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EVALUATION OF ANTIOXIDANTS AND PHYTOCHEMICAL SCREENING OF *Caulerpa peltata* LAMOUR AND *Centroceras clavulatum* (Ag.)(Mont.)

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ABSTRACT The main objective of the present study was to determine the nutritional value of *Caulerpapeltata* and *Centroceras clavulatum* collected from Thikkodi coast by analysing their biochemical, phytochemical and antioxidant activity.The samples were extracted using different solvents of acetone,ethanol,water and petroleum ether. In this study the phytochemical constituents such as alkaloids, steroids, flavonoids, phenols, coumarin, cardiac glycoside, tannins, terpenoids, saponins and biochemical constituents such as protein, carbohydrate and the DPPH free radical activity of selected seaweeds collected from Thikkodi coast were analysed.The present study provides useful information of all phytochemicals present in both the algae, *Caulerpa peltata* and *Centroceras clavulatum*. It shows variations in different solvents because of its differential solubility. Most of the phytochemicals were found absent in its petroleum ether solution because they are less soluble in petroleum ether. Compared to *Centroceras clavulatum*, *Caulerpa peltata*showed high amount of protein and carbohydrate. Both these algae have antioxidant activity. In the present study, the seaweed extracts have high DPPH scavenging capacity, which increased with increasing concentration.A positive relation has been documented between antioxidation capabilities and total flavonoid content for *Caulerpa peltata* and *Centroceras clavulatum*. Their antioxidant activity shows an inverse relation with the phenolic content. The results suggest that both the algae can be used as antioxidants in the field of medical science.

KEYWORDS : Caulerpa peltata, Centroceras clavulatum, antioxidants, phytochemicals

INTRODUCTION

Marine environment supplies many kinds of habitat that supports marine life. Marine life depends on the salt water present in sea. Marine ecosystem mainly includes algal vegetation and marine environment proves to be a rich source of biological and chemical diversity and thisecosystem is a rich reservoir of biologically active compounds which has the potential to supply ingredientsas foods. Marine algae are the richest producers of marine environment (Bhadury andWright 2004). Marine macroalgae are rich in bioactive compounds with antimicrobial, antiviral, anti-tumoral, anti-inflammatory, and antioxidant activity (Mayer *et al*., 2011). The compounds responsible for the antioxidant, antimicrobial, anti-cancer and antiviral activity are phenolic compounds, sulphated polysaccharides, and organic acids (Wijffels 2008).Seaweeds are commercially important as source of food, medicineandfertilizer (Rasmussen and Morrissey 2007). Alkaloids, Catechin, Flavonoids, phenols, saponin, steroids, tannins, terpenes, sugars, amino acids, are the common phytochemicals that present in different macro algae. It has a greater role in human health.

Free radicals are chemical species capable of existing independently with one or more unpaired electrons in their outer most shell, which capture electrons from other substances and maintain the neutral stage. Initial attack causes the free radical to become neutral. Another free radical is formed in this process, resulting in a chain reaction. If the two radical meet, they can combine with unpaired electrons and produce covalent bonds. Relative oxygen species (ROS) is derived from molecular oxygen. It will damage the DNA, proteins etc. and causes different diseases like cancer, respiratory ailment etc.

Antioxidant capacity of different compounds prevents several diseases like cancer, coronary heart diseases, neurological problems etc.

The main objective of present study was to determine the nutritive value of *Caulerpapeltata* and *Centroceras clavulatum* collected from Thikkodi coast by analyzing their biochemical, phytochemical, and antioxidant activity.

MATERIALS AND METHODS

The samples of Caulerpa peltata and Centroceras clavulatum were collected from Thikkodi coast ((11^{0}29`N lat&75^{0}37' E long), Kerala. The healthy plants were collected and stored in polythene bags. Samples were washed thoroughly with tap water to remove the adhered dirt particles and spreading on a blotting paper and dried at room temperature.Then the samples dried at 40^{0}C for two days and powdered.

50 gm dried crushed samples were extracted using 250 ml solvents of petroleum ether, acetone, ethanol and water. They are covered with aluminium foil and labelled. After twenty four hours extract was filtered through Whatsmans filter paper no.1 and evaporated to dryness at 40^{0}C on a heat incubator and kept in refrigerator.

