ORIGINAL RESEARCH PAPER

Botany

KEY WORDS: Phytochemical screening, Antioxidant activity of red algae

ANALYSIS OF PHYTOCHEMICALS AND ANTIOXIDENTS FROM RED SEAWEEDS(HALYMINIYA DILATE AND LIAGORA ALBICANCE) COLLECTED FROM RAMESWARAM, TAMIL NADU

P.Yogarajalakshmi Research scholar, Department of Botany, Queen Mary's college, Chennai-600004

T.V.Poonguzhali* Associate Professor, Department of Botany, Queen Mary's college, Chennai-600004*Corresponding Author

ABSTRACT

Seaweeds are marine macroscopic algae which form an important components of marine living organism. This study was carried out to detect presence of some secondary metabolites that they have the secondary metabolites like phytochemicals have been extensively investigated as a source of medicinal agents seaweeds are becoming a viable source of biologically active composites with a hopeful application as nutraceuticals, functional food components. Natural products from marine algae have attracted the attention of biologists and chemists the world over for the last five year **METHODS:** The aim of present study was to perform the qualitative and quantitative phytochemical analysis of red seaweed Halymiya dilate and Liagora albicance. DPPH Radical scavenging and cytotoxicity screening were carried out in methanol extract of 2 macro algae as per standard methods with few modifications **RESULT** Among the 2 seaweeds, H.dilata showed the maximum number of active constituents in the methanol extract Likewise. H.dilata showed maximum number of compounds in petroleum ether and methanol. L.albicance showed the maximum number of compounds acetone and methanol. The methonal extract showed the highest radical scavenging activity(IC 50 VALUE) was methanol extract when tested by DPPH in both H.dilata and L.albicance

INTRODUCTION

Macroscopic marine algae, popularly known as seaweeds, form one of the important living resources of the ocean. Seaweeds have been one of the richest and most promising sources of bioactive primary and secondary metabolites characterized by a broad spectrum of biological activities.

Halymenia dilatata belongs to the family Halymeniaceae and is found distributed in the coastal lines of Asia (Tamil nadu, India, China, Taiwan, South-west and South-east Asia), Pacific Islands, Africa, Australia and New Zealand. The algae look like clumps of several sheets, with 10-20cm long thallus which are translucent, flexible, slippery and slimy. Edges of the thallus are smooth often in regular ruffles and the surface may be plain or might have large paler blotches or mottles. Colour of the thallus may be brownish-orange, dark pink to reddish depending on the geographical location of occurrence.

Liagora albicance Eight species of the genus Liagora (Liagoraceae, Rhodophyta) are reported from the mandabam coast Rameswaram. It is the independent species The current concept of the genus Liagora J.V. Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development,including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott, 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have commented on the apparent inequity of Liagora amongst the numerous precisely defined, related genera in the Liagoraceae (e.g. Yamadaella I.A. Abbott, 1970), suggesting that, based on these reproductive differences, the genus should be sub divided. This process has now been underway for some time. Earlier studies were based on morphol- ogy (e.g. Huisman & Kraft, 1994; Huisman & Schils, 2002), but more recently DNA sequence analyses have been incorporated, these generally supporting interpretations based on morphology (e.g. Huisman et al., 2006). Thus far, three genera (Ganonema K.C. Fan & Yung-C. Wang, Izziella Doty and Titanophycus Huisman, G.W. Saunders & A.R. Sherwood) have been segregated from (or resurrected from the synonymy of) Liagora The current concept of the genus Liagora J.V. Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott, 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have commented on the apparent inequity of Liagora amongst the numerous precisely defined, related genera in the Liagoraceae (e.g. Yamadaella I.A. Abbott, 1970), suggesting that, based on these reproductive differences, the genus should be sub- divided. This process has now been underway for some time. Earlier studies were based on morphol- ogy (e.g. Huisman & Kraft, 1994; Huisman & Schils, 2002), but more recently DNA sequence analyses have been

