IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

DETERMINING THE COSMETIC POTENTIAL OF *Graciliria edulis* FOR THE FORMULATION OF SOAP

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Abstract: Seaweed is that the name given to species of marine plants and algae that grow in oceans, rivers, and lakes. Seaweed is probably the foremost helpful and abundantly available natural ingredient for skincare on the plant. Seaweeds are natural sources of a number of bioactive molecules that might be used as practical ingredients in many industrial applications, such as functional food, cosmetics and pharmaceutical industries. These organism area unit are exceptionally made of rich sources of bioactive molecules and secondary metabolites, like amino acids, peptides, proteins, polyphenols and polysaccharides. The present study characterised and analyzed the methanolic extract of *G.edulis*. The extract was subjected to varied phytochemical, antimicrobial, antioxidant, anti-inflammatory and anti-toxicity to see its cosmetic potential. Additionally, a group of phenol compounds found in seaweeds are referred to as phlorotannis, that functions as polymers of phloroglucinol are reported to act as robust antioxidant properties and their free radical scavenging ability is more powerful than that of different polyphenols compared to terrestrial plants. Because of huge variety of bioagents that posses Antimicrobial and Antioxidant properties, natural extracts have found their means into cosmetic product formulation.

Keywords: **Seaweed, *Graciliria edulis*,Phytochemical properties,Antiinflammatory response, Antioxidant properties, Cosmetic products.**

I. INTRODUCTION:

Biologically active substances are often known and extracted from a awfully heterogenous group within the oceans, the marine organisms, that have excellent reservoirs for this. Seaweed (or microalgae) are aquatic photosynthetic organisms belongs to the Eukaryotic domain and to the plantae (green and red algae) and Chromista (brown algae) kingdoms, respectively.(Leonel Pereira 2018) In cosmetics industries the skin has traditionally been used for the topical delivery of compounds, being a dynamic, complex, integrated arrangement of cells, tissues, and matrix parts that regulates body heat and water loss, while preventing the invasion of toxic substances and microorganisms.(Zubia *et al.*,2009)

Seaweeds are extravagantly found in oceans and have, therefore, drawn extended attention and interest. Additionally, seaweeds are noted for their richness in polysaccharides, carotenoids, dietary fibre, minerals, vitamins and other macro molecules like proteins, carbohydrates, lipids, essential fatty acids, and non-essential amino acids and polyphenol (Cheong *et al.*,2018, Khan *et al.*,2020).

The world population continues to grow, although a slower rate than within the recent past, and is predicted to succeed in 9.7 billion by 2050. Aging may be a natural and progressive declining physiological method, and oxidative stress incorporates a substantial role in aging, and several studies have prompt totally different mechanisms by which free radicals will injury biological systems, resulting in the development of chronic disease, cardio vascular disease, skin damage and several types of cancer. A group of phenol compounds found in seaweed has the high phenol compounds that possess antimicrobial and antioxidant properties, natural extracts have found their means into cosmetic product formulation. (Ahn *et al.*,2007)

II. OBJECTIVE

- Attain *Gracilaria edulis* red algae from sippikulam,Tuticorin,Tamilnadu.
- Phytochemical analysis of *Gracilaria edulis* extracts
- Antimicrobial evaluation of *Gracilaria edulis* extracts
- Analysis of free radical scavenging potencial of *Gracilaria edulis*
- Anti-inflammatory and cute toxicity evaluation of *Gracilaria edulis* on *Poecilia reticulat* fish
- Formulation of soap using *Gracilaria edulis* extract

III. MATERIALS AND METHODS

3.1 Sample Collection and Processing

The sample *Gracilaria edulis* (Red algae) were collected from intertidal zone of sipikulam, Tuticorin. (Lat. 8.9972° N; Lon. 78.2512°19E) of southeast coast 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, the sample was washed with fresh water and shade dried and stored for further uses.