Phytochemical screening was conducted by the method of Harborne(1998). Protein content of the samples were determined by Lowry *et al* 1951. Carbohydrate content was determined by following the method of Dubois *et a*l 1956. Estimation of phenol was determined by Singleton and Rossi 1965, and flavonoid content by Chang et al 2002. DPPH radical scavenging activity was determined by Yen and Chen 1955.

RESULT

Phytochemical analysis of various extracts of *Caulerpa peltata* is conducted and it is determined that alkaloids and steroids content are much abundant in its ethanolic extract. Phenolic and flavonoid content is very abundant in its ethanol and acetone extract, coumarin, cardiacglycoside, and saponin in water extract; Tannins are very abundant in ethanol and water extract; terpenoids in ethanol extract only. Absence of steroids,flavonoids, tannins,saponinsand terpenoids are reported in the petroleum ether extract and the absence of cardiac glycoside reported in ethanolic extract of *Caulerpa peltata*.

Phytochemical analysis of various extracts of *Centroceras clavulatum* is conducted. Here,alkaloids and steroids are very abundantly reported in ethanol extract only. Flavonoids and phenolic contents are reported very abundantly in acetone and ethanol extract.Abundance of coumarin is noticed in water and petroleum ether extract; cardiac glycoside in water extract; terpenoids in its ethanol and petroleum ether solution. Steroids and saponins are entirely absent in petroleum ether solution and absence of tannins noticed in its both acetone and petroleum ether solution.

Biochemical analysis of *Caulerpa peltata* is conducted and estimated that its ethanol solution has much more protein content (35.03±0.04%) and less protein is detected in petroleum ether extract (23.65± 0.09%). In the case of carbohydrate, *Caulerpa peltata* show higher value in acetone extract (6.75±0.01%) and least value in petroleum ether solution (3.15±0.06%). (Fig- 1).

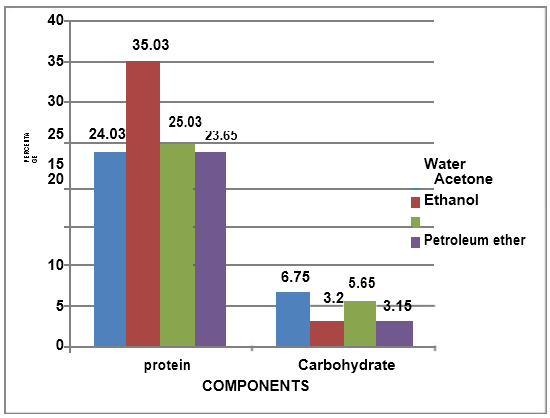
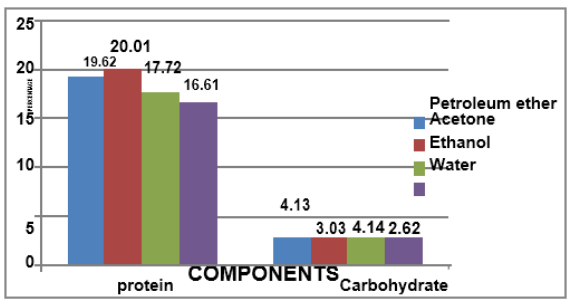


Figure 1

Biochemical analysis of *Centroceras clavulatum* is conducted and noticed that its ethanol extract has much more protein content (20.01±0.02%) and water extract has much more carbohydrate content (4.14±0.05%). Least amount of protein (16.61±0.01%) and carbohydrate (2.62±0.03%) is noticed in petroleum ether solution. (Fig-2)



In *Centroceras clavulatum* high phenolic content is noticed in water extract (12.48±0.46 mg/g) and flavonoid in acetone extract (43.43±1.80 mg/g). Least phenolic content is noted in petroleum ethersolution (6.66±0.08 mg/g) and flavonoid content in ethanol extract (15.81±0.28 mg/g) (Fig-3).