incorporated, these generally supporting interpretations based on morphology (e.g. Huisman et al., 2006). Thus far, three genera (Ganonema K.C. Fan & Yung-C. Wang, Izziella Doty and Titanophycus Huisman, G.W. Saunders & A.R. Sherwood) have been segregated from (or resurrected from the synonymy of) Liagora rrent concept of the genus Liagora J.V. Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott, 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have commented on the apparent inequity of Liagora amongst the numerous precisely defined, related genera in the Liagoraceae (e.g. Yamadaella I.A. Abbott, 1970), suggesting that, based on these reproductive differences, the genus should be sub- divided. This process has now been underway for some time. Earlier studies were based on morphol- ogy (e.g. Huisman & Kraft, 1994; Huisman & Schils, 2002), but more recently DNA sequence analyses have been incorporated, these generally supporting interpretations based on morphology (e.g. Huisman et al., 2006). Thus far, three genera (Ganonema K.C. Fan & Yung-C. Wang, Izziella Doty and Titanophycus Huisman, G.W. Saunders & A.R. Sherwood) have been segregated from (or resurrected from the synonymy of) Liagora The current concept of the genus Liagora J.V. Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott, 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have commented on the apparent inequity of Liagora amongst the numerous precisely defined, related genera in the Liagoraceae (e.g. Yamadaella I.A. Abbott, 1970), suggesting that, based on these reproductive differences, the genus should be sub- divided. This process has now been underway for some time. Earlier studies were based on morphol- ogy (e.g. Huisman & Kraft, 1994; Huisman & Schils, 2002), but more recently DNA sequence analyses have been incorporated, these generally supporting interpretations based on morphology (e.g. Huisman et al., 2006). Thus far, three genera (Ganonema K.C. Fan & Yung-C. Wang, Izziella Doty and Titanophycus Huisman, G.W. Saunders & A.R. Sherwood) have been segregated from (or resurrected from the synonymy of) Liagora The current concept of the genus Liagora J.V. Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott, 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have commented on the apparent inequity of The current concept of the genus Liagora J.V. Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott, 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have commented on the apparent inequity of The current concept of the genus Liagora J.V. Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott, 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have commented on the apparent inequity of The current concept of the genus Liagora J.V. Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have commented on the apparent inequity of The current concept of the genus Liagora J.V. Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott, 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have commented on the apparent inequity of The current concept of the genus Liagora J.V. Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott, 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have commented on the apparent inequity of The current concept of the genus Liagora J.V. Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott, 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have commented on the apparent inequity of The current concept of the genus Liagora J.V. Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott, 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have

