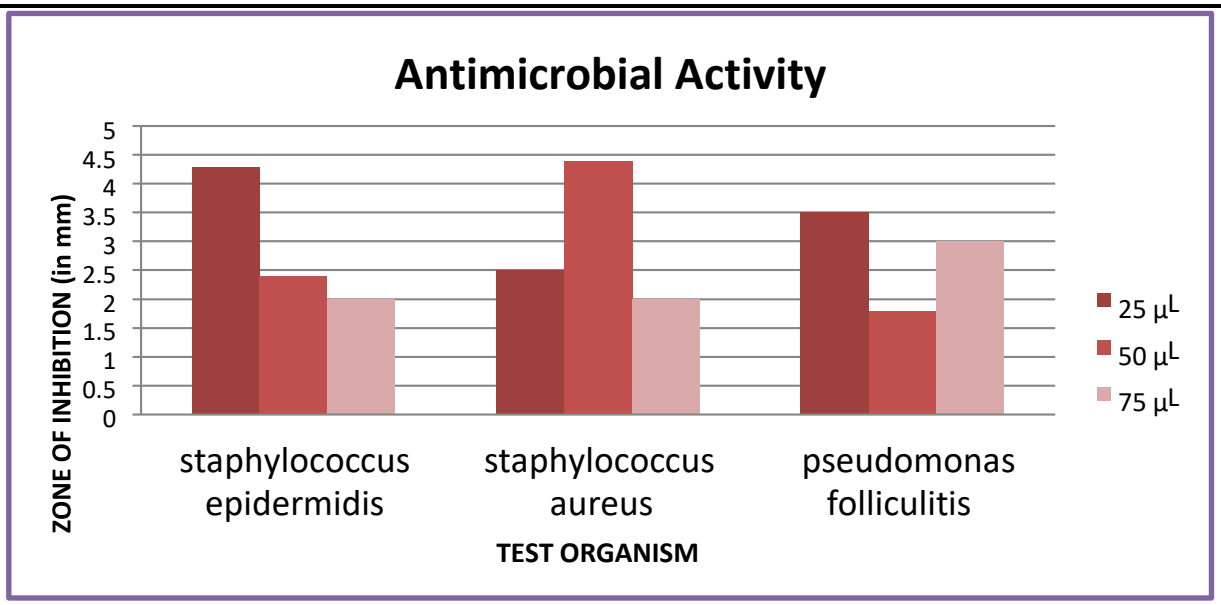

AQUEOUS EXTRACT
Water



3.2: Antimicrobial analysis of the Petroleum ether *Eucheuma spinosum* Extract:

The petroleum ether extract of the *Eucheuma spinosum* were studied for their Antimicrobial properties and their results were reported in terms of size of zone of inhibition produced by extract against the test organism.



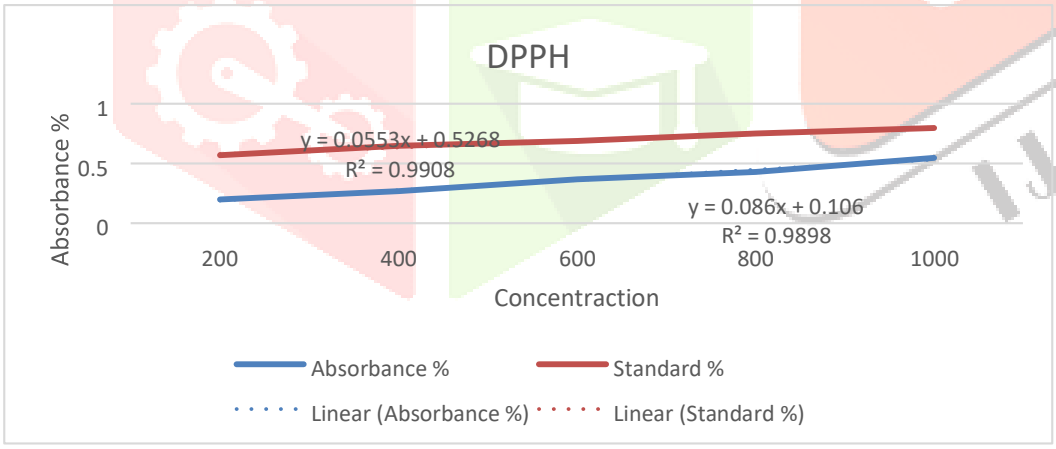


graphical representation of antimicrobial activity of petroleum ether extract of *E. spinosum.*