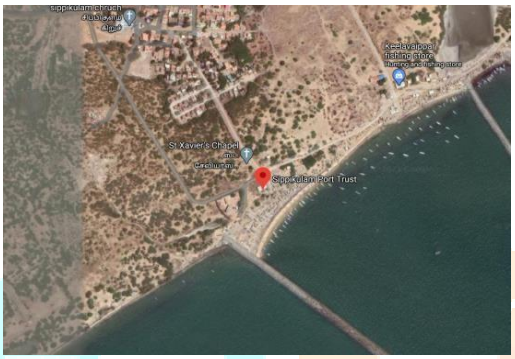

Figure 1. Location of the algal sample collection

Figure 2. Dried algal sample of *Gracilaria edulis*

3.2 Preparation of sample

After the completion of drying; 50 g of seaweed is measured and add 150 ml of methanol 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 extraction liquid was filtered by using Whattman filter paper. The extracted sample was condensed using Soxhlet extractor at 50°C and stored for further use.

3.3 Phytochemical analysis

The obtained crude extracts were subjected to preliminary phytochemical screening following the methodology of Harborne (1998) and Kokate (2001).

3.3.1 Test for alkaloids

To 5 ml of the crude extract 2 ml of hydrochloric acid was added. 1 ml of Dragendroff's reagent was added to this acidic medium. An orange or red precipitation was immediately produced which indicates the presence of alkaloids.

3.3.2 Test for flavonoids

To 1 ml of the crude extract, a few drops of dilute sodium hydroxide was added. An intense yellow colour appeared in the plant crude extract, which became colourless on the addition of a few drops of dilute acid which indicates the presence of flavonoids.

3.3.3 Test for saponins

The crude extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 min. The formation of 1 cm foam layer showed the presence of saponins.

3.3.4 Test for phenols

0.5ml of the extract was treated with 5% ferric chloride and the formation of deep blue or black colour indicates the presence of phenols.

3.3.5 Test for tannins

3ml of the extract and a few drops of 1% lead acetate were added. A yellow precipitate was formed indicates the presence of tannins.

3.4 Antibacterial activity

The antibacterial activity of *G. edulis* was tested against various Gram positive and Gram-negative strains using agar well diffusion technique with *Bacillus subtilusspizizenii, K. pnueumonia, Methyicillin resistant staphylococcus aureus (*MRSA) bacterial culture were swabbed is used to see the antibacterial activity. The well was punctured with a well cutter. The antibacterial activity was carried out using different concentrations such as 25 µL, 50 µL, and 75 µL, crude extract of *G. edulis* with the positive control value of 21 µL Zentamycin was used. These were allowed to dry under aseptic condition and incubated at 37°C for 24 h. The diameters of a clear zone (mm) was measured as antibacterial activity. (Vallinayagam., *et al* 2009).

3.5 In-vitro Antioxidant Activity

Method used for antioxidant activity were Hydrogen peroxide scavenging activity and DPPH assay (Ganesan *et al.*,2003)

3.5.1 Hydrogen peroxide scavenging activity

- **Principle**

The principle of this method is that there is a decrease in absorbance of H_2O_2 upon oxidation of H_2O_2.

- **Procedure**

The hydrogen peroxide scavenging was determined according to the method of Ruche et al. (1989). A solution of H_2O_2 was prepared in phosphate buffer and their concentration was determined spectrophotometrically from the absorption at 230 nm. Various concentrations *of Gracilaria edulis* (methanolic extract) were added to H_2O_2 and incubated for 10 min. The absorbance at 230 nm was determined against a blank containing phosphate buffer without H_2O_2. The percentage scavenging of H_2O_2acid was calculated using the formula,

$$\textbf{Inhibition (\%)} = A_0\text{-}A_1/A_0 \times 100$$

Where A_1 sample is absorbance of the test sample *Gracilaria edulis* (methanolic extract), A_0 control is absorbance of the control. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against various concentration of the extract used.

3.5.2 DPPH free radical scavenging activity

- **Principle**

DPPH is 2,2-diphenyl-1-picrylhydarzyl, a stable free radical scavenged by antioxidants from the extract through the donation of a proton to form reduced DPPH. The complete reduction of a proton to form reduced DPPH and the complete reduction reaction of this stable free radical is indicated by change in colour from purple to pale yellow. This reaction is spectrophotometrically measured at 517 nm.

