STUDIES ON SKIN PROTECTION PROPERTIES OF CAULERPA PELTATA.

ABSTRACT:-

Skin is the largest organ of the body. It plays a major role in protecting the body from environment stresses. Lack of melanin and exposure to intense UV rays both raise the risk of skin cancer. UVA penetrates the epidermis and dermis deeply. UV light causes the generation of reactive oxygen species (ROS). The UV protectants are prepared from the natural resources like marine sea weeds and secondary metabolites of fungi etc. Algae have a long history of producing lengthy fossils. biologically active photoprotective substances exhibit wide range of UV absorbing property, antioxidant capacity, matrix-metalloproteinase inhibitors, anti-ageing and immunomodulatory activities. UV spectrum was evaluated in the UV region (200–400 nm) using UV-Vis spectrophotometer (Shimadzu 1800,Japan) and The ultraviolet spectra of methanolic extract of *C.peltata* (500 µg/mL) in the wavelength range of 200–400 nm. The FTIR spectrum was utilised to identify the functional group of the active components present in the methanolic extract of the selected green seaweed based on the peak value in the infrared radiation region. The antibacterial activity of methanolic extract of *C.peltata* against *E.Coli, Salmonella typhi, Salmonella paratyphi, Pseudomonas aeroginosa*was investigated in this study at various doses (500µg/ml and 1mg/ml). The antioxidant activities of methanolic extract of *C.peltata* were shown to be related to its ability to scavenge hydroxyl and DPPH radicals in the DPPH radical assay. The Algae *Caulerpa peltata* shows inhibitory effect of tyrosinase enzyme which is a primary source of the production of melanin. It shows that *Caulerpa peltata* can be a prominent organic ingredient that can be used in skin lightening cosmetic product which can be a substitute for the harmful chemical products which causes severe side effects to skin.

Keywords: Skin,UV rays, Cancer, Anti-Tyrosinase, DPPH, Algae, Skin lightening, Anti-ageing

INTRODUCTION

The skin is the largest organ of the human body and plays a vital role in protecting the body from environmental stresses. Its major functions include regulation of body temperature, prevention of excessive loss of body fluids, and protection against the entry of toxic substances and microorganisms. The skin consists of three major layers, namely the epidermis, dermis, and hypodermis. The outermost layer, the epidermis, is composed primarily of stratified squamous epithelium containing keratinocytes and melanocytes (Grice.,2011). The dermis is the second layer and consists of a thick fibrous connective tissue containing elastic fibres, fibroblasts, fibrocytes, blood vessels, and lymphatic vessels. Approximately 70% of the dermis is composed of collagen, which contributes to the strength and elasticity of the skin. The innermost layer, the hypodermis, primarily functions in energy storage and is composed mainly of adipocytes and connective tissue septa containing nerves and blood vessels (Gilaberte.,2016).

The epidermis is generally organized into five distinct strata: the basal, spinous, granular, lucid, and corneal layers. Melanin, the principal pigment responsible for skin colour, also provides an important protective function against solar and ultraviolet (UV) radiation (Brenner and Hearing, 2007). Melanocytes located in the basal layer of the epidermis produce melanin and contribute significantly to protection against UV-induced damage (Cives et al.,2020). Individuals with albinism, who have deficient melanin production, are particularly susceptible to premalignant and malignant skin lesions at an early age. The absence or reduction of melanin combined with prolonged exposure to intense UV radiation considerably increases the risk of skin cancer (McBride & Leppard, 2002).

Skin ageing is a natural process that begins from an early stage of life and becomes increasingly apparent with advancing age. The skin is one of the most visible organs in which the physiological effects of ageing can be observed. Consequently, considerable attention has been directed towards preventing and reversing skin ageing, particularly through cosmetic and pharmaceutical interventions (Kazansi et al., 2016) (Baumann L.,2007). Skin ageing can be defined as a combination of physiological, morphological, and histological changes that occur in the skin with advancing age (Wong et al., 2021). It can broadly be classified into intrinsic and extrinsic ageing. Intrinsic ageing is a natural consequence of the passage of time

and occurs independently of external influences, whereas extrinsic ageing results from environmental and behavioural factors. Among these factors, chronic exposure to sunlight is considered one of the most important contributors to extrinsic ageing, and photo-ageing is therefore often considered synonymous with extrinsic ageing (Review, 2008).

Intrinsic skin ageing resembles the gradual functional decline observed in other organs. During this process, the stratum corneum remains relatively unchanged, while alterations occur in the epidermis and dermis, including flattening of the dermo–epidermal junction (Jenkins, 2002). Normal skin colour is determined primarily by the presence and distribution of melanin, carotene, oxyhaemoglobin, and reduced haemoglobin within the dermis, epidermis, and subcutaneous tissues. Alterations in the concentration or distribution of these substances, or the accumulation of other pigments, can result in changes in skin colour (Lerner et.al.,1988).

Regulation of skin pigmentation can become dysregulated, resulting in various pigmentary disorders. Intracellular pH is an important regulator of tyrosinase (TYR) activity. The pH within melanosomes influences the catalytic activity of tyrosinase, while an appropriate pH gradient is also necessary for the sorting and delivery of melanosomal proteins. Even when melanocytes produce normal levels of functional tyrosinase, melanin production may remain low, suggesting that intracellular pH may contribute to the regulation of pigmentation in different skin types (Yamaguchi et al., 2007).

Skin pigmentation is broadly classified into two major types:

- Hyperpigmentation
- Hypopigmentation

Hyperpigmentation refers to an increase in melanin pigmentation and may occur under several physiological and pathological conditions, including suprarenal Addison's disease, pregnancy, and various debilitating illnesses. Hyperpigmentation involving the dorsal surfaces of the fingers and toes has also been reported in South Indian adults and infants and has been associated with vitamin B12 deficiency (Ignatius & Vit, 1963).

Hypopigmentation is characterized by a reduction in skin colour relative to the surrounding skin but does not represent a complete absence of pigment. It differs from depigmentation, which refers to the complete loss of pigmentation. Hypopigmentation may result from a reduction in melanocytes or melanin production or from decreased availability of tyrosine, an

amino acid required for melanin biosynthesis. Genetic alterations involving the tyrosinase or OCA2 genes are among the major causes. Since melanin is also present in the eyes and hair, hypopigmentation may affect these tissues as well (Shinkai 2018)(Ferrier 2017)(Bolognia 2014)(Cross 2017).

Chemical- and drug-induced hypopigmentation occurs when the melanin content of the skin is reduced. Except in cases involving arsenic exposure, such changes are generally associated with topical application or localized injection of chemicals or drugs. Several mechanisms have been proposed, including immune-mediated destruction of melanocytes in sensitized skin, free-radical-mediated melanocyte damage, inhibition of tyrosinase activity, interference with melanosome formation, and disruption of melanosome transfer to keratinocytes (Lerner et.al.,1988).

Melanin is a macromolecular pigment synthesized by melanocytes. Tyrosinase catalyses the initial and rate-limiting reactions of melanogenesis by converting tyrosine into dihydroxyphenylalanine (DOPA). Deficiency of tyrosinase activity is associated with albinism. Melanin occurs primarily in two forms: eumelanin, which is black or dark brown, and phaeomelanin, which is red or yellow. The biosynthetic pathways of these pigments diverge downstream of DOPA, with the melanocortin receptor MC1R signalling pathway contributing to the regulation of the eumelanin–phaeomelanin balance. Because several intermediates generated during melanogenesis are potentially toxic, melanin synthesis occurs within specialized organelles known as melanosomes, which are modified lysosome-related organelles (Cordero & Casadevall, 2020) (Mort et al., 2015).

