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Research Article

A COMPARATIVE STUDY ON THE ANTI-TYROSINASE ACTIVITY OF *CHAETOMORPHA ANTENNINA CERATOPHYLLUM SUBMERSUM EXTRACTS*

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ABSTRACT

The diversity of marine macroalgae (seaweeds) species and their widely ranging biochemical composition means that they represent a source of potential bioactive compounds for applications in the agri-food industry, cosmetics and pharmacology. There is a trend in the cosmetic industry towards the development and manufacture of cosmetics with seaweed extracts. Seaweeds are rich in vitamins, essential amino acids, mineral macro elements and trace elements such as iodine. Melanin is the major pigment for color of human skin. It is secreted by melanocyte cells in the basal layer of the epidermis. Melanin may be overproduced with chronic sun exposure, melasma, or other hyper pigmentation diseases. Therefore, a number of depigmenting agents have been developed for cases of undesirable skin discoloration. Tyrosinase, a copper-containing monooxy-genase, is a key enzyme that catalyzes melanin synthesis in melanocytes. It catalyzes two major reactions, including hydroxylation of tyrosine and oxidation of the *o*-diphenol product, *l*-dopa. Dopa oxidation produces a highly reactive intermediate that is further oxidized to form melanin by a free radical-coupling pathway. If free radicals are inappropriately processed in melanin synthesis, hydrogen peroxide is generated, leading to production of hydroxyl radicals and other reactive oxygen species. Melanin biosynthesis can be inhibited by avoiding ultraviolet exposure, by inhibition of melanocyte metabolism and proliferation, by inhibition of tyrosinase, or by removal of melanin by corneal ablation. The present research is a Comparative study of the Anti-tyrosinase activity of *Chaetomorpha antennina* and *Ceratophyllum submersum* extracts.

INTRODUCTION

Tyrosinase, a copper-containing monooxy-genase, is a key enzyme that catalyzes melanin synthesis in melanocytes. It catalyzes two major reactions, including hydroxylation of tyrosine and oxidation of the *o*-diphenol product, *l*-dopa. Dopa oxidation produces a highly reactive intermediate that is further oxidized to form melanin by a free radical-coupling pathway (Meenakshi Bhattachaerjee, 2016). If free radicals are inappropriately processed in melanin synthesis, hydrogen peroxide is generated, leading to production of hydroxyl radicals and other reactive oxygen species (Mitali Priyadharshini Pati *et.al.,* 2016). Melanin biosynthesis can be inhibited by avoiding ultraviolet exposure, by inhibition of melanocyte metabolism and proliferation, by inhibition of tyrosinase, or by removal of melanin by corneal ablation (Rashed .K, Medda. R and Pintus. F, 2016).

Apart from avoiding UV exposure, application of tyrosinase inhibitors may be the least invasive procedure for maintaining skin whiteness; such agents are increasingly used in cosmetic products (Rui Manuel Santos Costa de Morais, 2015). Oxidative stress may be induced by increasing generation of ROS and other free radicals. UV radiation can induce formation of ROS in skin such as singlet oxygen and superoxide anion, promoting biological damage in exposed tissues via iron-catalyzed oxidative reactions (Soad M. Mohy El-Din *et.al.,* 2015). These ROS enhance melanin biosynthesis, damage DNA, and may induce proliferation of melanocytes (Subathraa K and TV Poonguzhali, 2013 and Sumathi. S and Krishnaveni. M, 2012). The aim of the present study is to identify and compare the effects of Chaetomorpha antennina and Cerotpohyllum submersum extracts and its polysaccharides as a potential antityrosinase agent.

Algotherapy is a science in which seaweed extracts are used in health or beauty treatments. Seaweed baths are used as a treatment for arthritis, rheumatism and other aches. Many companies produce seaweed powder for beauty and body care products (Chang, V.S. and Teo, S.S, 2016). A number of compounds extracted from seaweeds are thought to be of value in various cosmetic application and some are now becoming commercially important. Algae are an excellent choice for the

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preparation of cosmetic products because of the lipids they contain which can be used to produce oils (Harsha Kharal *et.al.,* 2012). *Chlorella* is the most used genus for the production of cosmetics, other than genera *Spirulina, Anabaena* etc. they are used in several forms such as algal oils, algal powders, algal flour and algal flakes. Products coming from these seaweeds comprise of algal soaps, algal clay masks, beauty serums and oils, scrubs, shampoos and so on (Meenakshi Bhattachaerjee, 2016). Thus Algae are easily accessible resources used for innovative formulations by the cosmetics industry. Excipients, active ingredients, and additives, all categories of raw materials that go into cosmetics, can come from the sea (Mitali Priyadharshini Pati *et.al.,* 2016 and Maria Filomena de Jesus Raposo *et.al.,* 2015).