- **Procedure**

The free radical scavenging activity of the *Gracilaria edulis* (methanolic extract) was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (Blois, 1958). Solution of DPPH in ethanol (0.1 mM) was prepared and 1.0 ml of this solution was added to 2.0 ml of *Gracilaria edulis* (methanolic extract) extract at different concentrations (100–500 µg/ml). After, thirty minutes, the absorbance was measured at 517nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

(AA%) = [control-sample]/control *10

3.6 Anti-inflammatory Activity

The anti-inflammatory analysis was performed by Protein Denaturation by Egg Albumin Method. The technique involved Mixture 0.2 ml of egg albumin (collected from fresh hen`s egg), phosphate – buffered saline (PBS, PH 6.4) 2.8ml and different concentrations (10,20,30,40,50µg/ml) of Diclofenac sodium (2ml each). Prepare a same volume with double-distilled water as control. Incubate the mixture at 37°C in BOD incubator for 15 min and heat at 70°C for 5 min. cool the solutions and measure absorbance at 660nm.Take the vehicle as blank. Calculate percentage of inhibition of denatured protein by following formula.

$$\%\ \textbf{inhibition of the sample} = \frac{(\mathbf{Vt - Vo})\mathbf{control} - (\mathbf{Vt - Vo})\mathbf{test}}{(\mathbf{Vt - Vo})\mathbf{control}}\ \mathbf{x\ 100}$$

The percent inhibition of edema for each group was calculated as:

Vt = paw volume after carageenan administration.

Vo = paw volume before carageenan administration.

The percentage of inhibition was carried out for different time interval.

3.7 Acute Toxicity

- **Principle**

The maximum loading of the sample for freshwater fish of 0.8 g wet weight fish/L for static and semi-static renewal testing is recommended. For flow-through systems, the recommended maximum loading is 0.5 g wet weight fish/L per 24 hours (example: in a 10 L tank with a flow rate of 5 tank volumes per 24 hours, a total of 50 L pass through the tank in 24 hours. With 25 g fish, this corresponds to 25 g in 50 L in 24 hours equivalent to 0.5 g/L in 24 hours). A loading not exceeding 5 g/L of solution at any time is recommended.

Another significant parameter is water temperature which should not differ by more than 2°C between test vessels or between successive days at any time during the exposure, and should be within the temperature ranges specified for the test species e.g. for zebra fish, Kapiswith a range of 21-25°C, the temperature selected could be 24°C and should not vary more than ± 1°C between test vessels and between successive days while staying in the recommended range of 21–25°C. The oxygen concentration should not be less than 60% of the air saturation value. Aeration can be used provided that it does not lead to a significant loss of test chemical as verified by analytical measurements of test concentrations. (Test Guideline No. 203 Fish, Acute Toxicity Testing.,2019).

3.8 Formulation of Soap

A cosmetic preparation was made using components such as Xanthum gum, bees wax, Shea butter, and coconut oil. All these components are mixed making it a Soap Base and then Essential oil is added.

Table 1. Components of the Soap.

COMPONENTS	FUNCTION	QUANTITY
Beeswax	Thickener	2 gm
Coconut oil	Foaming agent	3.5 gm
Xanthum Gum	Thickener	2 gm
Shea butter	Humectant	1 gm
Gracilaria edulis extracts	Antioxidant and Antimicrobial Agent	1 gm
Essential oil	Fragrance	Few drops

IV. RESULTS AND DISCUSSION

4.1 Phytochemical analysis

The Methanolic, Aqueous, acetone and chloroform extracts of *G. edulis* were subjected to phytochemical analysis. The analysis revealed that most-efficient extraction of the phytochemicals was found in the case of methanol extract. The pytochemical screening was carried out to find out secondary metabolite present in the Methanolic, Aqueous, acetone and chloroform extracts of G. edulis. The qualitative analysis of the extract confirmed the presence of alkaloids, flavanoids, phenols, tannins, and saponins. These results were found to be in harmony with the findings reported by Alireza *et al*, 2016. The detailed methods are described above and the results are also given in Table 2.