Melanocytes are specialized pigment-producing cells that are responsible for skin and hair pigmentation. In addition to determining pigmentation, melanocytes contribute to photoprotection by absorbing ultraviolet radiation and reducing UV-induced cellular damage. Studies of pigmentation mutations have provided valuable insights into genetic and developmental mechanisms, photoprotection, cancer susceptibility, and human evolution. Understanding melanocyte biology may therefore contribute to the development of novel approaches for the prevention and treatment of pigmentation disorders and other skin diseases. Melanocytes possess the ability to withstand ultraviolet radiation (UVR) and survive associated genotoxic stress (Lin & Fisher, 2007).

Melanogenesis is the biological process responsible for the synthesis of melanin pigments, primarily by melanocytes. Melanocytes are neuroectoderm-derived dendritic cells that

originate from melanoblasts, which arise from embryonic neural crest cells. Following closure of the neural tube, melanoblasts migrate to different regions of the developing body and differentiate into melanocytes as well as contributing to certain cells of the peripheral nervous system, craniofacial bone and cartilage, and the choroid of the eye.

Melanoblast-derived melanocytes are predominantly located in the basal layer of the epidermis and hair follicles. They can be identified by melanocyte-specific markers, including tyrosinase (TYR), tyrosinase-related protein 1 (TYRP1), DOPA chrome tautomerase or tyrosinase-related protein-2 (TYRP2), and premelanosome protein (D'Mello et al., 2016)(Bonaventure, J., 2013)(Schadendorf, D., 2015).

Melanin biosynthesis begins with the conversion of L-tyrosine to dopaquinone (DQ), either directly or through L-dihydroxyphenylalanine (L-DOPA). Dopaquinone serves as a common substrate for both eumelanin and pheomelanin synthesis (Fig. 1). Tyrosinase plays a critical rate-limiting role in these oxidation reactions. In the presence of cysteine, dopaquinone reacts to form 3- or 5-cysteinyl-DOPA, which subsequently undergoes oxidation and polymerization to produce pheomelanin, a group of red-yellow pigments (Pillaiyar et al., 2017) (Yamaguchi.,2007).

Melanin is an indolic polymeric pigment found in vertebrates and is composed of covalently linked indole-derived molecules. Eumelanin possesses considerable redox activity and metal-chelating capacity and has been investigated as a potential target in anti-melanoma therapy. The structural organization of melanin also contributes to its stability. The degree of skin pigmentation is primarily determined by the amount and type of melanin produced. Increased melanin production results in hyperpigmentation, whereas reduced production results in hypopigmentation (Riley P.A.,1997). Melanogenesis involves several stages in the melanocyte life cycle, including the formation of melanoblasts from embryonic neural crest cells, migration and proliferation of melanocytes, differentiation of melanoblasts into mature melanocytes, melanin production within melanosomes, transfer of mature melanosomes to keratinocytes, and eventual cell death (Miroslawa.,2015).

Tyrosinase is a copper-containing enzyme that acts as the rate-limiting enzyme in melanin biosynthesis and is also involved in the enzymatic browning of fruits and vegetables. Consequently, the identification and screening of effective tyrosinase inhibitors are important for both cosmetic and food applications. Tyrosinase inhibitors are widely investigated as depigmenting agents and anti-browning compounds. Phenolic compounds can inhibit

158 tyrosinase activity by competing with its substrates. Algal extracts containing phenolic and
159 flavonoid compounds have demonstrated tyrosinase-inhibitory activity, indicating that
160 phenolic composition may contribute substantially to their anti-tyrosinase potential.
161 However, non-phenolic compounds may also possess anti-tyrosinase activity (Baek et al.,
162 2021).

163 Matrix metalloproteinases (MMPs) are extracellular endopeptidases produced by various cell
164 types, including fibroblasts, keratinocytes, mast cells, macrophages, and neutrophils, and they
165 play important roles in extracellular matrix degradation and skin ageing (Sanjeewa
166 et.al.,20160). The MMPs are broadly categorized into three major functional groups.
167 Interstitial collagenases include MMP-1, MMP-8, and MMP-13, which have affinities for
168 collagen types I, II, and III. Stromelysins include MMP-3, MMP-10, and MMP-11, which act
169 on laminin, fibronectin, and proteoglycans, whereas gelatinases such as MMP-2 and MMP-9
170 effectively cleave type IV and V collagen. Two phlorotannins, namely dieckol and 1-(3′,5′-
171 dihydroxyphenoxy)-7-(2′,4′,6′-trihydroxyphenoxy) 2,4,9-trihydroxydibenzo-1,4,-dioxin,
172 isolated from the methanol extract of the marine brown alga *Ecklonia cava*, have been
173 reported to suppress both the protein and gene expression levels of MMP-1, MMP-3, and
174 MMP-13 in human osteosarcoma cells (MG-63) (Thomas & Kim, 2013).

175 UV radiation is generally classified into three wavelength ranges: UVC (100–280 nm), UVB
176 (280–320 nm), and UVA (320–400 nm). UV radiation is mutagenic and has the potential to
177 act as both a tumour initiator and promoter. The harmful effects of UV radiation depend on
178 the intensity and duration of exposure and the availability of protective measures
179 (Elwood.,1993).

180 Polyphenols are plant-derived bioactive compounds that have attracted considerable attention
181 because of their diverse biological properties, including antioxidant, anti-inflammatory,
182 anticancer, and anti-melanogenic activities. These properties have increased interest in their
183 potential applications in skin protection and cosmetic formulations.

184 Algae represent a diverse and polyphyletic assemblage of photosynthetic organisms occurring
185 in both aquatic and terrestrial environments. They range from unicellular microalgae such as
186 *Chlorella*, *Prototheca*, and diatoms to multicellular forms such as giant kelps. Most algae are
187 aquatic and autotrophic and lack several specialized tissues characteristic of terrestrial plants,
188 including stomata, xylem, and phloem. Seaweeds represent the largest and structurally most

complex marine algae, while charophytes, including *Spirogyra* and stoneworts, represent some of the most complex freshwater algae (Butterfield, N. J. (2000)(T.M. Gibson (2018))).

Marine algae produce a wide range of bioactive compounds, including phenolic compounds that may be synthesized in response to different environmental conditions. These compounds have been associated with antioxidant, antibacterial, anti-inflammatory, anticancer, anti-ageing, and pro-mineralogenic activities and may also contribute to the modulation of cardiovascular disease risk (Gager et.al.,2021).

Algal polysaccharides possess diverse structural and functional characteristics and have considerable potential for cosmetic applications. Depending on their chemical structure, they may exist as anionic, cationic, non-ionic, or amphoteric polymers. Their properties are influenced by temperature, concentration, and the surrounding environment. Fucoidans from brown algae, carrageenans from red algae, and ulvans from green algae represent important naturally occurring algal polysaccharides. These compounds have numerous cosmetic applications and can function as rheology modifiers, suspending agents, hair conditioners, and wound-healing agents. They may also contribute to moisturizing, hydrating, emulsifying, and emollient properties (Goddard and Gruber,1999)(Wang et al., 2015).

Natural products have attracted increasing interest because of their potential to provide beneficial biological activities, including antioxidant and anti-allergic properties. Consequently, diverse natural resources are being explored for the discovery of commercially valuable bioactive compounds. Microbial diversity represents a particularly important but relatively underexplored biological resource capable of producing a wide range of commercially significant metabolites. Cultivable microorganisms, including bacteria, yeasts, fungi, and algae, produce several compounds with potential cosmetic applications. In skincare formulations, antioxidants, enzymes, peptides, and anti-collagenase and anti-elastase compounds have considerable potential (Gupta et.al.,).

Phlorotannins are important polyphenolic secondary metabolites commonly found in marine algae. They have been reported to possess anticoagulant, anti-melanogenic, antifungal, anti-inflammatory, and anticancer activities (Balboa et.al.,2013). Octaphlorethol A, a phlorotannin isolated from the marine alga *Ishige foliacea*, has demonstrated anti-inflammatory activity (Manzoor et.al.,2013) and anti-diabetic effects, which have been associated with modulation of glucose absorption and reduction of oxidative stress (Lee SH et.al.,2012) (Lee SH et.al.,2013). Several chemical compounds used in cosmetics, including linoleic acid, kojic

acid, naturally occurring hydroquinone, and catechol, have been reported to inhibit melanin formation (Zawar VP et.al.,). However, limitations associated with the clinical efficacy, adverse effects, and potential toxicity of some existing skin-lightening agents have stimulated the search for safer and more effective natural alternatives. Plant-derived compounds are therefore being explored as potential ingredients for the development of cosmetic formulations with reduced adverse effects (Roh JS et.al.,2004).