MATERIALS AND METHODS

Collection, Processing and Extraction of Seaweeds

Chaetomorpha antennina and *Ceratophyllum submersum* were collected from the shores of Royapuram fishing harbour (N4beach) in Chennai. The samples were manually collected; epiphytes and debris were removed by washing in running tap water and washed again with distilled water. The samples were then allowed to shade dry for 7 days at room temperature and were finely powdered using an electric blender. The materials required for the extraction process are *Chaetomorpha antennina* and *Ceratophyllum submersum,* Solvent (Methanol) 500ml and Conical flask (500 ml). 10gms of the dried Green algae and aquatic plant were extracted separately in 100ml of Methanol (1: 10 ratio) for 3 days in a separate conical flask. The solvent were filtered using a muslin cloth or filter paper. The filtrates were stored in screw capped container for further analysis.

Extraction of Crude Polysaccharides (Silva et al)

The materials required for the extraction of Crude Polysaccharides are Dried powdered sample, Acetone, 0.25M Sodium chloride (Nacl), Sodium hydroxide (NaOH), Trypsin, Filter paper or cheese cloth and Centrifuge tubes. 10g of powder sample was incubated overnight with acetone to remove lipid and pigments. The residue was then dissolved in 5 volumes of 0.25M NaCl, and the pH was monitored periodically and adjusted to 8 using NaOH. 10mg of trypsin was added to the content for proteolysis and incubated for 24hours. After incubation, the content was filtered through cheese cloth or filter paper. The filtrate was precipitated using ice cold acetone under gentle agitation at 4°C. The precipitate formed was centrifuged at 10,000rpm for 20 minutes. The total polysaccharide extract was dried under vacuum. Extracted polysaccharide was re-suspended in distilled water and was used for further analysis.

Purification of Polysaccharides

Column Chromatography and Dialysis

The materials required for the Column Chromatography and Dialysis are Crude polysaccharides, DEAE Cellulose column (3×45cm), Sodium chloride (0-3M), Dialysis bag and Distilled water. 50mg of crude polysaccharides was dissolved in 10ml of distilled water. It was applied to a DEAE cellulose column pre equilibrated with water and eluted in NaCl gradient (0-3M) until no carbohydrate was detected. Each fraction was assayed for carbohydrate content by phenol sulphuric acid method. The carbohydrate-positive fractions were pooled together and dialyzed (MWCO 14,000) for 24 hours against distilled water.

Chemical Analysis

Estimation of Carbohydrates (Phenol-Sulphuric Acid- Dubois et al.,)

The materials required for the Estimation of Carbohydrates by Phenol-Sulphuric acid method are Polysaccharides, 5% Phenol, 96% Sulphuric acid, 2.5N Hydrochloric acid, Sodium carbonate, Glucose (standard), Stock- 100mg glucose dissolved in 100 ml of distilled water and Standard- 10ml of stock made upto 100ml. 100mg of the sample was weighed into the boiling tubes. They were hydrolysed by keeping in boiling water bath for 3 hrs with 5ml of 2.5N Hydrochloric acid and was cooled to room temperature. The solution was neutralized with solid sodium carbonate until the effervescence ceased. It was made to a volume of 100ml and was centrifuged. The standard in ranging concentration (0.2ml-1ml) was pipette into a series of test tubes.0.2ml of the extract was pipette in 2 separate test tubes. The volume was made up to 1ml in each tube with distilled water. 1ml of phenol solution was added to each tube followed by 5ml of 96% Sulphuric acid and was shaken well. After 10 minutes the contents in the tubes were shaken and were placed in a water bath at 25°-30° C for 20 minutes. A blank with 1 ml of distilled water was set. The colour was read at 490nm and the amount of total carbohydrates was calculated using the standard graph.