commented on the apparent inequity of The The current concept of the genus Liagora J.V. Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott, 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have commented on the apparent inequity of Liagora amongst the numerous precisely defined, related genera in the Liagoraceae (e.g. Yamadaella I.A. Abbott, 1970), suggesting that, based on these reproductive differences, the genus should be sub- divided. This process has now been underway for some time. Earlier studies were based on morphol- ogy (e.g. Huisman & Kraft, 1994; Huisman & Schils, 2002), but more recently DNA sequence analyses have been incorporated, these generally supporting interpretations based on morphology (e.g. Huisman et al., 2006). Thus far, three genera (Ganonema K.C. Fan & Yung-C. Wang, Izziella Doty and Titanophycus Huisman, G.W. Saunders & A.R. Sherwood) have been segregated from(or resurrected from the synonymy of) Liagora. Lin et al. (2011) undertook a morphological and molecular study of the species of Liagora and the closely related Izziella from Taiwan and reinforced The current concept of the genus Liagora J.V Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have commented on the apparent inequity of Liagora amongst the numerous precisely defined, related genera in the Liagoraceae (e.g. Yamadaella I.A. Abbott, 1970), suggesting that, based on these reproductive differences, the genus should be sub- divided. This process has now been underway for some time. Earlier studies were based on morphol- ogy (e.g. Huisman & Kraft, 1994; Huisman & Schils, 2002), but more recently DNA sequence analyses have been incorporated, these generally supporting interpretations based on morphology (e.g. Huisman et al., 2006). Thus far, three genera (Ganonema K.C. Fan & Yung-C. Wang, Izziella Doty and Titanophycus Huisman, G.W. Saunders & A.R. Sherwood) have been segregated from (or resurrected from the synonymy of) Liagora. Lin et al. (2011) undertook a morphological and molecular study of the species of Liagora and the closely related Izziella from Taiwan and reinforced The current concept of the genus Liagora J.V. Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused car- pogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse goni- moblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts inter- mingle with the involucral filaments, whereas they remain separate in the second type (Abbott, 1990; Huisman, 2002, 2006). Several authors (e.g. Huisman, 2002; Huisman & Schils, 2002; Lin et al., 2011) have commented on the apparent inequity of Liagora amongst the numerous precisely defined, related genera in the Liagoraceae (e.g. Yamadaella I.A. Abbott, 1970), suggesting that, based on these reproductive differences, the genus should be sub- divided. This process has now been underway forsome time. Earlier studies were based on morphol- ogy (e.g. Huisman & Kraft, 1994; Huisman & Schils, 2002), but more recently DNA sequence analyses have been incorporated, these generally supporting interpretations based on morphology (e.g. Huisman et al., 2006). Thus far, three genera (Ganonema K.C. Fan & Yung-C. Wang, Izziella Doty and Titanophycus Huisman, G.W. Saunders & A.R. Sherwood) have been segregated from (or resurrected from the synonymy of) Liagora. Lin et al. (2011) undertook a morphological and molecular study of the species of Liagora and the closely related Izziella from Taiwan and reinforcedThe current concept of the genus Liagora J.V.Lamouroux (Liagoraceae) is rather broadly defined and includes species displaying at least three types of carposporophyte development, including: (1) a diffuse gonimoblast with fused carpogonial branches; (2) a compact gonimoblast with fused carpogonial branches; and (3) a diffuse gonimoblast with unfused carpogonial branches. In the first and the third types, the gonimoblasts intermingle with the involucral filaments, whereas they remain separate in the second type (Huisman, et a.,l 2002, 2006). Several authors (e.g.Huisman, 2002; Huisman & Schils, 2002; Lin et al.,2011) have commented on the apparent inequity of Liagora amongst the numerous precisely defined, related genera in the Liagoraceae (e.g. YamadaellaI.A. Abbott, 1970), suggesting that, based on these reproductive differences, the genus should be subdivided but more recently DNA sequence analyses have been incorporated, these generally supporting interpretations based on morphology (e.g. Huisman et al., 2006). Thus far, three genera (Ganonema K.C. Fan & Yung-C. Wang, Izziella Doty and Titanophycus Huisman, G.W. Saunders& A.R. Sherwood) have been segregated from (or resurrected from the synonymy of) Liagora.Lin et al.(2011)

Algae is a living organism. It is eukaryotic in nature its naturally accouring photosynthetic pigment. Plants produce a vast number of natural compounds, called secondary metabolites.

Rhodophyta are red because of the presence of the pigment phycoerythrin. Pigments are chemical compounds which reflect only certain wavelengths of visible light. This is very colourfull more important then reflection of light is the ability of pigments to absorb certain wavelengths. Some Rhodophytes have very little phycoerythrin, and may appear green or bluish from the chlorophyll and other pigments present. A great number of modern medicines have been derived from plants that are considered as important sources of medicinal agents to treat different diseases Algae include four main divisions; namely, Red algae (Rhodophya), Brown Algae (Phycophyta), Green Algae (Chlorophyta). Although, Seaweeds which are macroscopic, multicellular, and marine algae, are divided into three categories; red, green and brown organisms comprises about 30000 species. In most of Asian countries, seaweeds are traditionally traded as food items including sushi wrappings, seasonings, condiments, and vegetables (El Gamal, 2010; Mark et al., 2016).