In recent decades, environmental and atmospheric changes have influenced the biologically active UV radiation reaching the Earth's surface. UV radiation is generally categorized into UVA, UVB, and UVC. UVA represents the longest wavelength range, while UVB occupies an intermediate range and UVC possesses the shortest wavelength and highest energy. Fortunately, UVC radiation is largely absorbed by the ozone layer and therefore does not normally reach the Earth's surface in significant amounts (Solano, 2020).

UVA penetrates deeply into the epidermis and dermis and, together with UVB, contributes significantly to photo-induced skin damage. Prolonged UVA exposure can cause skin ageing, wrinkling, sagging, UV-induced immunosuppression, and burns. UVB absorption by DNA can lead to the formation of cyclobutane pyrimidine dimers (CPDs), while UVA can contribute to oxidative stress and mutation formation. UV exposure can also induce rapid, persistent, or delayed pigmentation, with delayed pigmentation associated with increased tyrosinase activity and new melanin synthesis (Kaur et al., 2014).

UV radiation induces the generation of reactive oxygen species (ROS) and can deplete endogenous antioxidant defenses. The resulting oxidative stress may contribute to hyperpigmentation, accelerated skin ageing, dryness, and other skin-related disorders (Jesumani et al., 2019). Epidemiological evidence indicates that repeated exposure to sunlight, particularly from childhood, is a major risk factor for skin cancer. UVB represents a particularly potent component of solar radiation responsible for carcinogenesis in Fig 2 . UV-induced DNA damage, including the formation of cyclobutane pyrimidine dimers (CPDs) and related photoproducts, can result in mutations in epidermal cells and contribute to the development of cancer (Ichihashi et al., 2003).

The efficacy of sunscreen products is commonly expressed in terms of the sun protection factor (SPF), which is defined as the ratio between the UV energy required to produce a minimal erythema dose (MED) on sunscreen-protected skin and that required to produce the same response on unprotected skin.

SPF = Minimal erythema dose in sunscreen-protected skin / Minimal erythema dose in non-sunscreen-protected skin

The minimal erythemal dose (MED) is defined as the lowest dose or duration of UV irradiation sufficient to produce a minimally perceptible erythema on unprotected skin. A higher SPF generally indicates greater protection against UV-induced sunburn; however, standardized methods are required for accurate SPF determination (Dutra et al., 2004).

Substances that protect the skin against the damaging effects of UV radiation are generally referred to as UV protectants. Organic compounds derived from natural resources are of particular interest because of their UV-absorbing and antioxidant properties. Commonly used UV-protective compounds include benzophenones, para-amino benzoic acid (PABA), and cinnamates. Natural UV-protective compounds can also be obtained from marine seaweeds and secondary metabolites produced by fungi and other microorganisms.

Algae have a long evolutionary history and comprise numerous groups classified according to their pigmentation, morphology, physiology, and other characteristics. They are photosynthetic organisms capable of inhabiting both aquatic and terrestrial environments and produce a wide range of bioactive compounds with applications in several industrial sectors (Rastogi et al., 2017).

Seaweeds contain fibre, proteins, lipids, vitamins, minerals, and polysaccharides such as carrageenan and alginate. They are also rich in bioactive compounds, including carotenoids, steroids, terpenoids, phenolic compounds and their derivatives, flavonoids, coumarins, and vitamins, making them valuable sources of natural antioxidants. Based on their chemical structures, major algal pigments include chlorophylls, phycobilipigments, and carotenoids such as carotenes and xanthophylls (Rosaline et al., 2012) (El Gamal, 2010) .

Algae have received considerable attention as sources of natural photoprotective agents because of their unique bioactive compounds. Important algal photoprotective substances include mycosporine-like amino acids (MAAs), sulphated polysaccharides, carotenoids, and polyphenols. These compounds exhibit UV-absorbing properties and may possess antioxidant, matrix-metalloproteinase-inhibitory, anti-ageing, and immunomodulatory activities.

Methanolic extracts of seaweeds have demonstrated considerable antioxidant activity, particularly following prolonged incubation. Bioactive compounds obtained from algae have

also been associated with antibacterial, antioxidant, antifungal, anti-inflammatory, and antitumour properties. Fucosterol, isolated from several *Sargassum* species, including *Sargassum micracanthum, Sargassum tenerrium, Sargassum fusiforme, Sargassum horneri,* and *Sargassum linearifolium*, has been reported to possess antioxidant, antidepressant, anticonvulsant, and other biological activities (Ham et.al.,2010; Terasaki et.al.,2009; Zhen et.al. 2015;Majik et.al.2015;Perumal et.al.,2017).

Marine macroalgae have also demonstrated antibacterial activity against a broad range of human pathogenic microorganisms. Crude extracts obtained from numerous macroalgal species have been reported to inhibit the growth of harmful microorganisms (Rosaline et al., 2012). Similarly, the methanolic extract of *Caulerpa sertularioides* contains catechin-related compounds, including gallocatechin, epicatechin, and catechin gallate, and has demonstrated considerable antioxidant activity. The antioxidant defence properties of algae extend beyond lipid peroxidation inhibition and may also involve mechanisms such as metal-ion chelation (Santoso et al., 2004).

The present study aims to evaluate the skin-protective effects of *Caulerpa peltata* and to investigate its potential as a natural alternative to conventional chemical-based cosmetic ingredients.

MATERIALS AND METHODS:-

Collection of algal sample:-

The Algal sample was collected from Mandapam, Rameshwaram coast**.**

Identification of algal sample:-

The Identification of Algal sample was done by Botanical Survey of India, West Bengal, Kolkata.

Preparation of methanolic extract of selected algae :-

The collected algal sample was washed thrice with distilled water and allow it to dried in room temperature. The dried algal sample was made to powdered form and the extract was taken by adding Methanol. The Methanolic extract of *Caulerpa peltata* was then centrifuged at 7000rpm for 20 min. After that the supernatant from the centrifuge tube was allowed to dried for 3-4 days. After that the dried sample was taken by scraping out from the Petri plate.

For further phytochemical investigation, the extracts were collected and stored in the refrigerator at 4°C.

Yield of methanolic extract of *Caulerpa peltata* :-

The dried extract was weighed and its yield was calculated by following formula

$$\text{Yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of algae used for the study}} \times 100$$

QUALITATIVE ANALYSIS OF PHYTOCHEMICALS PRESENT IN ALGAE:-

Test for Carbohydrates:-

Anthrone test:-To 10ml of sulphuric acid 20mg of anthrone reagent was dissolved. 4ml of anthrone reagent prepared before was added to 1ml of methanolic extract of *Caulerpa peltata*. The appearance of green colour complex indicates the presence of carbohydrates.(Blenkinsopp, 1999).

Molisch test:-Add 2 drops of Molisch reagent (prepared dissolving 0.1g of ∞-naphthol in 2 ml of ethanol) to 2 ml of the methanolic extract of *Caulerpa peltata* to be tested and mix.Incline the tube, and gently add 2 ml of concentrated sulfuric acid down the side of the test tube. A purple colour at the interface between the sugar and acid indicates a positive result.(I. Elzagheid, 2018)

TEST FOR PROTEIN:-

Biuret test:-To 2ml of methanolic extract of *Caulerpa peltata* 2ml of 10% NaOH and 2 drops of 0.1% $CuSO_4$ solution was added. The appearance of pink or violet colour indicates the presence of protein.(Janairo et al., n.d.)