3.3Antioxidant Analysis

3.3.1 DPPH Assay:

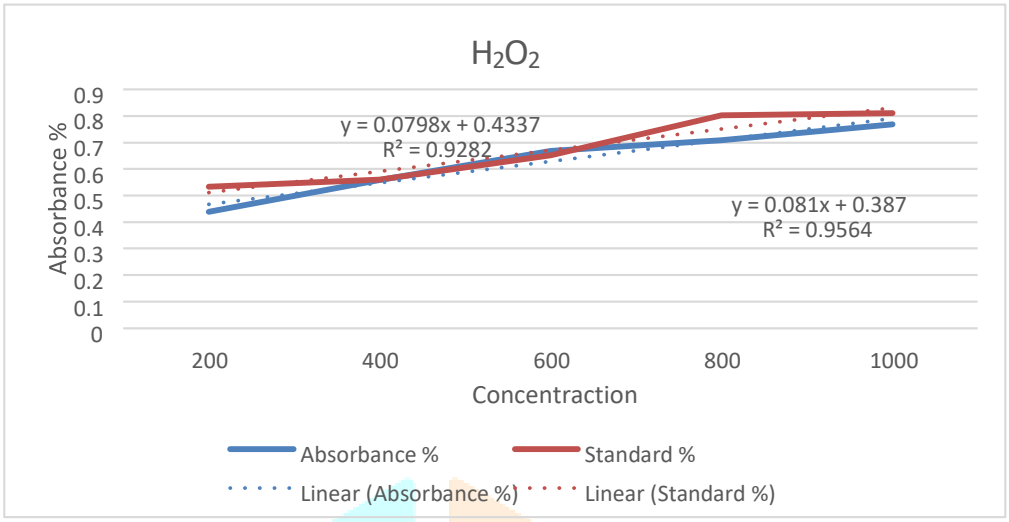
The Antioxidant activity of the *Eucheuma spinosum* extracts was measured using the scavenging activity of the stable DPPH free radical. It shows the antioxidant activity increases as the concentration of crude petroleum ether extract increases.



Graphical Representation of Antioxidant Activity by DPPH assay.

3.3.2: H_20_2Assay:

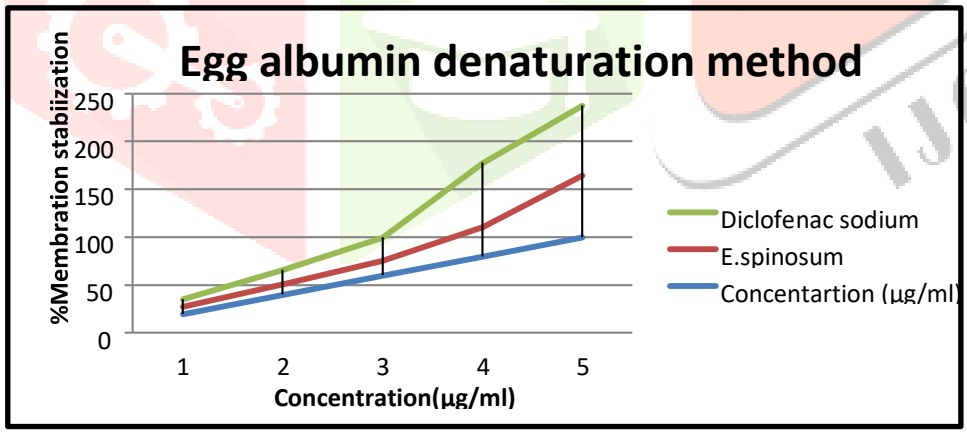
The Antioxidant activity of the *Eucheuma spinosum* extracts was also analysed on the basis of the Hydrogen Peroxide Scavenging by the petroleum ether extract.



Graphical representation of the Antioxidant Activity by H_2O_2 Scavenging assay.

3.4 Anti-inflammatory Activity:

In vitro anti-inflammatory activity of Petroleum ether extract of by egg albumin denaturation method at concentration of (20µl-100µl) compared with standard drug Diclofenac showed inhibition of (Fig 5.6) egg albumin denaturation method.



Graphical representation of the anti-inflammatory observed by Egg Albumin Denaturation method.

3.4 Characterization of the Product:

The result of formulation of face serum is presented below. The overall evaluation report reveals an ideal tropical formulation containing extracts of *Eucheuma spinosum*

PARAMETER	**OBSERVATION FOR SOAP**
Appearance	Liquid
Color	Transparent yellow
Odour	Pleasant
Viscosity	Medium
Ph	6

Evaluation of the formulated cosmetics.

FACE SERUM

4.CONCLUSION

The present study was characterized and analyzed the petroleum ether extract of *Eucheuma spinosum.* The extract was subjected to various phytochemical, antimicrobial, antioxidant and anti-inflammatory analyze to determine its cosmetic potential.

5.ACKNOWLEDGMENT

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