Seaweeds have been reported to contain secondary metabolites which include alkaloids, glycosides, flavonoids, saponins, tannins, steroids, and related active metabolites, and have been extensively used in the drug and pharmaceutical industry (Eluvakkal et al., 2010). Recently, researches have proved that compounds originating from marine algae exhibit various biological activities (Kim and Wijesekara, 2010; Wijesekara and Kim, 2010; Wijesekara et al., 2010 and (Wijesekara et al., 2011).Therefore, there is a new trend to isolate novel bioactive compounds and constituents from edible seaweeds (Li et al., 2011).

Algae are used for many purposes: Algae is a mixed group of plants with an extensive fossil history. The macroscopic marine algae have been closely associated with human life and are being exhaustively used in

numerous ways as a source of food, feed, fertilizer, medicine and chiefly for economically important phycocolloids

The phytochemicals from marine algae are extensively used in various industries such as food, confectionary, textile, pharmaceutical, dairy and paper mostly as gelling, stabilizing and thickening agents. Seaweeds are marine macroalgae which are the renewable living resources.

There are also many secondary ways for using algae: producing alginates and derivate of algae used in industry. Nowadays researchers use to analyze algae for medical purposes, because they have a strong potential against many diseases, in alimentation because they act like protective and functional additives (New Man et al., 2003; Horincar et al.,2011) Algae are found everywhere: in the sea, rivers, lakes, soil, walls, and as symbiotic in animal and plants.

Marine algae have been consumed. They are invitro culture medium are food fodder and rich in vitamins, minerals, dietary fiber, protein, and various functional polysaccharides (Kuda et al. 2005). Moreover, seaweeds are considered to be a rich source of antioxidant. Hence, many types of seaweeds have been examined to identify new and effective antioxidant compounds, as well as to elucidate the mechanisms of cell proliferation and apoptosis (Siriwardhana et al. 2004; Athukorala et al. 2005; Heo et al. 2005a, b, c; Park et al. 2005). Among all the substance contain in seaweed antioxidant has been a major attraction. Seaweed are exposed to free radical and strong oxidizing agents due to there reaction between sun light and oxygen Therefore seaweeds are considered as an important source of antioxidant substances that may also correspond to protect human body against reactive oxygen species (Plaza et al., 2008). A variety of dietary antioxidants can be considered as effective agents to reduce oxidative stress which can give a major impact in development of diseases including cancer Generation of reactive oxygen species and other free radical by ultraviolet radiation are obstructed by antioxidants to prevent oxidative stress. Excessive reactive oxygen species is associated with carcinogenesis due to DNA damage and mutation (Khan et al., 2008). The main mechinism of food antioxidant also is the free radical scavenging(Pokorny et al., 2001). DPPH free radical scavenging assay is a rapid, simple, cheap and widely used methods to measure the capacity of a substance as a hydrogen donor or scavenge free radical. It is used to assess the antioxidant activities in food. DPPH is a stable dark purple free radical (Kedare and Singh, 2011). The addition of DPPH with hydrogen donor substance will reduce the stable DPPH free radical hence will cause the purple decolorization (Matsukawa et al., 1997) to produce yellow DPPH-H (Lu et al., 2010).The % inhibition data were plotted against log concentration fitted a graph and half –maximal inhibitory concentration (IC50) value was calculated by linear regression analysis. Recently, the active antioxidant compounds were identified as fucoxanthin, phlorotannins, and other polyphenolic compounds (Yan et al. 1996; Yan et al. 1999; Yoshie et al. 2002).

MATERIALS AND METHODS

COLLECTION AND AUTHENTICATION OF SAMPLE

Halymenia dilatata and Liagora albicance were collected from Rameswaram coast, Tamilnadu. Algal material were washed well with tap water. They were dried under shade further dried at room temperature and were made into fine powder and kept in a air tight container and stored. (The samples were authenticated from BSI (Botanical Survey of India, Coimbatore).The samples were air dried at room temperature and ground to powder and stored in plastic bags in a dry place until use.

Preparation of the extracts

Ten grams of powdered samples were packed in Soxhlet apparatus and extracted with (1:10) solvents like methanol, aqueous for 8 h, and the filtrate was collected (crude extracts) and stored in the refrigerator until further use.