TEST FOR FAT:-

Dichromate test:-To 3ml of methanolic extract of *Caulerpa peltata* few drops of5% of Potassium dichromate solution and 5ml of Concentrated Nitric acid was added. The appearance of blue colour indicates the presence of lipids.(*Estimation of Lipids*, n.d.)

SOLUBILITY TEST:-

The solubility of the methanolic extract of *Caulerpa peltata* in various solvents was tested. The solvent used are; DI water, DI water: ethanol (ratio of 1:1, 1:2, 2:1), methanol and hexane. The methanolic extract of *Caulerpa peltata* was added in each solvent at the ratios of 1:10, 1:15, 1:20, 1:30 and 1:50. These were mixed with vortex mixer and kept at room temperature and 60°C for 10 min. Then, the tested solutions were observed for their solubility and compatibility.(Paper, 2014)

SPECTRAL CHARACTERIZATION:-

ULTRAVIOLET(UV) ANALYSIS:-

The extract was diluted in 100 µg/ mL methanol and its UV is measured in a UV-Vis spectrophotometer (Shimadzu 1800,Japan) against blank of methanol.(Bhuvana et al., 2019).

FOURIER TRANSFORM INFRARED SPECTROSCOPY(FTIR) :-

Fourier transform infrared spectroscopy (MODEL JASCO 4700; Wavelength range 4000nm to 400nm) was carried out to determine the functional group in the methanolic extract of *Caulerpa peltata*. The spectra were measured in the frequency range of 4000-400cm^{-1} using JASCO 4700 Fourier transform infrared spectroscopy.(Muthu Lakshmi and Subha., 2019)

Anti-bacterial activity of methanolic extract of *Caulerpa peltata*:-

The bacterial strains taken for the antibacterial activity are *Escherichia coli, Salmonella typhi, Salmonella Para typhi, Pseudomonas aeruginosa.*

Caulerpa peltata extract was taken in two concentrations(500µg, 1mg).Four plates were prepared for each concentration for each organism. Agar plates were sterilized by UV before performing the experiment.200µg of microbial samples were introduced . for each organism two plates were given for two concentrations. Discs are created in each Petri plates . 500µg concentration of algal sample were introduced in four Petri plates of four organisms. 1mg concentration of algal sample were introduced in four Petri plates of four organism. Incubate it for 24 hrs in room temperature .After 24hrs measure the zone of inhibition created surrounding the algal sample.

Anti-oxidant activity of methanolic extract of *Caulerpa peltata* by DPPH method:-

0.1 Mm solution of DPPH in methanol is prepared. 1ml of this DPPH(0.1mM solution) is added to 3ml of the solution of extracts in methanol at different concentrations of 50µg, 100µg, 200µg, 400µg, 800µg /ml. The mixtures were vigorously shaken and allowed to stand

at room temperature for 30 minutes. Then the absorbance was measured at 517nm using UV-VIS Spectrophotometer. Low absorbance value of reaction mixture indicates higher free radical scavenging activity.

DPPH scavenging activity (%) = $\{(A_0 – A_1)/A_0\} \times 100$,

Where.,

A0 is the absorbance of the blank (without extract) and

A1 is the absorbance of the test sample.

The IC50 values of the DPPH radical by the extracts were evaluated at 50% scavenging activity(Wu et al., 2018).

Anti-tyrosinase activity of methanolic extract of *Caulerpa peltata*:-

The tyrosinase inhibitory effect of methanolic extract of *Caulerpa peltata*was determined spectrophotometrically with the degree of inhibition of mushroom tyrosinase catalysed oxidation of L-DOPA. The reaction mixture contained 25mM phosphate buffer (pH 6.8) with or without sample and L-dopa (1.25mM). Then, tyrosinase (100 U/mL) was added into the mixture and the activity was determined by following the increase in absorbance at 475nm resulting from the formation of the dopachrome product. The inhibition of tyrosinase activity was calculated with the following formula:

Inhibition(%)=[1-(A_{475} in sample/A_{475} in control)]×100%

The mode of concentration was determined by different concentration of 0.2mg, 0.4mg, 0.6mg, 0.8mg, 1mg(Pintus et al.,2015).

RESULT AND DISCUSSION

COLLECTION OF ALGAE:-

The Algal sample was collected at Mandapam coast, Rameshwaram during the month of September 2021**.** The Fig 3 shows the satellite map of collected area.

Caulerpa species has a thick rhizome and compact clusters of rounded or flat-topped uprights. This algae thrives in low-light environments such as shadowed seawalls, under ledges, and in very deep water. It is an excellent nutrient consumer in the aquarium, to the point where it must be closely monitored to ensure that it does not outgrow the available nutrients. Its qualities vary greatly depending on where it is obtained. Although it's unclear whether they're

400 separate species, there are two unique types. The smooth spherical caps on one are different
401 from the concave shape with thin edges on the other. The uprights become significantly taller
402 in shallow water, resembling*Caulerpa racemosa.*

403

The classification of *Caulerpa peltata*

Kingdom: Plantae

Sub kingdom: Viridiplantae

Phylum: Chlorophyta

Subphylum: Chlorophytina

Class: Ulvophyceae

Order: Bryopsidales

Family: Caulerpaceae

Genus: *Caulerpa*

Species: *peltata*

Scientific Name: *Caulerpa var. peltata*

Common Name: Saucer Algae, Mushroom Algae

Origin: Gulf of Mexico, Atlantic, Caribbean

Depth Collected: 3-200 Feet

Maximum Height : 6″

Growth Rate: Fast

Light: Moderate

Temperature: 72-86

Propagation: Fragmentation, Sporulation.(Kronenwetter Russ.,2021)

IDENTIFICATION OF ALGAE:-The Identification of Algal sample was done by Botanical Survey of India, West Bengal, Kolkata. It is evident in Fig 4 that *Caulerpa*

chemnitzia (Esper) J.V.Lamouroux-Caulerpaceae (Family) Green seaweed (Synonyms – Caulerpa peltata J.V.Lamourox).

Habitat of *Caulerpa peltata*:-

Caulerpa peltata is also known as sea grapes shown in the Fig 5 thrives in low-light environments such as shadowed seawalls, under ledges, and in very deep water. A unique variety found in low-light environments such as reef ledges and deep-sea habitats. If given strong bright light, it will grow in tall strands, but if exposed to a strong current and a partially shaded habitat, it will stay more compact.(Macroalgae for Marine Reef Aquariums: C*aulerpa racemosa var peltata* (live-plants.com).The thallus of *Caulerpa peltata* is a horizontal, branched stolon which is 1-2mm in diameter and erect and rhizoids which are finely branched of height 1-5mm. Numerous disc like branchlets (1.5-2.5mm diameter) called blades and peltate was bear on stalk (1-2mm long) by *Caulerpa chemnitzia* (Esper) J.V.Lamouroux- (Synonyms – Caulerpa peltata J.V.Lamourox)(Trono, G.C. Jr. 2001).it grows on low intertidal and upper subtidal of dead coral colonies of 1-2m depth of shores which are moderately exposed(algaebase.,2015).These little umbrellas emerge along the length of a horizontal root that creeps over the surface. Some form a loose cluster. It appears in bright yellow-green to bluish green.

Yield of methanolic extract of *Caulerpa peltata*:-

The dry weight of algal sample was half kilo. The extract was taken by using methanol. The final yield of methanolic extract of *Caulerpa peltata*was 0.4%.

Physical appearance and pH of methanolic extract of *Caulerpa peltata*:-

The methanolic extract of *Caulerpa peltata*was dark green in colour, sticky in nature. The pH of methanolic extract of *Caulerpa peltata*was 6 which shows that it is acidic in nature.

Phytochemical analysis of methanolic extract of *Caulerpa peltata*:-

The results of phytochemical analysis of methanolic extract of *Caulerpa peltata* are shown in the Table1

TEST FOR CARBOHYDRATES:-

Anthrone test:-

The methanolic extract of *Caulerpa peltata* shows the appearance of green colour which indicates the presence of Carbohydrates.

