A COMPARATIVE STUDY ON THE PHYTOCHEMICAL ANALYSIS OF *CHAETOMORPHA ANTENNINA* AND *CERATOPHYLLUM SUBMERSUM*

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ABSTRACT

Macroalgae or seaweed the most accessible marine resources of the coastal zone occupy potentially important place as a source of biomedical compounds. Seaweeds are the source of amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones, alkenes, cyclic polysulphides, acrylic acid, saturated and unsaturated fatty acids. Many bioactive and pharmacologically important compounds such as alginate, carrageen and agar as phycocolloids have been obtained from seaweeds and is used in medicine and pharmacy. Quality of protein and lipid in seaweeds are most acceptable for consumption compared to other vegetable mainly due to their high content in essential amino acid and relatively high level of unsaturated fatty acid. Fresh and dried seaweeds are utilized as human food. In recent years, many marine resources have attracted attention in the search for bioactive compounds to develop new drugs and health foods. Researchers found that algae contain remarkable amount of components valuable for human health. Synthetic drugs are not only expensive but are also often with adulterations and side effects. Therefore, there is a need to search for new strategies to control microbial infections. Now-a-days, the use of antibiotics has increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs due to indiscriminate use of antibiotics. Decreased efficiency and resistance of pathogen to antibiotics has necessitated the development of new alteration. The present study is aimed at identifying and comparing the Phytochemical properties of seaweed extracts of *Chaetomorpha antennina* and *Ceratophyllum submersum*.

KEYWORDS: *Macroalgae, Proteins, Lipids, Phytochemical Analysis, Chaetomorpha antennina* and *Ceratophyllum submersum.*

INTRODUCTION

Seaweeds have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential (**Emasushan Minj *et.al.*, 2017)**. Antibiotic treatment of microbial diseases has been applied for many years. The prevention and treatment of these infectious diseases by applying products from macroalgae appears as a possible alternative (**Manchu *et.al.*, 2016)**. Hence, the interest in macroalgae has been increased during the last years. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. From the literature, it is observed that the edible seaweeds contain a significant amount of the protein, vitamins and minerals, which are essential nutrition for human. Seaweed extracts are considered to be a rich source of phenolic compounds. The large majority of these are terpenes, but fatty acids are also common with nitrogenous compounds (**Sujatha *et.al.*, 2015)**.

Numerous substances are identified as antimicrobial agents from algae such as Chlorellin derivatives, acrylic acid, halogenated aliphatic Phenolic inhibitors etc. Nowadays there is an increasing demand for biodiversity in screening programmes for selecting therapeutic drugs from natural products, the marine organisms; especially seaweeds are of immense interest since they have a broad range of biological activities such as antibacterial, antifungal, antiviral, antitumour, anti-inflammatory and antioxidants. Seaweeds have been recognized as potential sources of antibiotic substances. The production of antimicrobial activities was considered to be an indicator of the bioactive secondary metabolites ((**Subathraa K and TV Poonguzhali, 2013 and M. S. Leelavathi and Prasad M. P, 2015)**.

The antibacterial agents found in the algae include terpenoid, phlorotannins, acrylic acid, phenolic compounds, steroids, halogenated ketone and alkaline, cyclic polysulphides and fatty acids (**Soad *et.al.*, 2015)**. In a number of marine algae antibacterial activities are attributed to the presence of acrylic acid (**Elayarani D, 2016)**. Researches show that the antibacterial activity of algae is due to their ability to synthesize respectively nitrogen compounds and diterpenes in Chlorophyceae, mixed halogenated terpenes in Rhodophyceae and metabolites of aromatic origin in Phaeophyceae (**Tofighi, *et.al.*, 2015)**. Among the macroalgae Chlorophyceae members form an important group of seaweeds having rich source of potential new drugs (**Vinoth Kumar *et.al.*, 2015)**.

Thus Marine Seaweeds draws an extraordinary wealth of mineral elements from the sea that can account for up to 36% of its dry mass. The mineral nutrients present in seaweeds are diverse, the main elements being iodine and calcium. The mineral macronutrients include sodium, calcium, magnesium, potassium, chlorine, sulfur and phosphorus and the micronutrients include iodine, iron, zinc, copper, selenium, molybdenum, fluoride, manganese, boron, nickel and cobalt. Research also depicts the important functional activities of marine seaweeds, such as antioxidant, anti-mutagen and anticoagulant effect, antitumor activity, and an important role in the modification of lipid metabolism in the human body shown by the marine seaweeds. The chemical composition of seaweeds varies with species, habitat, maturity and environmental conditions. The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contain complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans (**Tejavathi DR and Jayasree DR, 2013, Rashed .K, Medda. R and Pintus.F 2016, Valentina *et.al.*, 2015 and Sanjay *et.al.*, 2013)**.