Estimation of Protein (Lowry's Method)

Protein was estimated using the Lowry's Method. The materials required are

- Fiolin-ciocalteau reagent
- Reagent A- 20% sodium carbonate in 0.1N Sodium hydroxide
- Reagent B- 0.5% copper sulphate in 1% potassium sodium tartarate
- Reagent C- Alkaline copper solution (50ml of A and 1ml of B reagents).
- Stock solution- 50mg of Bovine serum albumin dissolved in distilled water and made upto 50ml in standard flask.
- Standard solution- 10ml of the stock solution was diluted to 50ml with distilled water in standard flask. 1.0ml of this solution contains200µg of protein
- Polysaccharides

The standards in ranging concentration (0.2ml-1ml) were transferred into a series of test tubes. 0.2ml of sample extract was also transferred into two other test tubes. The volume was made upto 1.0ml in all the test tubes. 5ml alkaline copper solution was added to each tube including the blank. It was mixed well and was allowed to stand for10mins. 0.5ml of Fiolin's-ciocalteau reagent was added**.** It was mixed well and was incubated at room temperature in the dark for 30 minutes till Blue colour developed. The Absorbance was read at 620nm.

Production of Enzyme Tyrosinase (William H. Heidcamp Method)

The Materials required for the production of Enzyme Tyrosinase are Potatoes, Paring knife, Blender, 0.1M Sodium

Fluoride (NaF), Rubber gloves, Saturated Ammonium sulphate (4.1M at 25°C), Volumetric cylinders (50ml, 100ml, 250ml), Cheesecloth, Beakers (100ml, 250ml), Chilled centrifuge tubes (30-50ml), 0.1M Citrate buffer, pH 4.8 and Glass stirring rod. A small potato was peeled and was cut into pieces about 1 inch square. 100gms of the potato was added to a blender, along with 100ml of sodium fluoride. It was homogenized for about one minute at high speed. The homogenate was poured through several layers of cheese cloth and finally into a beaker. The volume of the homogenate was measured and equal volume of saturated ammonium sulphate was added (i.e., if the fluid volume of homogenate is 150ml, 150 ml of ammonium sulphate is taken). A flocculent white precipitate will appear as many of the previously soluble potato proteins becomes insoluble. The enzyme tyrosinase is one of these proteins and thus will be found in the subsequent precipitate. The homogenate is treated with ammonium sulphate in chilled centrifuge tubes and is centrifuged at 1,500 × g for 5 minutes at 4°C. after centrifugation the supernatant is discarded and the Pellet was sustained.

All the Pellets were collected in a 100ml beaker to which 60ml of citrate buffer, p H 4.8 was added to the pooled pellet and the contents were stirred well. A glass rod was used to break up the pellets. The stirring was continued for 2 minutes while keeping the solution cool. The solution was again transferred into centrifuge tubes and was re-centrifuged at 300×g for 5 minutes at 4°C. The supernatant was collected and stored. The enzyme was placed in an ice bucket. The enzyme tyrosinase is insoluble in 50% ammonium sulphate, but is solube in the citrate buffer. The extract was maintained chill. Tyrosinase is stable for about an hour.

Tyrosinase Inhibition Activity (Yen and Kim, 2012)

The Materials required for the production of Tyrosinase Inhibition Activity are 50mMSodium Phosphate buffer, Tyrosinase enzyme, L-DOPA Substrate, Aquatic weed extract and Polysaccharides. 1.9 ml of 50mM sodium phosphate buffer was mixed with 100 µL of sample extract solution and 20 µL of tyrosinase (1000 unit /mL in 50 mM phosphate buffer, pH 6.8) into a test tube. This was followed by 20µL of 0.1 mM L-DOPA substrate addition in the test tube. The reaction mixture was incubated at 37°C for 10 minutes in a water bath. Absorbance was measured at 475 nm. The 50mM sodium phosphate solution was used as as a blank solution in Spectrophotometer.

RESULTS AND DISCUSSION

Collection, Processing and Extraction of Seaweeds

10gms of the dried Green algae and aquatic plant were extracted separately and was placed in 100ml of Methanol (1: 10 ratio) for 3 days in a separate conical flask. The solvent were filtered using a muslin cloth or filter paper. The filtrates were stored in screw capped container for further analysis (Figures 1 & 2).

Extraction of Crude Polysaccharides (Silva et al)

The total polysaccharides extract were dried under vacuum. Extracted polysaccharides were re-suspended in distilled water and were used for further analysis (Figures 3& 4).