Molisch test:-

The methanolic extract of *Caulerpa peltata* shows the appearance of purple colour at the interface between the sugar and acid which indicates a positive result of presence of carbohydrates.

TEST FOR PROTEINS:-

The methanolic extract of *Caulerpa peltata*does not shows the appearance of pink or violet colour which indicates the absence of protein**.**

TEST FOR FATS:-

The methanolic extract of *Caulerpa peltata* does not shows appearance of blue colour which indicates the absence of lipids.

Solubility test of methanolic extract of *Caulerpa peltata*:-

The methanolic extract of *Caulerpa peltata*soluble in solvents like distilled water, methanol, ethanol and hexane.

Spectral analysis of methanolic extract of *Caulerpa peltata*:-

UV SPECTROSCOPY:-

UV spectrum was evaluated using UV-Vis spectrophotometer (Shimadzu 1800,Japan) and the Ultraviolet spectra of methanolic extract of *Caulerpa peltata* (500 µg/mL) in the wavelength range of 200–400 nm. Fig6 which shows the absorbs UVB rays. As a result the methanolic extract of *Caulerpa peltata* absorbs UVB rays which is a harmful rays and it is the primary factor to cause anti-wrinkling and anti-ageing of human skin.

The *Caulerpa peltata* is a prominent organic source which can be used in the cosmetic products as an anti-aging and anti-wrinkling agent.

FOURIER TRANSFORM INFRARED SPECTROSCOPY:-

The FTIR spectrum was utilised to identify the functional group of the active components present in the methanolic extract of the selected green seaweed based on the peak value in the

infrared radiation region(Paul, 2018). The FTIR results of methanolic extract of *Caulerpa peltata* showed in the Fig 7& Table 2 different peaks represents the existence of different functional groups. The broad absorption peak at 3361 cm^{-1} indicates the presence of OH stretching alcohol(Paul, 2018), peak at 2951 cm^{-1} indicates the presence of C-H methylene medium(C=H) to strong bounded, peak at 2838 cm^{-1} indicates the presence of OH carboxylic acid strong, O-H alcohol weak, peak at 1671 cm^{-1} indicates the presence of C=C alkane disubstituted, peak at 1454 cm^{-1} indicates the presence of OH bending carboxylic acid, peak at 1420 cm^{-1} indicates the presence of OH bending carboxylic acid, peak at 1120 cm^{-1} indicates the presence of C-O secondary alcohol stretching, C-O alkyl, peak at 1022 cm^{-1} indicates the presence of Aryl ether stretching (Pandya et.al.,2017), peak at 625 cm^{-1} indicates the presence of Alkyl halides shown in the Table 2(Deepak.et al., 2018).

Anti-bacterial activity of methanolic extract of *Caulerpa peltata*:-

The antibacterial activity of methanolic extract of *Caulerpa peltata* against different bacterial strains(250µg) like*Escherichia Coli, Salmonella typhi, Salmonella paratyphi, Pseudomonas aeruginosa*was investigated in this study at various doses (500µg/ml and 1mg/ml), and the findings are given in Fig8 , Fig 9& Table 3.

In previous studies on Antibacterial activity of *Caulerpa peltata* shows minimal antibacterial activity of 500µg of methanol extract of Caulerpa Chemnitzia(Synonyms-*Caulerpa peltata*) against *Escherichia coli* (Zone of inhibition-10.3±0.57)(Raj et al., 2015)but our studies on Antibacterial activity of methanolic extract of *Caulerpa peltata*of 500µg/ml concentration against *Escherichia coli* showed maximal zone of inhibition of 28mm.Antibacterial activity of methanolic extract of *Caulerpa peltata*of 1mg/ml concentration against *Escherichia coli* showed zone of inhibition of 32mm.

Other previous studies of Antibacterial activity of *Caulerpa peltata*shows no antibacterial activity against any bacterial strains(Movahhedin et al., 2014).

Antibacterial activity of methanolic extract of *Caulerpa peltata*of 500µg/ml concentration against *Salmonella typhi* showed no zone of inhibition .Antibacterial activity of methanolic extract of *Caulerpa peltata*of 1mg/ml concentration against *Salmonella typhi* showed zone of inhibition of 20mm.

Antibacterial activity of methanolic extract of *Caulerpa peltata*of 500µg/ml concentration against *Salmonella paratyphi* showed zone of inhibition of 10mm.Antibacterial activity of

methanolic extract of *Caulerpa peltata*of 1mg/ml concentration against *Salmonella paratyphi* showed zone of inhibition of 13mm.

Antibacterial activity of methanolic extract of *Caulerpa peltata*of 500µg/ml concentration against *Pseudomonas aeruginosa* showed zone of inhibition of 20mm.Antibacterial activityof methanolic extract of *Caulerpa peltata*of 1mg/ml concentration against *Pseudomonas aeruginosa* showed zone of inhibition of 16mm.

Maximal level was found with a high zone of inhibition in *Escherichia Coli* (28mm for 500µg/ml & 32mm for 1mg/ml) and a minimal level of antibacterial activity in *Salmonella paratyphi* (10mm for 500µg/ml & 13mm for 1mg/ml). This results shows that the methanolic extract of *Caulerpa peltata* has high antibacterial activity against gram-negative bacteria especially *Escherichia c*

Anti-oxidant activity of methanolic extract of *Caulerpa peltata*:-

An Antioxidant is defined as "any chemical that considerably delays or inhibits oxidation of the oxidizable substrate when present at low concentrations compared to those of the oxidizable substrate." (Antolovich et al., 2002).

Anti-oxidant activity of methanolic extract of *Caulerpa peltata* ability to scavenge DPPH free radicals was demonstrated in Fig10& Table 4. Methanolic extract of *Caulerpa peltata*of 50µg/ml concentration shows 26.9% of DPPH scavenging activity.Methanolic extract of *Caulerpa peltata*of 100µg/ml concentration shows 29.09% of DPPH scavenging activity.

Methanolic extract of *Caulerpa peltata*of 200µg/ml concentration shows 35.71% of DPPH scavenging activity.Methanolic extract of *Caulerpa peltata*of 400µg/ml concentration shows 36.76% of DPPH scavenging activity.Methanolic extract of *Caulerpa peltata*of 800µg/ml concentration shows 62% of DPPH scavenging activity. This result shows that as the *concentration* of methanolic extract of *Caulerpa peltata* increases, the DPPH inhibition activity also increases.

The scavenging ratio rise from 29.90 percent to 62 percent when methanolic extract of *Caulerpa peltata* concentration was increased from 50µg/ml to 800 µg/ml. The antioxidant activities of methanolic extract of *Caulerpa peltata* were shown to be related to its ability to scavenge hydroxyl and DPPH radicals in the DPPH radical assay. The IC_{50} value of Anti-oxidant activity of methanolic extract of *Caulerpa peltata* is 650µg/ml concentration.

The algae *Caulerpa peltata* inhibits DPPH molecule which is a free radicals which causes anti-aging and anti-wrinkling. So it can be used as an anti-oxidant agent in cosmetic industry and it is a substitute for chemical cosmetics.

Anti-tyrosinase activity of methanolic extract of *Caulerpa peltata*:-

Tyrosinase is an important enzyme in the biosynthesis of melanin. They acts as hypo pigmenting agents. The hydroxylation of tyrosine is catalyses by the copper-containing enzyme called tyrosinase.

The effect of methanolic extract of *Caulerpa peltata* on mushroom tyrosinase activity using L-DOPA as substrate are reported in Fig 11& Table 5. this results shows that the methanolic extract of *Caulerpa peltata* exhibiting stronger inhibiting effect against mushroom tyrosinase with respect to increased concentration of algal extract.