CLASSIFICATION - GREEN ALGAE

Binomial name : *Chaetomorpha antennina* (Kutzing), 1847
Kingdom : Plantae
Division : Chlorophyta
Class : Ulvophyceae
Order : Cladophorales
Family : Cladophoraceae
Genus : Chaetomorpha
Species : antennina

Ceratophyllum submersum, is commonly known as soft hornwort. It is a submerged, free-floating aquatic plant (**Gunasekara *et. al.*, 2017)**. *Ceratophyllum* is a cosmopolitan genus of flowering plants commonly found in ponds, marshes and quiet streams in tropical and in temperate regions (**Seedevi *et. al.*, 2013)**. It is the only genus in the family Ceratophyllaceae. They are usually called as coontails (**Sahaa *et. al.*, 2008)**. *Ceratophyllum* extracts have been reported to possess antidiarrheal, antipyretic and carminative effect (**Rashed .K, Medda. R** and **Pintus. F, 2016)**. Traditionally *Ceratophyllum* is used to cure ulcer, diarrhea and wound besides having novel kidney protective action (**Aseer Manilal *et. al.*, 2012)**.

AQUATIC WEEDS

Binomial name : *Ceratophyllum submersum*
Kingdom : Plantae
Clade : Angiosperms
Order : Ceratophyllales
Family : Ceratophyllaceae
Genus : Ceratophyllum
Species : submersum

Many disorders in human organism such as atherosclerosis, arthritis, Alzheimer disease, Cancer etc.**,** may be the result of increased concentrations of free radicals in an organism (**Meenakshi Bhattachaerjee, 2016).**

Reactive oxygen species (ROS) and nitrogen (RNS) species are the most frequent pro-oxidants, either originating from normal metabolism or induced by UV radiation and different pollutants (**Maria Filomena *et.al.*, 2015)**. Harmful effects of disturbed antioxidant-prooxidant balance can be largely prevented by the intake of antioxidant substances. Antioxidants have already been found in seaweeds (**Bharathiraja *et.al.*, 2015)**. Due to their natural origin, the antioxidants obtained from seaweeds are of greater benefit in comparison to synthetic ones (**M. S. Leelavathi** and **Prasad M. P, 2015).** The use of natural antioxidants from seaweeds does not induce side effects, while synthetic antioxidants are found to have genotoxic effect (**Mitali Priyadharshini Pati *et. al.*, 2016)**. The present study is aimed at identifying and comparing the Phytochemical properties of seaweed extracts of *Chaetomorpha antennina* and *Ceratophyllum submersum*.

MATERIALS AND METHODOLOGY

COLLECTION, PROCESSING AND EXTRACTION OF SEAWEEDS

Chaetomorpha antennina and *Ceratophylum submersum* aquatic plants were collected from a pond in Ajax (Thiruvottiyur) 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 made into a fine powder using an electric blender. The materials required for the extraction of Seaweeds are Aquatic plant, *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.

PHYTOCHEMICAL ANALYSIS- QUALITATIVE METHOD

The following tests such as Tests for Carbohydrates (Bial's test), Protein (Xanthoproteic test), Amino acids (Ninhydrin test), Glycosides, Cardiac Glycosides (Keller Kiliani test), Alkaloids (Dragendroff's test), Tannin, Flavonoids, Phenols, Terpenoids (Salkowshi's test), Saponins (Froth test), Resins, Steroids, Emodins, Lipids (Solubility test) and Vitamin C were carried out using standard protocols.

RESULTS AND DISCUSSION

COLLECTION OF SAMPLE

Green algae *Chaetomorpha antennina* was collected from the shore of Royapuram beach.

Figure 1 : *Chaetomorpha antennina*

The aquatic weeds *Chaetomorpha antennina* and *Ceratophyllum submersum* were washed with water, the calcareous, stones and epiphytes were removed by manual setting and intensively washed with tap water and then again with distilled water. The aquatic weeds were shade dried for 7-10 days. The bioactive compounds from seaweeds were obtained by using the solvent extraction. Methanol was taken in a conical flask and the weeds were weighed using an electronic weighing balance and added to the solvents in the ratio of 1:10 and left for 2-3 days and the extract was filtered using a muslin cloth **(Figures 1 - 4)**.