Table 2. The phytochemical analysis of *G. edulis* extracts

Phytochemical compounds	Methanolic extract	Aqueous extract	Acetone extract	Chloroform extract
Alkaloids	+	+	+	-
Flavonoids	+	+	+	-
Saponins	+	+	-	-
Tannins	+	+	-	+
Phenols	+	-	-	-

Key: (+) Present;(-) Absent.

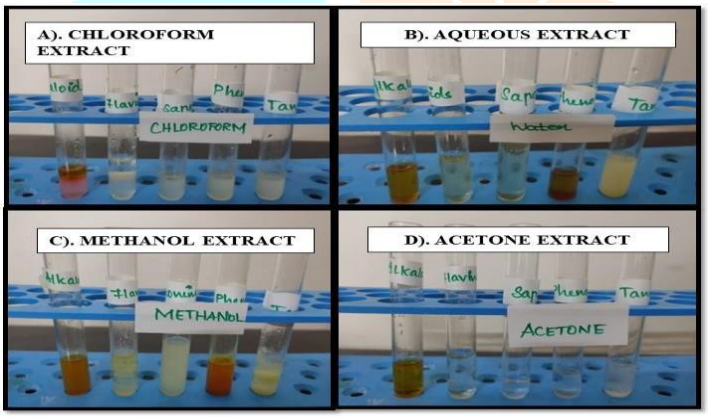

Figure 3. Phytochemical Analysis of *G. edulis* extracts

4.2 Antimicrobial analysis of *G. edulis* Methanolic Extract

Various extracts of *G. edulis* were subjected to antimicrobial analysis by well diffusion method. It was observed that acetone, chloroform and aqueous extract were not effective towards the tested pathogens. The methanolic extracts possessed promising activity against the tested bacteria and fungi with zone of inhibition ranging from 14 mm to 18 mm in diameter. Among the tested pathogens, the most susceptible bacterial strains were *Bacillus spizizini* with 28 mm in diameter of inhibitory zone. This result shows that the extract provides consistent antimicrobial activity as compared to that of the standard drugs.

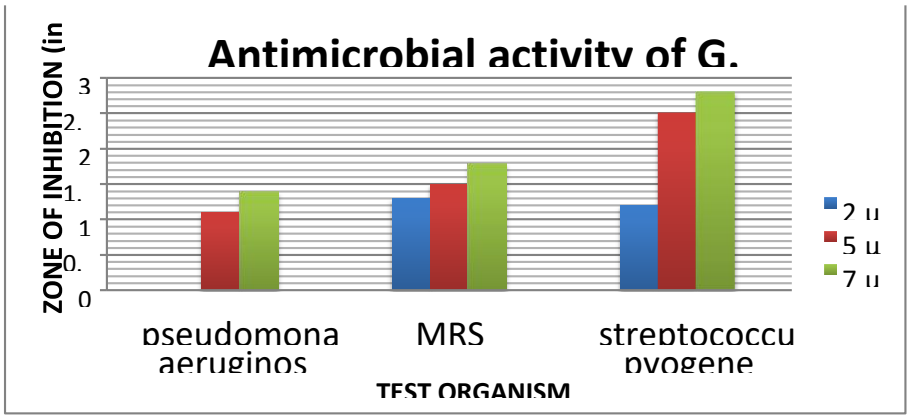

Figure 4. Graphical representation of the antimicrobial activity of the algal extract

Figure 5. Antimicrobial efficacy of *G.edulis*

4.3 Antioxidant Analysis

4.3.1 DPPH Assay

The Antioxidant activity of the *G. edulis* extracts was measured using the scavenging activity of the stable DPPH free radical. It shows the antioxidant activity increases as the concentration of crude Methanolic extract increases.