The control shows no inhibitory effect against mushroom tyrosinase. 0.2mg concentration of methanolic extract of *Caulerpa peltata*shows inhibitory effect of 5.96%, 0.4mg concentration of methanolic extract of *Caulerpa peltata*shows inhibitory effect of 10.97%, 0.6mg concentration of methanolic extract of *Caulerpa peltata*shows inhibitory effect of 14.42%, 0.8mg concentration of methanolic extract of *Caulerpa peltata*shows inhibitory effect of 18.50%, 1mg concentration of methanolic extract of *Caulerpa peltata*shows inhibitory effect of 23.62%. Kojic acid is used as a standard inhibitor of tyrosinase which is used in cosmetic industry as a whitening agent. The IC_{50} value is 2mg.

The Algae *Caulerpa peltata* shows inhibitory effect of tyrosinase enzyme which is a primary source of the production of melanin. It shows that *Caulerpa peltata*can be a prominent organic ingredient that can be used in skin lightening cosmetic product which can be a substitute for the harmful chemical products which causes severe side effects to skin.

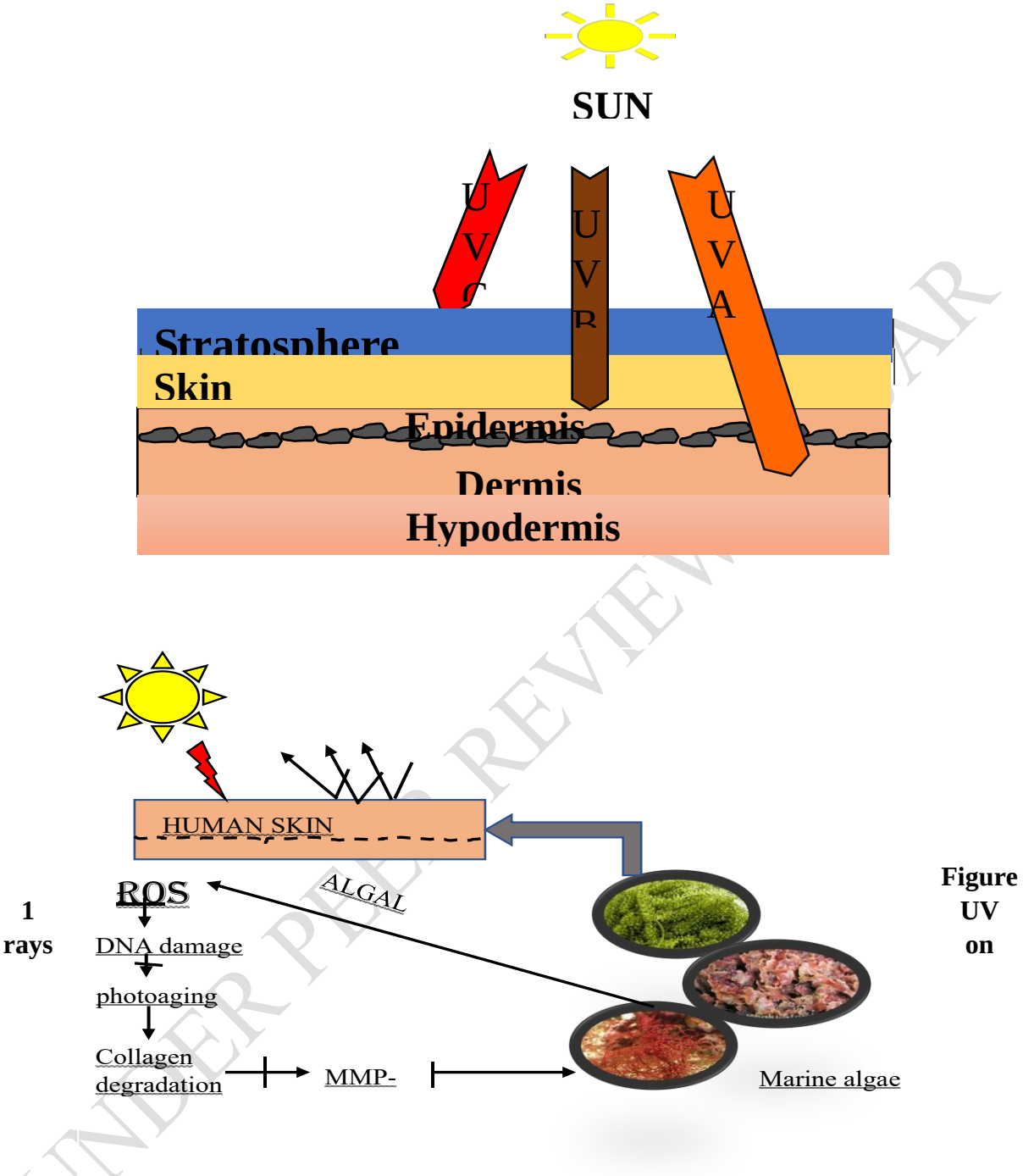

Figure 1 UV rays on Human skin

Figure 2 Algal Photo protectivity

Figure 3 Satellite map of collected area of algal sample

Fig 5 Morphology of *Caulerpa peltata*

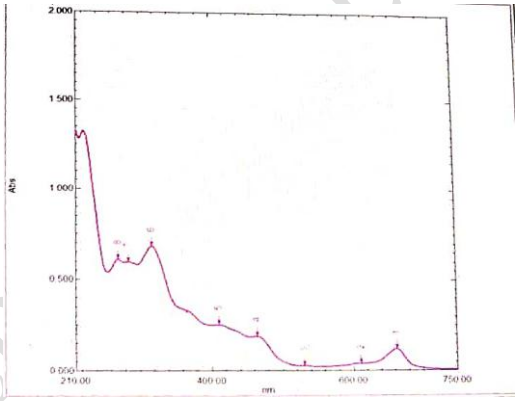

Figure 6 UV spectrum of methanolic extract of *Caulerpa peltata*

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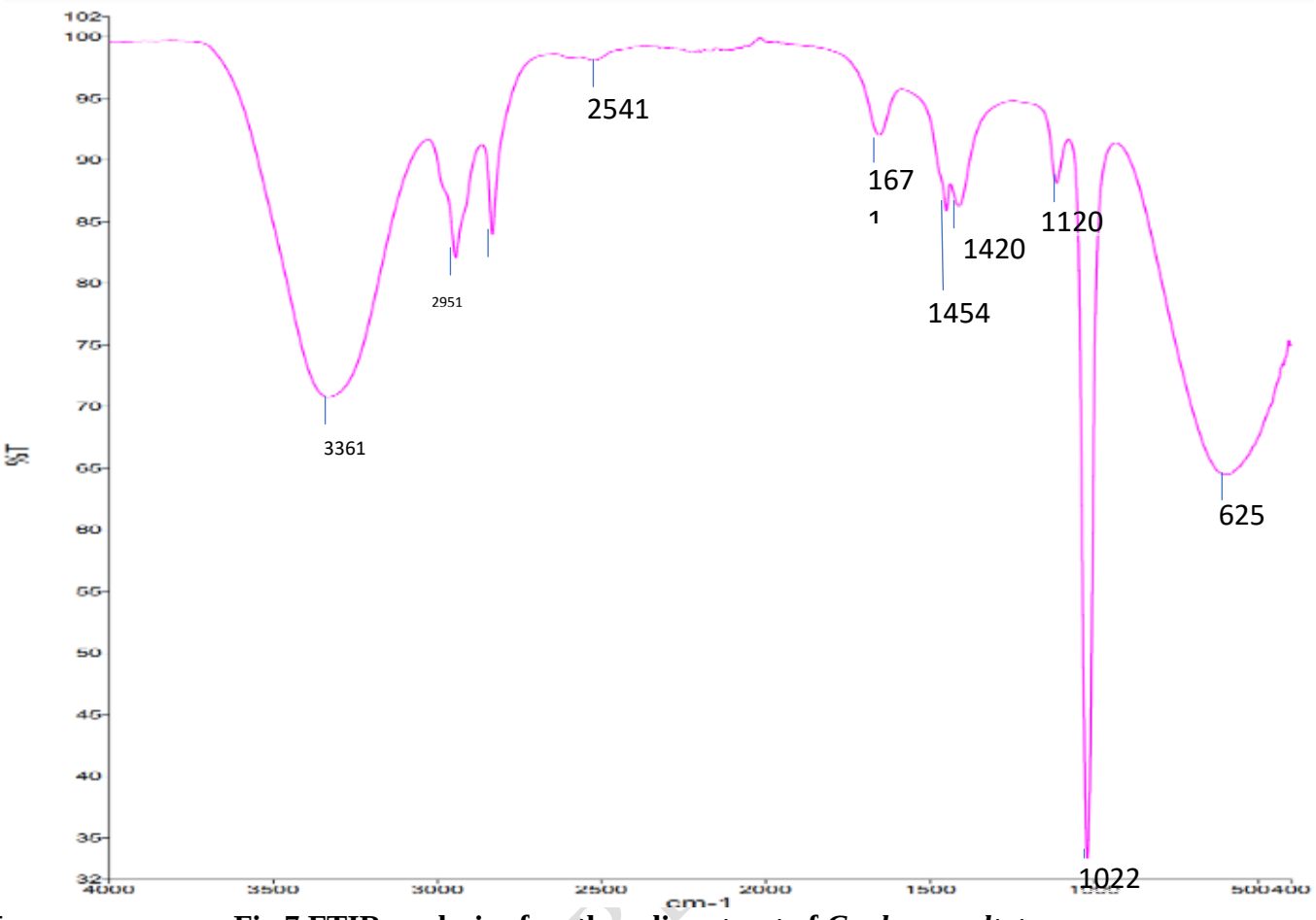