Figure 3 *Chaetomorpha* extract

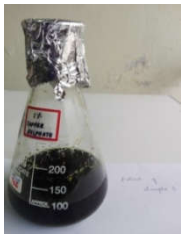


Figure 4 *Ceratophyllum* extract

Extraction of Crude Polysaccharides

Extraction resulted by yielding 0.5g of green solid crude polysaccharides from 10g of *Chaetomorpha antennina* and 0.4g of brownish green crude polysaccharides from 10g of *Ceratophyllum submersum* (Figures 5 & 6).

Figure 5 Polysaccharides after centrifuge

Figure 6 Dry crude polysaccharides

Column Chromatography and Dialysis

A few grams of crude polysaccharides were dissolved in 10ml of distilled water. From that 3ml of diluted samples were added to DEAE- Cellulose Column and were eluted with different gradients of NaCl (0-3M). Different fractions which contain polysaccharides were separated based on their ionic character at different molarity. 50ml of partially purified polysaccharides from *Chaetomorpha antennina* and 35ml of partially purified polysaccharides *Ceratophyllum submersum* were collected. The partially purified polysaccharides were subjected to dialysis. 26ml of purified polysaccharide from *Chaetomorpha antennina* and 17ml of purified polysaccharide from *Ceratophyllum submersum* were obtained (Figures 7 - 10).

Figure 7 Column Chromatography of *Chaetomorpha antennina*

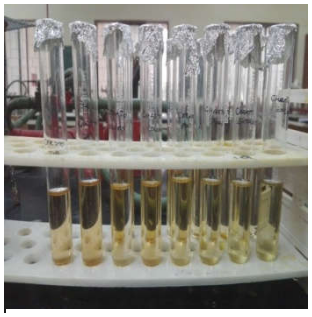


Figure 8 Partially purified Polysaccharides of *Chaetomorpha antennina*

Figure 9 Column Chromatography of *Ceratophyllum submersum*

Figure 10 Partially purified Polysaccharides of *Ceratophyllum submersum*

Chemical Analysis

Estimation of Carbohydrates

Glucose (Standard)

By phenol sulphuric acid method, 64mg/ml of carbohydrates in *Chaetomorpha antennina* and 68mg/ml of carbohydrates in *Ceratophyllum submersum* were estimated. Chemical composition of the purified polysaccharide from *Chaetomorpha antennina* and *Ceratophyllum submersum* were determined as carbohydrate content (Tables 1-3 and Graphs 1 & 2).

Table 1 Total Carbohydrate Content of *C. antennina*

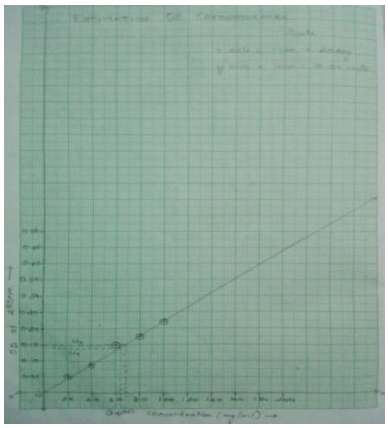
SAMPLE	O.D (490nm)	Concentration of Glucose (mg/ml)
C.antennina	0.14	64

Table 2 Total Carbohydrate Content of *Ceratophyllum submersum*

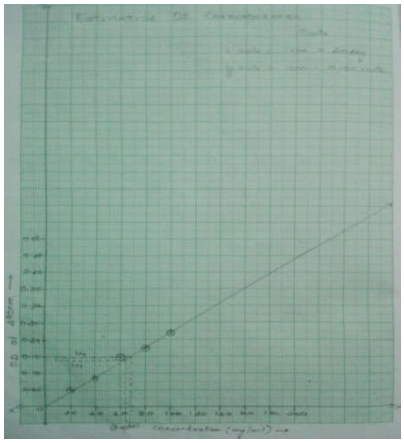
Sample	O.D (490nm)	Concentration of Glucose (mg/ml)
C.submersum	0.15	68

Table 3 Results for Glucose Standard Curve

S.NO	Concentration of Glucose (mg/ml)	O.D (490nm)
1	20	0.05
2	40	0.09
3	60	0.15
4	80	0.18
5	100	0.22



Graph 1 Chemical Analysis for Carbohydrates of *Chaetomorpha antennina*



Graph 2 Chemical Analysis for Carbohydrates of *Ceratophyllum submersum*

Estimation of Proteins

Bovine serum Albumin- BSA (Standard)

By Lowry's method, 4mg/ml of protein content was estimated both in *C.antennina* and *Ceratophyllum submersum* (Tables 4 - 6). In the chemical composition of purified polysaccharides only a small concentration of protein were present.