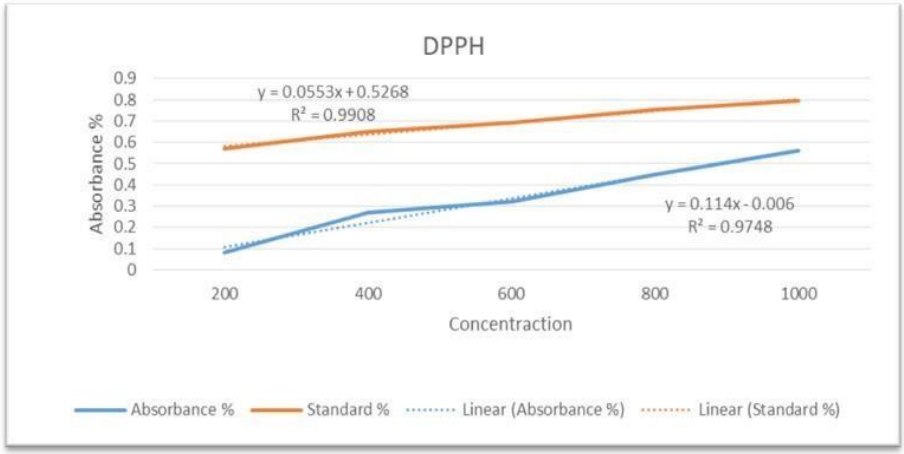

Figure 6. Representation of Antioxidant Activity by DPPH assay

4.3.2 Hydrogen Peroxide Scavenging Assay(H_2O_2):

The Antioxidant activity of the *G. edulis* extracts was also analysed on the basis of the Hydrogen Peroxide Scavenging of the methanolic extract.

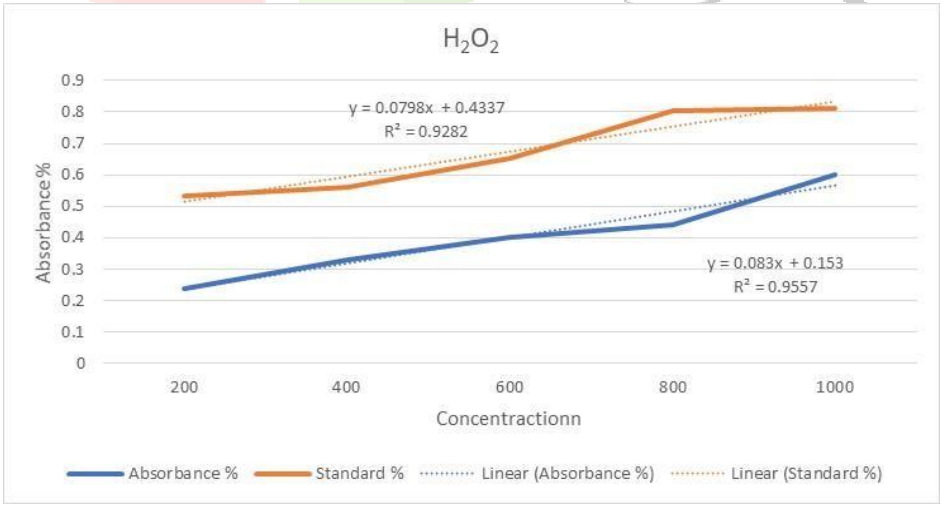

Figure 7 Graphical representation of the Antioxidant Activity by H_2O_2 Scavenging assay

4.4 Anti-inflammatory Activity:

In in vitro anti-inflammatory activity of methanol extract of *G. edulis* by Egg Albumin Denaturation Method at concentration of (20µl-100µl) compared with standard drug Diclofenac showed inhibition of (Fig.8) egg albumin denaturation method.

Table 3. Percentage inhibition of protein denaturation.

Concentration (µg/ml)	Methanolic extract of G. edulis	Standard Diclofenac sodium
20	5	8
40	8	15
60	14.7	24
80	28.7	67
100	63.7	73