Fig 7 FTIR analysis of methanolic extract of *Caulerpa peltata*

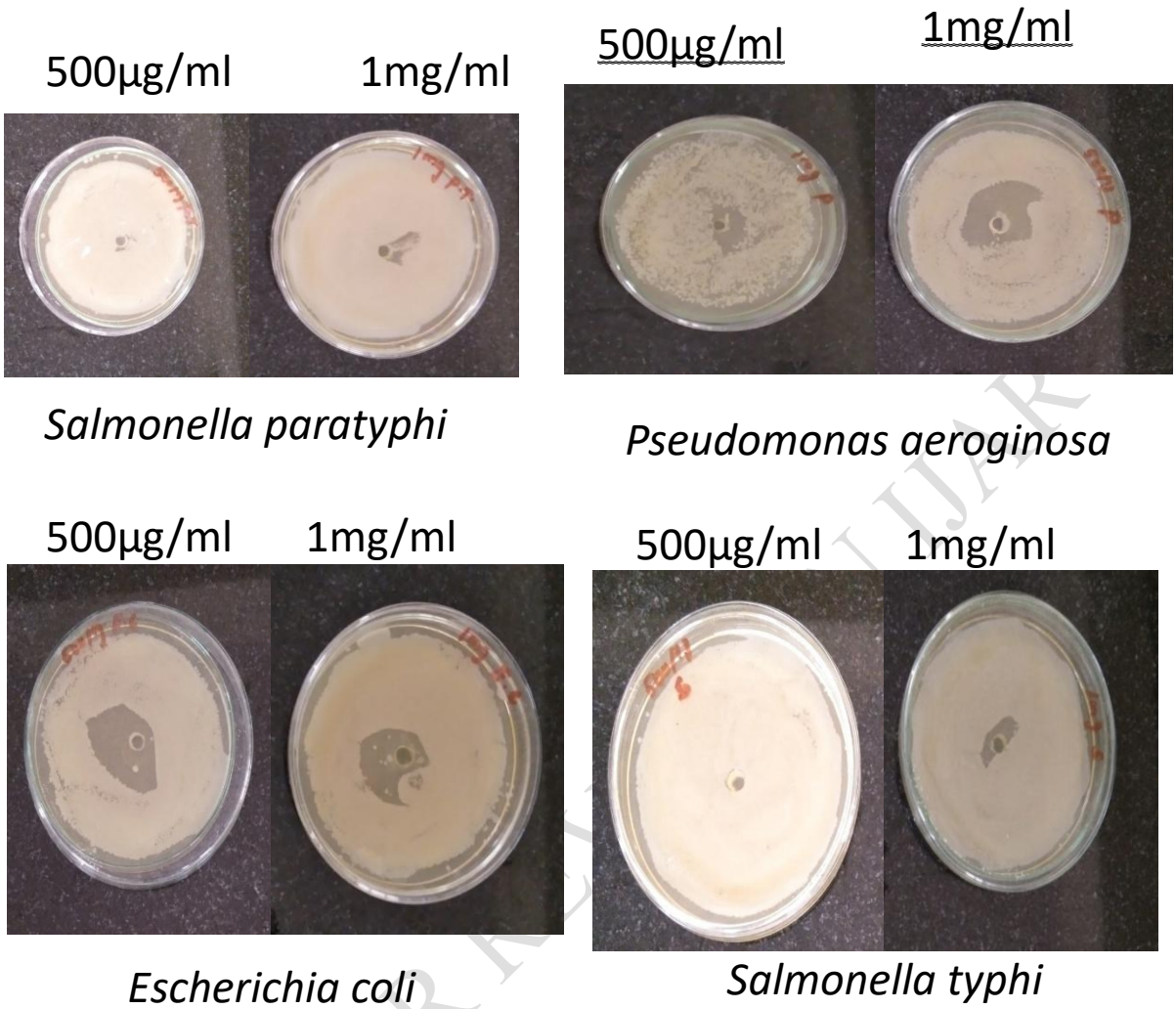

Fig 8Antibacterial activity of methanolic extract *Caulerpa peltata*

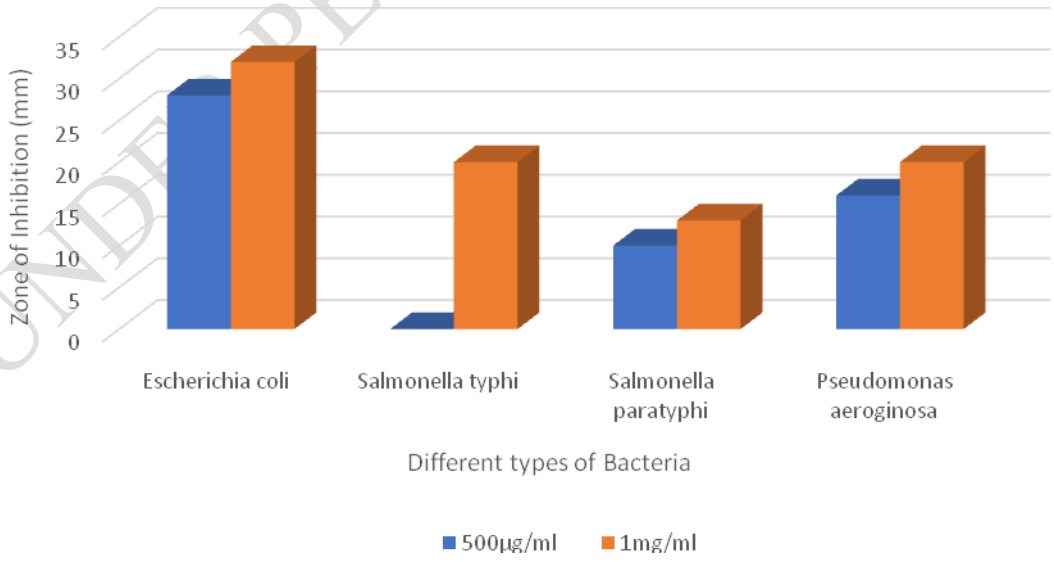

Figure 9Antibacterial activity of methanolic extract of *Caulerpa peltata*

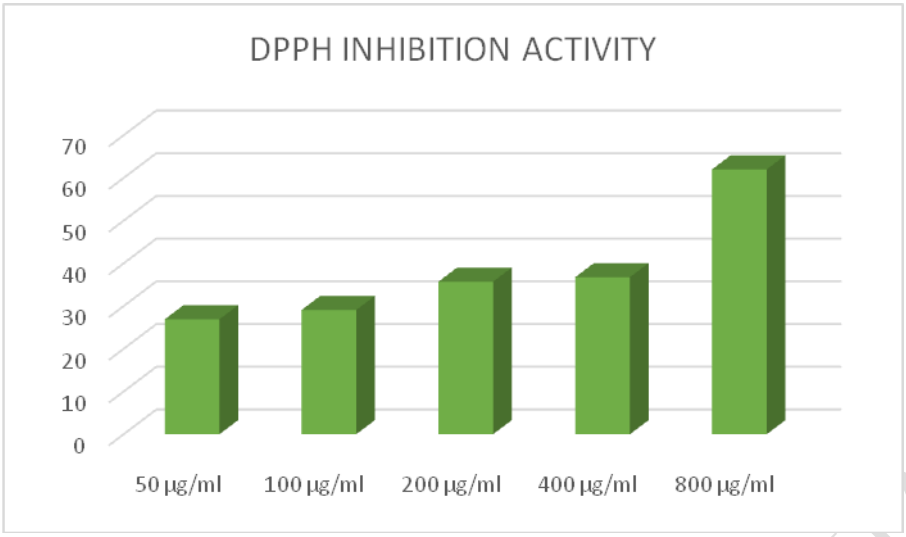

Figure 10Antioxidant activity of methanolic extract of *Caulerpa peltata*

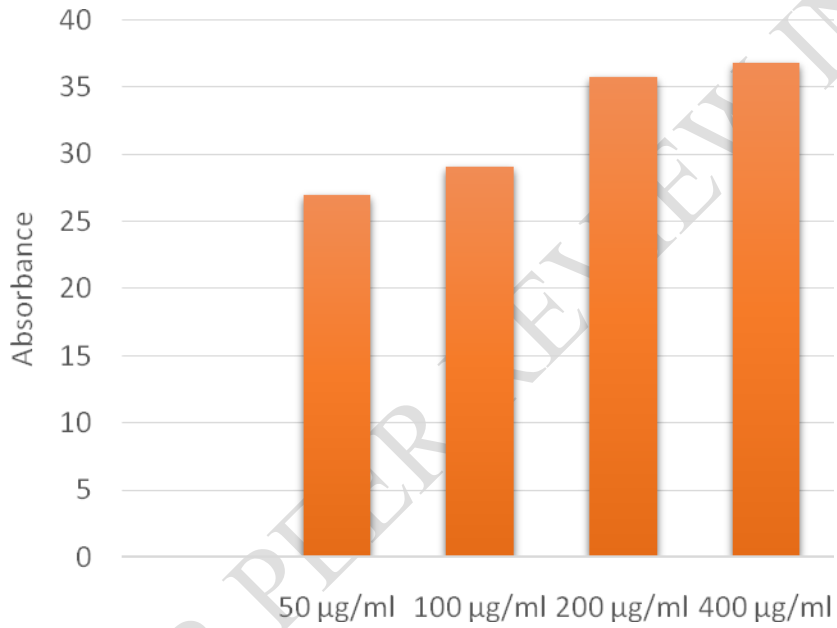

Figure 11Anti-tyrosinase activity of methanolic extract of *C.peltata*:-

TEST	RESULT
Carbohydrates(Anthrone test)	+++
Carbohydrates(Molisch test)	+++
Proteins	---
Fat	---

Table 1

Phytochemical analysis of methanolic extract of *Caulerpa peltata*

695 **Table 2FTIR analysis of methanolic extract of *Caulerpa peltata***

S.NO	BACTERIAL STAINS	ZONE OF INHIBITION(mm)	
		500µg/ml	1mg/ml
1.	*Escherichia coli*	**28 mm**	**32 mm**
2.	*Salmonella typhi*	**0 mm**	**20 mm**
3.	*Salmonella paratyphi*	**10 mm**	**13 mm**
4.	*Pseudomonas aeroginosa*	**20 mm**	**16 mm**

S.NO	ABSORBANCE	COMPOUND	REFERENCE
1.	3361	OH stretching alcohol.	(Paul, 2018)
2.	2951	C-H methylene medium(C=H) to strong bounded.	(Pandya et.al.,2017)
3.	2838	OH carboxylic acid strong, O-H alcohol weak.	(Pandya et.al.,2017)
4.	1671	C=C alkane disubstituted	(Pandya et.al.,2017)
5.	1454	OH bending carboxylic acid .	(Pandya et.al.,2017)
6.	1420	OH bending carboxylic acid .	(Pandya et.al.,2017)
7.	1120	C-O secondary alcohol stretching, C-O alkyl	(Pandya et.al.,2017)
8.	1022	Aryl ether stretching	(Pandya et.al.,2017)
9.	625	Alkyl halides	(Deepak et al., 2018)

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698 **Table 3Anti-bacterial activity of methanolic extract of *Caulerpa peltata***

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S.NO	CONCENTRATION OF METHANOLIC EXTRACT OF *Caulerpa peltata*	DPPH INHIBITION ACTIVITY (%)
1.	**50** µg/ml	**26.90**
2.	**100** µg/ml	**29.09**
3.	**200** µg/ml	**35.71**
4.	**400** µg/ml	**36.76**
5.	**800** µg/ml	**62**

S.NO	CONCENTRATION OF METHANOLIC EXTRACT OF *Caulerpa peltata*	TYROSINASE INHIBITION (%)
1.	Control	0.00
2.	0.2 mg	5.96
3.	0.4 mg	10.97
4.	0.6 mg	14.42
5.	0.8 mg	18.50
6.	1 mg	23,62

700 **Table 4Anti-oxidant activity of methanolic extract of *Caulerpa peltata***

701 **Table 5Anti-tyrosinase activity of methanolic extract of *Caulerpa peltata***