Table 4 Total Protein Content of *C.antennina*

SAMPLE	O.D (620nm)	Concentration of protein (mg/ml)
C.antennina	0.03	4

Table 5 Total Protein Content of *Ceratophyllum submersum*

SAMPLE	O.D (620nm)	Concentration of protein (mg/ml)
Ceratophyllum submersum	0.03	4

Table 6 Results for Bovine Serum Albumin Standard Curve

S.No	Concentration of BSA (mg/ml)	O.D (620nm)
1	20	0.10
2	40	0.27
3	60	0.43
4	80	0.56
5	100	0.70

FT-IR Spectrum for Chaetomorpha Crude Extract

The FTIR spectrum for the *Chaetomorpha* extract was analysed (Figure 11). The absorbance band were in the region of 3437cm^{-1} corresponds to the hydroxyl stretching vibration of

the polysaccharides and that at 2923cm^{-1} corresponds to a weak C-H bonds. The intense peak at 1636cm^{-1}were equivalent to that of galactans. The region at 1415cm^{-1} indicates the carboxylic acid. The peaks around 1324cm-1 are the skeleton of galactans. The most important band were found at 1253^{-1}cm which indicated sulphatic groups (S=O). The most important band was found at 1028.06cm^{-1}.The band at 825cm^{-1} shows the mannuronic units.

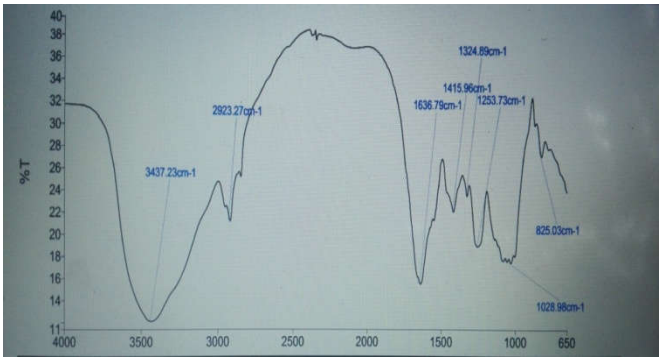


Figure 11 FT-IR Image of *Chaetomorpha* Crude Extract

FT-IR Spectrum for** Ceratophyllum **Crude Extract

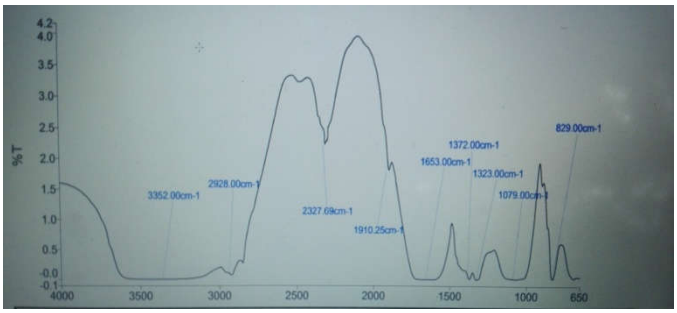


Figure 12 FT-IR image of *Ceratophyllum* Crude Extract

The FTIR spectrum for the *Ceratophyllum* extract was analysed (Figure 12). The absorbance band in the region of 3352cm^{-1} corresponds to the hydroxyl stretching vibration of the polysaccharides and that at 2928cm^{-1} corresponds to a weak C-H bonds. The region at 2327cm^{-1}are equivalent to the alkyl group. The range at 1910cm^{-1} indicates the carbonyl group. The peak around 1653cm^{-1} are the C=H bonds. The band found at 1372cm^{-1} indicated carboxylic acid. The band at 1323cm^{-1} shows the galactan units. The most important band in the region 1079cm^{-1} was indicated as carbohydrates.

FTIR Spectrum for Polysaccharide of Chaetomorpha

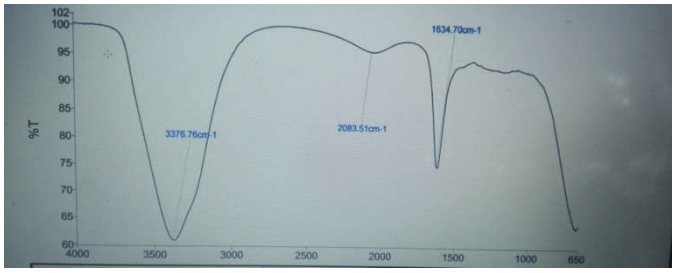


Figure 13 FT-IR Image for *Chaetomorpha* Polysaccharide

The FTIR spectrum for the polysaccharide was analysed (Figure 13). The intense band at the region of 3376cm^{-1} indicated the hydroxyl group. The vibration at the region of 2083cm^{-1} shows the alkenes groups (C=C). The narrow steep range at 1634cm^{-1} represents the galactans.