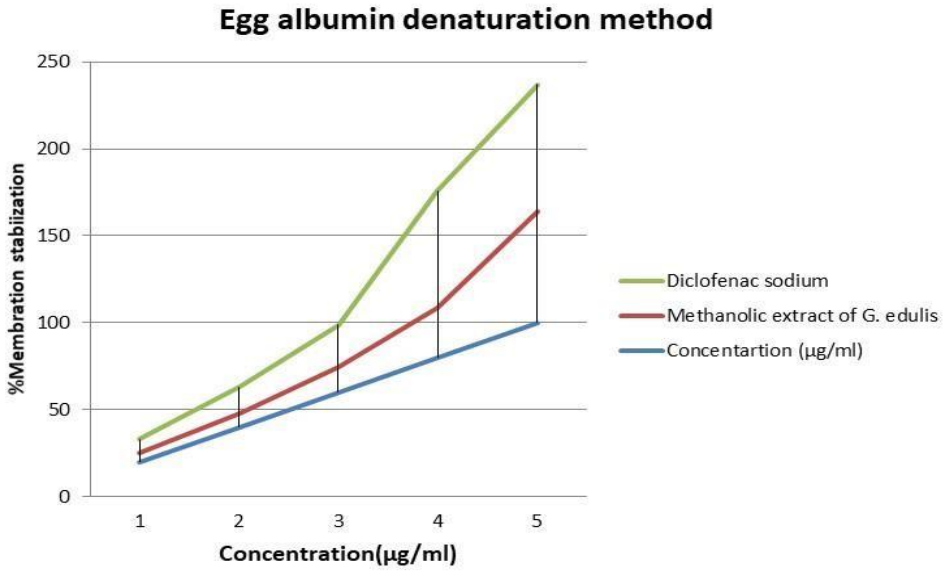

Figure 8. Graphical representation of the anti-inflammatory observed by Egg Albumin Denaturation method.

4.5 Anti-toxicity Analysis

The Anti-Toxicity analysis of the sample was conducted for two concentrations 50mg and 100 mg. The initial length and weight of the *Poecilia reticulata*were noted as 2.3 cm and 0.23gm respectively. The changes in the fish as a result of exposure to the sample were recorded and reported in the table below. The control for this experiment was taken as distilled water.

Table 4. Clinical signs observed after the administration of the sample 1 (50mg).

Study & Tank details									
Test day/ observation	Day 0, 2-3hrs	Day0, 5-6hrs	Day1 morning	Day1 afternoon	Day2 morning	Day 2 afternoon	Day3 morning	Day3 afternoon	Day 4 morning
Approximate observation from start	2.5 hrs	5.5 hrs	24 hrs	30 hrs	48 hrs	54 hrs	72 hrs	78 hrs	96 hrs
Date/time	10 March, 2021/ 11:21 am	10 March, 2021/ 02:21 pm	11 march 2021/ 10:00 am	11 march 2021/ 3:00 pm	12 march 2021/ 10:05 am	12 march 2021/ 3:00 pm	13 march 2021/ 10:30 am	13 march 2021/ 3:00 pm	14 march 2021/ 10:30 am
No. of live fish for scoring	7	7	7	7	7	7	7	7	7
No. of moribund removed after scoring	0	0	0	0	0	0	0	0	0
No. od dead removed	0	0	0	0	0	0	0	0	0
LAWS OF EQUILIBRIUM									
Abnormal horizontal orientation	-	-	-	-	-	-	-	-	-
Abnormal vertical orientation	-	-	-	-	-	-	-	-	-
Loss of buoyancy control	-	-	-	-	-	-	-	-	-
ABNORMAL SWIMMING BEHAVIOUR									
Hyperactivity	+	+	+	-	-	-	-	-	-
Hypoactivity	+	+	+	-	-	-	-	-	-
Corkscrew swimming	-	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-
Tetany	-	-	-	-	-	-	-	-	-
Irritated skin	-	-	-	-	-	-	-	-	-