Phytochemical analysis

To detect the presence of following biochemicals by standared qualitative and quantitative phytochemical procedure

PHYTOCHEMICAL SCREENING

QUALITATIVE PHYTOCHEMICAL SCREENING

The algal extracts were dissolved in ethanol which were qualitatively tested for the presence of various phytochemical constituents (Brain and Turner, 1975; Sofowora, 1982; Treas and Evans, 1983, Kim, 1986; Harborne, 1991).

Detection of Flavonoids

a. Ferric chloride test:

A few drops of neutral ferric chloride solution were added to one mL each of the above ethanolic solution. Formation of blackish red colour indicates the presence of flavonoids.

b. Alkaline reagent test

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

c. Lead acetate test :

The extract was treated with a few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids. Orange to crimson colour shows the presence of flavonones.

1. Test for Glycosides

50 mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests

Borntrager's test

To 2 mL of filtered hydrolysate, 3 mL of choloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presence of glycosides.

2. Test for steroids

LibermannBurchard test

To 2 mL of test solution and minimum quantity of chloroform are added with 3-4 drops of acetic anhydride and one drop of concentrated H_2SO_4. Formation of purple colour changes into green color that indicates the presence of steroids.

3. Test for Phenolic compounds

a. Ferric Chloride test

The extract (50 mg) is dissolved in 5 mL of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compound.

b. Lead acetate test

The extract (50 mg) is dissolved in 100 ml of distilled water. To this 3 mL of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

4. Detection of Terpenoids

Salkowski's test

About 100 mg of dried extract was dissolved in 2mL of

chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface was an indicative of the presence of terpenoids.

5. Test for Saponins
Froth test
The extract (50 mg) is diluted with distilled water and made upto 20 mL. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins.

6. Detection of Tannins
Ferric chloride test
To the filtrate, a few drops of ferric chloride solution were added. A blackish precipitate indicates the presence of tannins

7. Detection of Anthraquinones
To 50 grams of the algal 10ml distilled water was dissolved 2mL of extract was taken to which 1mL of dilute ammonia solution was added and shaken vigorously. Pink colour in ammonia layer indicated the presence of anthraquinones.

8. Cardiac Glycosides
Keller Killiani's test
To 100mg of extract in 1ml of glacial acetic acid containing one drop of ferric chloride solution was dissolved. This was then under layed with 1mL of conc. sulphuric acid. A brown ring obtained at the interface indicated the presence of a cardenolides.

9. Detection of Quinones
One mL of each of the various extracts was treated separately with alcoholic potassium hydroxide solution. Quinines give coloration ranging from red to blue.

QUANTITATIVE PHYTOCHEMICAL
Estimation of total Tannin
Total Tannin in algal extract were determind by
To 100 l of extract from 1mg/Ml solution 900 l methonal was added.

Then 1ml of 1:10 diluted folin ciocalteau reagent was added.

To this 1ml of 20% sodium carbonate solution was also added & Shaked well and incubated at room temperature for 30 mins in dark

Estimation of flavonoids
Total Flavonoids in algal extract were determind by To 500ul of extract from 1mg/ML solution, 500 l methonal was added To this 1ml of 5% sodium nitrite solution is added Then 1ml of 10% aluminium chloride is added & shaken well It was incubated for further 5 min at room temperature To this 1 mL of 1 M NaOH solution was added and then incubated for 30 mins in room temperature.

ESTIMATION OF PROTEIN
Total protein in algal extract were determind by To 100 ul of extract, 400 l Double Distilled water was added. To this 2.5 ml of CBB dye was added.

ESTIMATION OF CARBOHYDRATE
To 50 l of extract, 450 ul Distilled water was added. Then 1ml of concentrated sulfuric acid was added. To this 1ml of 50% phenol solution was also added & shaken well and incubated at room temperature for 15 mins. Absorbance of supernatant was taken at 490nm

ESTIMATION OF TOTAL PHENOL:
To 100 ul of extract from 1mg/ml solution 900 ul methonal was added. Then 1ml of 1:10 diluted folin ciocalteau reagent was added.