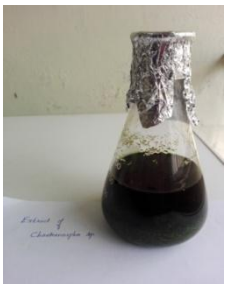

Figure 2 : *Chaetomorpha* Extract

Figure 3 : *Ceratophyllum submersum*

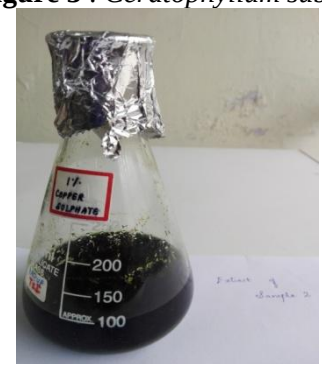

Figure 4 : *Ceratophyllum submersum* extract

PHYTOCHEMICAL ANALYSIS- QUALITATIVE ANALYSIS

In present study, the green algae *Chaetomorpha antennina* and *Ceratophyllum submersum* were used to screen for the presence of secondary metabolites. The phytochemicals such as carbohydrates, protein, alkaloids, phenols, tannin, flavonoids, lipids, terpenoids were the secondary metabolites present in the seaweeds. The sample showed the presence of Phytochemicals. Maximum phytochemicals were are present in the aquatic weeds **(Table 1)**.

S.NO	PHYTOCHEMICALS	*Chaetomorpha extract*	*Ceratophyllum extract*
1.	Carbohydrates	+	+
2.	Proteins	+	+
3	Aminoacids	+	+
4	Glycosides	+	+
5	Cardiac glycosides	+	+
6	Alkaloids	+	+
7	Tannin	+	+
8	Flavonoids	+	+
9	Phenols	+	+
10.	Terpenoids	+	+
11.	Saponin	+	+

12	Resins	-	+
13	Steroids	+	+
14	Emodins	-	+
15	Vitamin C	+	-
16	Lipids	+	+

Table 1: Phytochemical analysis of methanol extract of *Chaetomorpha antennina and Ceratophyllum submersum*

QUANTITATIVE ANALYSIS

ESTIMATION OF CARBOHYDRATES - GLUCOSE (STANDARD)

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

Table 2: Results for Glucose standard curve

SAMPLE	O.D (490nm)	Concentration of Glucose (mg/ml)
C.antennina	0.10	46
C.submersum	0.12	54

Table 3 : Total carbohydrate contents of *C.antennina* and *C.submersum*

The standard values of Glucose is shown in **Table 2**. By phenol sulphuric acid method, 46mg/ml of carbohydrates in *C.antennina* and 54mg/ml of carbohydrates in *C.submersum* were observed (**Table 3**).

ESTIMATION OF PROTEINS

BOVINE SERUM ALBUMIN- BSA (STANDARD)

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

Table 4: Results for Bovine serum albumin standard curve

SAMPLE	O.D (620nm)	Concentration of protein (mg/ml)
C.antennina	0.52	74
C.submersum	0.50	70

Table 5: Total protein contents of *C.antennina* amd *C.submersum*

The standard values of BSA is shown in **Table 4**. By Lowry's method, 74mg/ml of protein content in *C.antennina* and 70mg/ml in *C.submersum* were observed in **Table 5**.

ESTIMATION OF AMINOACIDS - LEUCINE (STANDARD)

S.NO	CONCENTRATION OF AMINOACID (mg/ml)	O.D (570nm)
1	20	0.02
2	40	0.04
3	60	0.06
4	80	0.08
5	100	0.1

Table 6: Results for Aminoacid standard curve

SAMPLE	O.D (620nm)	Concentration of aminoacid (mg/ml)
C.antennina	0.09	90

Table 7: Total Aminoacid contents of *C.antennina* and *C.submersum*

The standard values of Leucin is shown in **Table 6**. By Ninhydrin method, 90mg/ml of aminoacid content in *C.antennina* and 70mg/ml of aminoacid in *C.submersum* were estimated **Table 7**.

ESTIMATION OF ALKALOIDS

SAMPLES	Conc.of alkaloids (gm/ml)	Percentage of Alkaloids
Chaetomorpha antennina	**0.3**	**30%**
Ceratophyllum submersum	**0.7**	**70%**

Table 8 : Alkaloid contents of *C.antennina* and *C.submersum*

The dry weight of alkaloids were estimated in percentage**.** 0.3gm/ml of alkaloids in *C.antennina* and 0.7gm/ml of alkaloids in *C.submersum* were recorded.