FTIR spectrum for Polysaccharide of Ceratophyllum

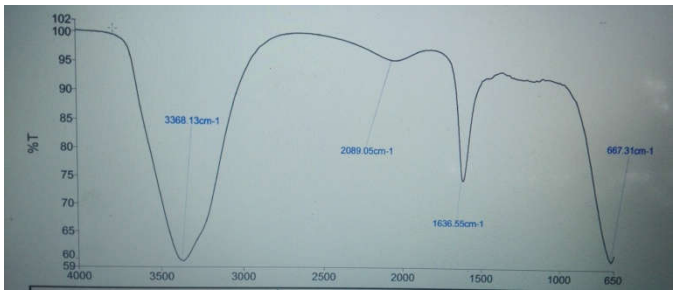


Figure 14 FT-IR Image for Ceratophyllum polysaccharide

The FTIR spectrum for the polysaccharide of *Ceratophyllum* was analysed (Figure 14). The maximum absorbance at the region of 3368cm^{-1} was indicated as hydroxyl group stretching vibration of polysaccharides. The mild vibration at region of 2089cm^{-1} represents alkenes (C=C). The band at the region of 1636cm^{-1} indicates carboxylate O-CO bonds. The intense peak at the region of 667cm^{-1} represents the sulphate ester. The polysaccharide samples show a maximum absorption peak at 2900cm$^{-1.}$ Many intense peaks represents C=O, C-H, carboxylic bond, mannuronic unit, galactans and OH bonds which are evident to show there is presence of carbohydrates.

Production of Tyrosinase and Tyrosinase Inhibitory Activity

The homogenate was prepared from 100g of potato and 100ml of Sodium fluoride. From the homogenate the enzyme was precipitated by adding equal volume of ammonium sulphate. After centrifugation citrate buffer was added to the pellets (Figure 15, Tables 7 - 8 & Graphs 3 - 4). About 50 ml of crude tyrosinase enzyme was obtained from both the extracts.

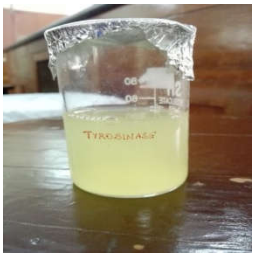


Figure 14 Crude Tyrosinase Enzyme

Tyrosinase Inhibitory Activity

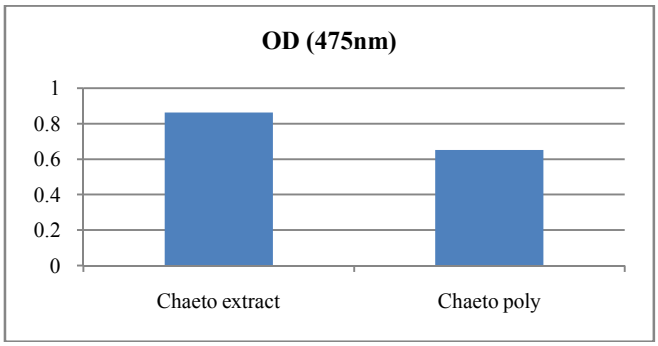
Table 7 Anti - tyrosinase Inhibitory Activity

S.NO	SAMPLE	O.D (475nm)
1	Ascorbic acid (Standard)	0.954
2	*Chaetomorpha* extract	0.862
3	*Chaetomorpha* polysaccharide	0.652