To this 1ml of 20% sodium carbonate solution was also added & Shaked well and incubated at room temperature for 30 mins in dark Absorbance of supernatant was taken at 765 nm.

Result and discussion
In the present study preliminary phytochemical screening 12 different chemical compound (alkaloids, steroids, tannins, saponins, flavonoids, phenols, protiens and glycosides) Were performed with methonal extract $DPPH^{\cdot}$ radical scavenging activity:The radical scavenging capacity of the ethanol extracts was measured based on DPPH (1, 1- diphenyl 2-picrylhydrazyl) radical scavenging activity. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (20-120 g/mL) of samples. The mixture was then allowed to stand for 30 min incubation in dark. One mL methanol mixed with 1mL DPPH solution was used as the control. The decrease in absorbance was measured at 517 nm. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated. All experiment were repeated three times independently the degree of decolourization of DPPH from purple to yellow indicated the scavenging effect of DPPH was calculated using the following equation

Percentage scavenging effect = 1-[(AControl-Asample/Acontrol]* 100
Asample = Absorption of algal extract with DPPH in methanol,
Acontrol= Absorption of DPPH in Methanol

3.0 Result and Discussion
Seaweed contribution to its efficacy as neutraceutical and traditional medicine based on the presence of their chemical compounds.Some seaweeds like climatic condition, season, species, subspecies. Harvesting and the method used for extraction of compounds will devastate the chemical composition of the extract. They contain different vitamins, minerals, trace elements, proteins and bioactive substance. In our present study three different extract methanol and petroleum ether highest number of compounds whereas acetone are having the least possible number of phytochemical compounds

In the present study quantitative and qualitative phytochemical screening was carried out with methanol pertrolium ether and chloroform extract was the selected from the marine algal species Preliminary phytochemical screening of 10 different chemical compounds were tested in four different extracts of H.dilata and L.albicance extract with the presence of phytochemical constituents like alkaloids, glycosides, flavonoids, saponins, tannins, steroids, quinines, Cardiac Glycosides, Terpenoids Anthraquinones .

The preliminary phytochemical test H.dilata Flavonoids and glycosides are presented in methanol, chloroform, petroleum ether. Steriods are presented in petroleum ether and methonal. Steriods absent of acetone. Phenol is presented in the petroleum ether and methanol. Phenol is absent in acetone. Terbinoids is presented in the petroleum ether, acetone and methonal. Saphonin and anthroquenen is absent. Tannin is presented in the petroleum ether and methonal. Tannin is absent in acetone. Quinones is absent in petroleum ether and acetone. Quinones is present in methanol.

The preliminary phytochemical test for L.albicance about the presence of flavonoids and glycosides in methanol, chloroform, petroleum ether. Steriods are present in acetone and methanol. Steroids absent in petroleum

ether.While Phenol is presented in the petroleum ether and acetone. Terpenoids is present in the petroleum ether, acetone and methonal. Saphonin and anthroquenen is totally absent. Tannin is present in the petroleum ether methanol and acetone. Quinone is Absent in petroleum ether and acetone. Quinones is presented in methonal.

Quantitative analysis of phytochemical substance of H.dilata and L.albicance Tannin flavonoids carbohydrate protein phenol H.dilata were estimated quantitatively varied according to solvents used in extraction processes. The highest quantitative value of carbohydrate(751.3 mg GAE/g) L.albicance were estimated quantitative value of carbohydrate (688.30 mg GAE/g)