behaviors									
Abnormal surface distribution	+	+	+	-	-	-	-	-	-
Abnormal bottom distribution	-	-	-	-	-	-	-	-	-
Over reactive to stimulus	+	+	-	-	-	-	-	-	-
Under reactive to stimulus	-	-	-	-	-	-	-	-	-
Loss of schooling	-	-	-	-	-	-	-	-	-
Dense schooling	-	-	-	-	-	-	-	-	-
ABNORMAL VENTILATORY FUNTION									
Hyperventilation	-	-	-	-	-	-	-	-	-
Hypoventilation	-	-	-	-	-	-	-	-	-
Irregular ventilation	-	-	-	-	-	-	-	-	-
Coughing	-	-	-	-	-	-	-	-	-
Gulping	-	-	-	-	-	-	-	-	-
Head shaking	-	-	-	-	-	-	-	-	-
ABNORMAL SKIN PIGMENTATION									
Darkening	-	-	-	-	-	-	-	-	-
Lightening	-	-	-	-	-	-	-	-	-
Mottled	-	-	-	-	-	-	-	-	-
OTHER VISIBLE ABNORMALITIES									
Oedema	-	-	-	-	-	-	-	-	-
Hemorrhage	-	-	-	-	-	-	-	-	-
Mucus secretion	-	-	-	-	-	-	-	-	-
Fecal cast	-	-	-	-	-	-	-	-	-
Aggression/cannibalism	+	+	-	-	-	-	-	-	-
If not listed above please describe.	-	-	-	-	-	-	-	-	-

Table 5. Clinical signs observed after the administration of the sample 2 (100mg).

Study & Tank details									
Test day/ observation	Day 0, 2-3hrs	Day0, 5-6hrs	Day1 morning	Day1 afternoon	Day2 morning	Day 2 afternoon	Day3 morning	Day3 afternoon	Day 4 morning
Approximate observation from start	2.5 hrs	5.5 hrs	24 hrs	30 hrs	48 hrs	54 hrs	72 hrs	78 hrs	96 hrs
Date/time	10 March, 2021/ 11:21 am	10 March, 2021/ 02:21 pm	11 march 2021/ 10:00 am	11 march 2021/ 3:00 pm	12 march 2021/ 10:05 am	12 march 2021/ 3:00 pm	13 march 2021/ 10:30 am	13 march 2021/ 3:00 pm	14 march 2021/ 10:30 am
No. of live fish for scoring	7	7	7	7	7	7	7	7	7
No. of moribund removed after scoring	1	1	1	1	1	1	1	1	1
No. od dead removed	1	1	1	1	1	1	1	1	1
LAWS OF EQUILIBRIUM									
Abnormal horizontal orientation	-	-	-	-	-	-	-	-	-
Abnormal vertical orientation	-	-	-	-	-	-	-	-	-
Loss of buoyancy control	-	-	-	-	-	-	-	-	-
ABNORMAL SWIMMING BEHAVIOUR									
Hyperactivity	+	+	-	-	-	-	-	-	-
Hypoactivity	+	+	-	-	-	-	-	-	-
Corkscrew swimming	-	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-
Tetany	-	-	-	-	-	-	-	-	-
Irritated skin	-	-	-	-	-	-	-	-	-

behaviors									
Abnormal surface distribution	+	+	+	-	-	-	-	-	-
Abnormal bottom distribution	-	-	-	-	-	-	-	-	-
Over reactive to stimulus	+	+	-	-	-	-	-	-	-
Under reactive to stimulus	-	-	-	-	-	-	-	-	-
Loss of schooling	-	-	-	-	-	-	-	-	-
Dense schooling	-	-	-	-	-	-	-	-	-
ABNORMAL VENTILATORY FUNTION									
Hyperventilation	-	-	-	-	-	-	-	-	-
Hypoventilation	-	-	-	-	-	-	-	-	-
Irregular ventilation	-	-	-	-	-	-	-	-	-
Coughing	-	-	-	-	-	-	-	-	-
Gulping	-	-	-	-	-	-	-	-	-
Head shaking	-	-	-	-	-	-	-	-	-
ABNORMAL SKIN PIGMENTATION									
Darkening	-	-	-	-	-	-	-	-	-
Lightening	-	-	-	-	-	-	-	-	-
Mottled	-	-	-	-	-	-	-	-	-
OTHER VISIBLE ABNORMALITIES									
Oedema	-	-	-	-	-	-	-	-	-
Hemorrhage	-	-	-	-	-	-	-	-	-
Mucus secretion	-	-	-	-	-	-	-	-	-
Fecal cast	-	-	-	-	-	-	-	-	-
Aggression/cannibalism	+	+	-	-	-	-	-	-	-
If not listed above please describe.	-	-	-	-	-	-	-	-	-