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CONCLUSION

The Algal sample was collected from mandapam, Rameshwaram coast. It was identified as *Caulerpa chemnitzia*(Esper) J.V. Lamouroux (Synonyms- *Caulerpa peltata* J.V. Lamouroux) by Botanical Survey of India, West Bengal, Kolkata. The Algal extract was prepared by using the solvent methanol. The yield was 0.4%

In Phytochemical analysis, The methanolic extract of *Caulerpa peltata* shows positive results for Molisch's test and anthrone test for carbohydrates while it shows negative results for biuret test for protein and dichromate test for fats. It shows that our algal sample contains carbohydrates. It is soluble in solvent like distilled water, methanol, ethanol and hexane.

The methanolic extract of *Caulerpa peltata* shows a prominent peak between 200-400nm which is a wavelength of UVB rays in UV spectrometer analysis. In FTIR analysis , different compounds were identified by different absorbance.

The methanolic extract of *Caulerpa peltata* high antibacterial activity against Gram-negative bacteria bacterial strains like against *Escherichia Coli, Salmonella typhi, Salmonella paratyphi, Pseudomonas aeruginosa.* The maximum zone of inhibition shown against

Escherichia coli.

The methanolic extract of *Caulerpa peltata* shows high inhibition activity against DPPH with respect to the increased concentration of the extract. The highest DPPH inhibition activity 62% showed in 800µg concentration of methanolic extract of *Caulerpa peltata.* The IC_{50} value is 650 µg/ml.

The methanolic extract of *Caulerpa peltata* shows tyrosinase inhibition activity with respect to increase of concentration of the extract. The highest inhibition activity of 23.62% showed in 1mg concentration of algal extract.

To conclude the algae *Caulerpa peltata* absorbs UVB rays which is responsible for anti-ageing, It shows antioxidant activity i.e. it inhibits DPPH which is a free radical and at last it also shows inhibitory effect against tyrosinase which place a vital role in melanin production. On seeing the results above, the algae *Caulerpa peltata* has UVB inhibition activity, Anti-oxidant activity and Anti-tyrosinase activity it would be a greater substitute for a chemical skin lightening products which is a very harmful to human skin used in cosmetic industry.

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