ESTIMATION OF TOTAL LIPIDS

SAMPLE	WEIGHT OF THE BEAKER (1)	WEIGHT OF BEAKER WITH RESIDUE (2)	WEIGHT OF LIPIDS (2-1)
C.antennina	**70.5**	**70.8**	**0.3**
C.submersum	**73.8**	**74.3**	**0.5**

Table 9 : Alkaloid contents of *C.antennina* and *C.submersum*

The total lipid content of the aquatic weed extracts obtained in the study is tabulated in **Table 9)**. 0.3mg/ml dry weight of lipid residue in *C.antennina* and 0.5mg/ml dry weight of lipid residue was present in *C. Submersum.*

ESTIMATION OF PHENOL CONTENT

SAMPLES	OD at 765nm
C.antennina	0.306
C.submersum	0.886

Table 10 : Phenolic content of of *C.antennina* and *C.submersum*

The total phenolic content was measured using Gallic acid equivalent (GAE)g of the extracts. The total phenolic content of the *C.antennina* was 0.3gm/ml of GAE/g and of *C.submersum* was about 0.88gm/ml of GAE/g. Phenols are known as carbolic acid. The antioxidant activity of phenols plays an important role in the absorption or neutralization of free radicals.

ESTIMATION OF TANNIN

TANNIC ACID (STANDARD)

S.NO	CONCENTRATION	O.D(720nm)
1	20	0.24
2	40	0.50
3	60	0.66
4	80	0.87
5	100	1.20

Table 11 : Standard curve value of Tannic acid

SAMPLES	O.D(720nm)	Conc.(mg/ml)
C.antennina	0.85	72
C.submersum	0.96	82

Table 12: Total Tannin contents of *C.antennina* and *C.submersum*

The total tannin content was measured in the sample. Tannic acid was used as a standard **(Table 11)**. The total tannin content in *C.antennina* was found to be 72 mg/ml and in *C.submersum* was found to be 82 mg/ml (**Table 12**).

ESTIMATION OF FLAVONOIDS

SAMPLES	O.D(415nm)
C.antennina	0.393
C.submersum	0.395

Table 13 : Total Flavonoids contents of *C.antennina* and *C.submersum*

The samples were read at 415nm to estimate Flavonoids. *C.antennina* has 0.393mg/ml of Flavonoids and *C.submersum* contained 0.395mg/ml of Flavonoids (**Table 13)**.

ESTIMATION OF STEROIDS

CHOLESTROL (STANDARD)

S.NO	CONC. OF STANDARD	O.D (540nm)
1	50	0.24
2	100	0.52
3	150	0.77
4	200	0.88
5	250	1.09

Table 14 : Standard value of Cholesterol

SAMPLE	O.D(540nm)	CONC.OF STEROIDS (mg/ml)
C.antennina	0.24	50
C.submersum	0.40	80

Table 15 : Steroid contents of *C.antennina* and *C.submersum*

The steroid content was measured in both the samples. Cholesterol was used as a standard (**Table 14)**. The steroid content in *C.antennina* was found to be 50mg/ml and in *C.submersum* to be 80mg/ml **(Table 15)**.

ESTIMATION OF GLYCOSIDES

SAMPLE	O.D(495nm)	Percentage of Glycosides
C.antennina	0.73	4.29%
C.submersum	0.84	4.94%

Table 16 : Glycoside contents of *C.antennina* and *C.submersum*

Using Baljet's reagent, the glycoside content of the samples were estimated. *C.antennina* contains 4.29% of glycosides and *C.submersum* contained 4.94% of glycosides **(Table 16)**.

ESTIMATION OF TERPENOIDS

SAMPLE	WEIGHT OF DRY RESIDUE	PERCENTAGE OF TERPENOIDS
C.antennina	0.15	1.5%
C.submersum	0.13	1.3%

Table 17 : Percentage of Terpenoids of *C.antennina* and *C.submersum*

The sample soaked in alcohol was extracted using petroleum ether. 1.5% of terpenoids was present in *C.antennina* and 1.3% in *C.submersum* (**Table 17**).

CONCLUSION

Bacteriostatic and bactericidal activity of marine algae has been extensively studied by several researchers. The antibacterial agents found in the algae include terpenoid, phlorotannins, acrylic acid, phenolic compounds, steroids, halogenated ketone and alkaline, cyclic polysulphides and fatty acids. In a number of marine algae, antibacterial activities are attributed to the presence of acrylic acid. Researches show that the antibacterial activity of algae is due to their ability to synthesize respectively nitrogen compounds and diterpenes in Chlorophyceae, mixed halogenated terpenes in Rhodophyceae and metabolites of aromatic origin in Phaeophyceae. Among the macroalgae Chlorophyceae members form an important group of seaweeds having rich source of potential new drugs. Hence the present study was aimed at identifying and comparing the Phytochemical properties of seaweed extracts of *Chaetomorpha antennina* and *Ceratophyllum submersum*.

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