- Cumulative mortality at each concentration was reported as 1.4 per 10 population over the recommended observation times
- No mortality in the control
- The LC_{50} values at 24, 48, 72 and 96 hours with 95% confidence limits, if possible;

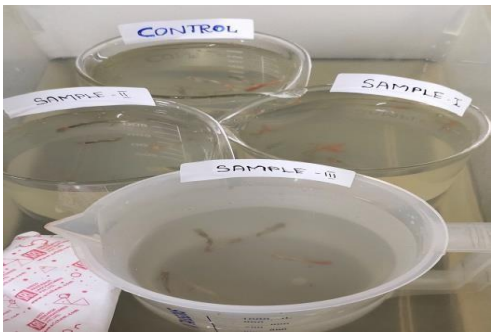

Figure 9. Acute toxicity test

4.6 Characterization of the Product:

The result of formulation of soap is presented in (Table 6). The overall evaluation report reveals an ideal tropical formulation containing extracts of *G. edulis.*

Table 6. Evaluation of the formulated cosmetics.

PARAMETER	OBSERVATION FOR SOAP
Appearance	Solid
Color	Green
Odor	Pleasant
pH	6.3
After feel	No residue left behind
Washability	Easily washed off when placed under running water
Moisture content (in percentage)	9.94%
Total Fatty Matter	0.17%

Figure 10. Seaweed soap

V. CONCLUSION

The present study characterized and analyzed the methanolic extract of *G. edulis*. The extract was subjected to various phytochemical, antimicrobial, antioxidant, anti-inflammatory, and anti-toxicity to determine its cosmetic potential. Due the vast variety of bioagents that possess Antimicrobial and Antioxidant properties, natural extracts have found their way into tropical formulation.

The pytochemical screening was carried out to find out secondary metabolite present in the *G.edulis* extracted with various solvents. The qualitative analysis of the leaf extract confirmed the presence of alkaloids, flavanoids, phenols, tannins, and saponin in the methanol extract. The analysis also revealed poor extraction of metabolites in case of aqueous, chloroform and acetone.

Seaweed extract of *G.edulis* were subjected to antimicrobial analysis by well diffusion method. It was observed that acetone, chloroform and aqueous extract were not effective towards the tested pathogens. The methanolic extracts possessed promising activity against the tested bacteria with zone of inhibition ranging from 18 mm to 22 mm in diameter. Among the tested pathogens, the most susceptible bacterial strains is *Bacillus spizizenii* with 21 mm in diameter of inhibitory zone. This result shows that the seaweed extract provides consistent antimicrobial activity as compared to that of the standard drugs.

The antioxidant study carried out showed potent activity of the extracts. The DPPH, Hydrogen peroxide, activity was performed and determined the IC_{50} values for each assay and were compared with the standard ascorbic acid. The results indicate that methanolic *G.edulis* extract of have potent antioxidant activity by scavenging ability observed against different activity of the methanolic extract .

Anti-inflammatory was studied using egg albumin protein denaturation technique. The results were reported as percentage membrane stabilization with corresponding to the concentration.

The Acute toxicity evaluation of *Gracilaria edulis* was performed on *Poecilia reticulat* fish respectively. The changes in the fish as a result of exposure to the sample were recorded and reported with no mortality rate.

Based on the result observed the extract was deemed to be suitable for the formulation of soap. The soap was characterized based on the color, appearance, pH, odour, tfm, moisture, density.

VI. ACKNOWLEDGEMENT

The authors are thankful and gratefully acknowledge to our college funding department DBT-Star scheme, DSTFIST scheme, and to the management of Dr.N.G.P. Arts and science college, Coimbatore, Our college principal, Deans of Dr. N.G.P. Arts and science college, Coimbatore as well as all faculty members and our guide, Department of Microbiology, Dr.N.G.P. Arts and science college, Coimbatore for providing constant support for the entire work (Communication number:Dr.N.G.P.Asc BS070).

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