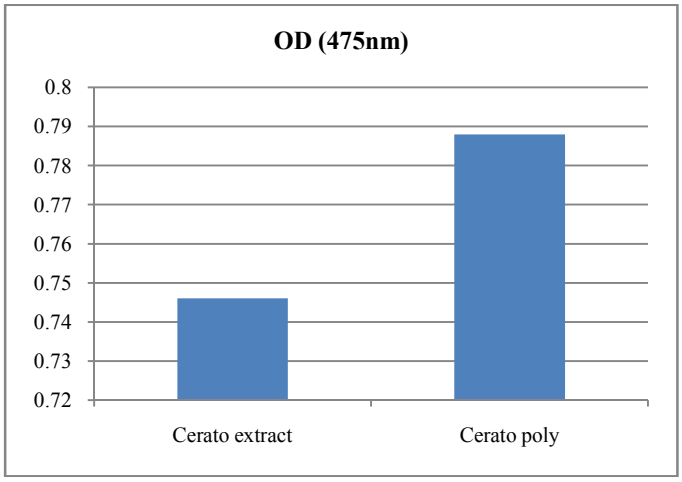
Tyrosinase Inhibitory Activity

Table 8 Anti- tyrosinase Inhibitory Activity

S.NO	Sample	O.D (475nm)
1	Ascorbic acid (Standard)	0.954
2	*Ceratophyllum* extract	0.746
3	*Ceratophyllum* polysaccharide	0.788



Graph 3 Anti - tyrosinase activity in the aquatic extracts and its polysaccharides



Graph 4 Anti - tyrosinase activity in the aquatic extracts and its polysaccharides

CONCLUSION

Seaweeds are considered as a source of bioactive compounds to produce secondary metabolites. Their bio-stimulant property is explored for use in agriculture and antimicrobial activities for the development of novel antibiotics. Traditional herbal medicines provide an interesting, largely unexplored source for development of potential new drugs. The potential use of traditional herbal medicines for development of new skin-care cosmetics is increasing. It is of great interest to know whether preparations used in cosmetics in folk medicine have activities that might be useful in modern formulations. The anti-tyrosinase effects of 95% ethanol extracts of some traditional Chinese herbal medicines used for skin-care in ancient books are recorded in cultures of human melanocytes. Their antioxidant abilities and phenolic contents are also tested. Thus the present study is a Comparative analysis of the Anti-tyrosinase activity of *Chaetomorpha antennina* and *Ceratophyllum submersum* extracts.

References

1. Meenakshi Bhattachaerjee (2016). Pharmaceautically valuable bioactive compounds of algae. *Asian journal of Pharmaceutical and Clinical Research,* Volume 9, Issue 6, Pages 24 - 30.
2. Mitali Priyadharshini Pati, Satyabrata das Sharma, Lakshman Nayak, Chita Ranjan Panda (2016). Uses of seaweeds and its application to human welfare: A review. *International Journal of Pharmacy and Pharmaceutical Sciences,* Volume 8, Issue 10, Pages 0975-1491.
3. Rashed .K, Medda. R and Pintus. F (2016). Evaluation of antioxidant, anti-tyrosinase potentials and phytochemical composition of four Egyptian plants. *International Food Research Journal* 23(1):203-210.
4. Rui Manuel Santos Costa de Morais (2015). Marine Polysaccharides from Algae with Potential Biomedical Applications. *Marine drugs* Pages 2967- 3028.
5. Soad M. Mohy El-Din, Amani M.D and El-Ahwany (2015). Bioactivity and phytochemical constituents of marine red seaweeds (*Jania rubens, Corallina mediterranea and Pterocladia capillacea*). *Journal of Taibah University for Science*, Pages 471–484.
6. Subathraa K and TV Poonguzhali (2013). Effect of different extracts of Chaetomorpha antennina and their phytochemical screening. *International Journal of Current Sciences*, Pages 35-39.
7. Sumathi. S and Krishnaveni. M (2012). Preliminary Screening, Antioxidant and Antimicrobial potential of *Chaetomorpha antennina* and *Caulerapa scalpelliformis* invitro study. *International Journal of Environmental Sciences*, Volume 2, Pages 567-560.
8. Chang, V.S. and Teo, S.S (2016). Evaluation of heavy metal, antioxidant and anti-tyrosinase activities of red seaweed (*Eucheuma cottonii*). *International Food Research Journal*, Volume 23(6), Pages 2370-2373.
9. Harsha Kharal, DD Joshi, Preethi Panthari, Manish Kant Pant and Amit C Kharkwal (2012). Algae as future drugs. *Asian Journal of Pharmaceutical and Clinical Research,* Volume 5, Issue l4, Pages 23-30.
10. Maria Filomena de Jesus Raposo, Alcina Maria Bernardo de Morais and Rui Manuel Santos Costa de Morais (2015). *Marine Polysaccharides from Algae with Potential Biomedical Applications.* Marine drugs 2967-3028.

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