The marine macro algae H.dilata were used for evalution of antioxidant affect by DPPH assay method, various concentration of extract was checked. Methanol was taken as standard and the absorbance was read out, it changed with the concentration. Lipid Preoxidation and generation of free radicals often occur in biological and food systems. In biological systems, antioxidative damage (madhavi Et Al.,1996) and free radical generation by prooxidative from environment such as ultraviolet radiation, cigarette smoke and air pollutant (Khan et al.,2008). Solvent extraction is a process designed to isolate soluble antioxidant compound through diffusion from the solid matrix (plant tissue) using liquid matrix (solvent).The solvent used to extract the antioxidants are the methanol solvent combination with water Different polarities of organic solvents greatly influence the selection of a particular solvent for the extraction of bioactive compounds (Musa et al 2011).The total TPC value of H.dilata and l.albicans antioxidant extract showed in Table 2.This current study on the red seaweed showed the presence of antioxidant using the concentration of methanol (20%,40%,60%,80%,100%) for 72hrs could maximize the phenolics yield compare to 24hrs extraction (Matanjun et al.,2008). Many researchers have found seaweeds to be a rich source of antioxidents. Antioxidents play a major role against many diseases. Generally marine algae has compounds that has chemical defense system facilitating their survival. Antioxident are compounds that inhibit oxidation. Marine macro algae has anticancer, antimicrobial, antifungal and antioxidant activites (Asheini et al.,2017) From our results it is predicted that antioxidant activity of H.dilata was higher TPC concentration with 100 µg/ml concentration with 245.5% and L.albicance 100 µg/ml concentration 198.8% the high TPC value when may be due to the presence of both hydrophilic and hydrophobic antioxidants.

Table3 shows the result of the percentage of DPPH free radical scavenging activity of both seaweeds extracts. The percentage of DPPH freeradical scavenging activity by H.dilata extract were ranged between the 120% methanol extract showed the highest percentage of DPPH Scavenging activity present 36.17. Mean while the percentages of DPPH free radical scavenging by L.albicance extract highest percentage of DPPH free radicals Scavenging activity was showed by 100% methanol extract 35.1.

Table 1. preliminary phytochemical analysis of various solvent extract of halyminiya dilata

S.NO	phytochemical characters	Name of the test	Petroleum ether	Acetone	Methanol
1	Flavonoide	Ferric chloride test	+	+	+/-
		Alkaline reagent test	+	-	+
		Lead acetate test	+	+	+
2	Test for Glycosides	Borntrager's test	+	+	+
3	Test for steroids	Libermann Burchard test	+	_	+
4	Test for phenolic compounds	Ferric chloride test	+	+	+
		Lead acetate test	+	+	+
5	Detection of Terpenoids	Salkowski's test	+	-	+
6	Test for saponins	Froth test	_	-	_
7	Detection of Tannins	Ferric chloride test	+	+	+
8	Detection of Anthraquinones	Anthraquinones	_	_	-
9	Cardiac Glycosides	Killer killianis test	++	-	+
10	Test for Quinones	Quinones	-	-	+

Table 2. Preliminary phytochemical analysis of various solvent extract of Liagora albicance

S.NO	Phytochemical Test	Name of the test	Petroleum ether	Acetone	Methanol
1	Flavonoide	Ferric chloride test	+	+	-
		Alkaline reagent test	+	-	+
		Lead acetate test	-	+	+
2	Test for Glycosides	Borntrager's test	+	+	+
3	Test for steroids	Libermann Burchard test	-	+	+
4	Test for phenolic compounds	Ferric chloride test	+	+	+
		Lead acetate test	+	+	+
5	Detection of Terpenoids	Salkowski's test	-	-	+
6	Test for saponins	Froth test	_	-	_
7	Detection of Tannins	Ferric chloride test	+	+	+
8	Detection of Anthraquinones	Anthraquinones	_	_	-
9	Cardiac Glycosides	Killer killianis test	+/-	-	+
10	Test for Quinones	Quinones	-	-	+

Table 1Qulitative phytochemical screening of halyminiya dilata and liagora albicance

Solvent	Methonal				
	Tannin	Flavanoid	Protein	Carbohydrate	Phenol
Halyminiya dilate	657.07	10.02	430.97	751.3	245.5
Liagora albicance	688.30	12.17	376.97	570.2	198.8

Table 1: Antioxidant activity of halyminiya dilata and liagora albicance

S. No	Concentration (µg/mL)	Absorbance @517nm		% of inhibition	
		Sample Halyminiya dilate	Sample liagora albicance	Sample Halyminiya dilate	Sam liagora albicance
1	20	0.332	0.306	268.6	427.3
2	40	0.326	0.270	177.3	376.2
3	60	0.274	0.266	170.9	184.3
4	80	0.266	0.257	158.0	170.9
5	100	0.244	0.250	149.2	142.4
6	120	0.249	0.240	138.2	193.8

Conclusion :

The qualitative phytochemicals showed the presents of 10 phytochemicals in 3 solvents in both seaweeds. Quantitative analysis showed presence of tannins and carbohydrates was more then protein in quantitative analysis. Antioxidant studies showed that the methanol extract of halyminiya dilate had more antioxidant potential that liagora albicance

REFERENCES

1. Matsukawa, R., Dubinsky, Z., Kishimoto, E., Masaki, K., Masuda, Y., Takeuchi, T., Chihara, M., Yamamoto, Y., Niki, E. and Karube, I. 1997. A comparison of screening methods for antioxidant activity in seaweeds. Journal of Applied Phycology 9:29–35.
2. Khan, N., Afaq, F. and Mukhtar, H. 2008. Cancer chemoprevention through dietary antioxidants progress and promise. Antioxidants & Redox Signaling 10(3):475-510.
3. Matanjun, P., Suhaila, M., Noordin, M. M., Kharidah, M. and Ming, C.H. 2008. Antioxidant activities and phenolic content of eight species of seaweeds from north Borneo. Journal of Applied Phycology 20:367-373.
4. Musa, K. H, Abdullah, A., Jusoh, K. and Subramaniam, V. 2011. Antioxidant activity of pink-flesh Guava (Psidium guajava L.): Effect of extraction techniques and solvents. Food Analytical Method 4:100–107.
5. Lu, J. M., Lin, P. H., Yao, Q. and Chen, C. 2010. Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems. Journal of Cellular and Molecular Medicine 14(4):840–860.
6. Kedare, S. B. and Singh, R. P. 2011. Genesis and development of DPPH method of antioxidant assay. Journal of Food Science and Technology 48(4):412–422.
7. Pokorny, J., Yanishlieva, N. and Gordon, M. 2001. Antioxidants in food: Practical applications. Boca Raton: CRC Press.
8. Khan, N., Afaq, F. and Mukhtar, H. 2008. Cancer chemoprevention through dietary antioxidants progress and promise. Antioxidants & Redox Signaling 10(3):475-510.
9. Plaza, M., Cifuentes, A. and Ibáñez, E. 2008. In the search of new functional food ingredients from algae. Trends in Food Science & Technology 19(1):31-39.
10. Kuda T., Tsunekawa M., Goto H. and Araki Y. 2005.Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. J. Food Comp. Anal. 18:625-633.Heo S.J., Cha S.H., Lee K.W., Cho S.K. and Jeon Y.J. 2005a.Antioxidant activities of Chlorophyta and Phaeophyta
11. Heo et al.: Antioxidant Activities of Red Algae Park P.J., Heo S.J., Park E.J., Kim S.K., Byun H.G., Jeon B.T. and Jeon Y.J. 2005. Reactive oxygen scavenging effect of enzymatic extracts from Sargassum thunbergii. J. Agric. Food Chem. 53: 6666-6672.Siriwardhana N., Lee K.W., Kim S.H., Ha J.W. Park G.T. and Jeon Y.J. 2004. Lipid peroxidation inhibitory effects of Hizikia fusiformis methanolic extract on fish oil and linoleic acid. Food Sci. Tech. Int. 10: 65-72.
12. Yan, X., Chuda Y., Suzuki, M. and Nagata, T., 1999.Fucoxanthin as the major antioxidant in Hijikia fusiformis, a common edible seaweed. Bioscience,Biotechnology, and Biochemistry. 63 (3), 605-607.
13. Yen, G.C. and Chen, H.Y., 1996. AntioxidantActivity of Various Tea Extracts in Relation to Their Antimutagenicity. Journal of agriculture and food chemistry. 43, 27-32.