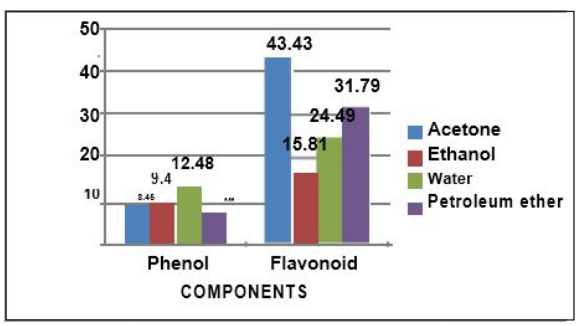


Figure 3

In caulerpa peltata high phenolic content is obtained in aceton solution and high flavonoid content obtained in petroleum ether solution.(figure 4)

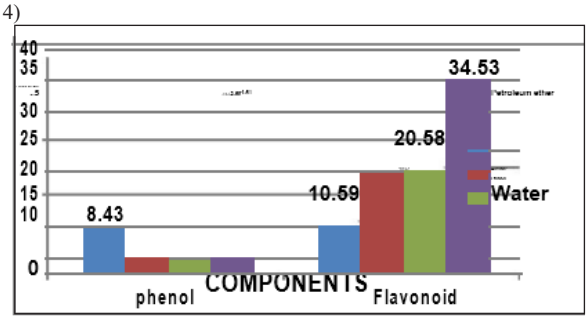


Figure 4

DPPH free radical scavenging activity of acetone extract of *Caulerpa peltata* shown higher at the concentration 500µg/ml (33.14±0.018%) and least in 100µg/ml (27.18±0.64%). (Fig-5). And in *Centroceras clavulatum* higher activity shown in 500µg/ml (40.43±0.61%) and least in 100µg/ml (31.10±0.35%)(Fig-6)

DPPH radical scavenging activity of ethanol extract of *Caulerpa peltata* reported higher value at 500µg/ml (40.08±0.12%) and lower at 100µg/ml (34.47±1.27%) (Fig-5). In *Centrocerasclavulatum* also higher activity shown in 500µg/ml (11.33±0.25%) and less activity in 100µg/ml (4.74±0.14%) (Table 6, Fig-6).

DPPH radical scavenging activity of water extract of *Caulerpa peltata* shown high activity in 500µg/ml (42.38±0.33%) and less at 100µg/ml (35.91±1.06%), (Fig-5). In *Centroceras clavulatum* higher activity detected in 500µg/ml (15.8±0.21%) and least active at 100µg/ml (8.41±0.71%) (Fig-6).

DPPH radical scavenging activity of petroleum ether extract of *Caulerpapeltata*show higher value at 500µg/ml (93.22±0.16%) and low value at 100µg/ml (87.16±0.57%) (Fig-5) and in *Centroceras clavulatum* also, higher value shown in 500µg/ml (22.7±0.14%) and low at 100µg/ml (19.76±0.91%) (Fig-6).

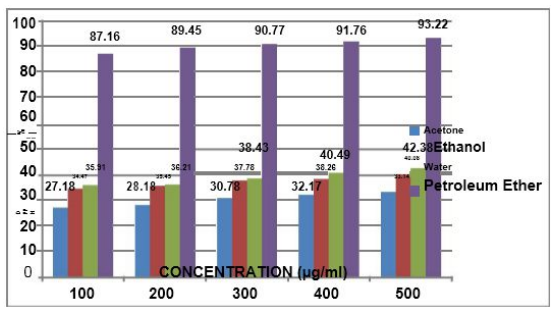


Figure 5

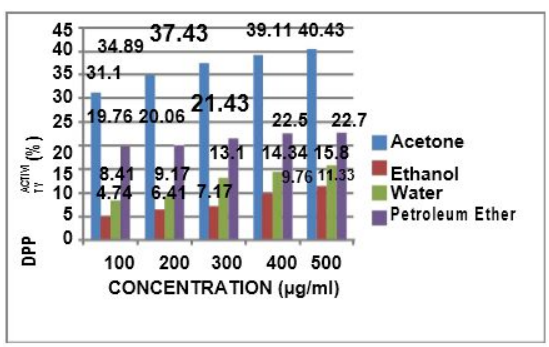


Figure 6

DISCUSSION

In the phytochemical analysis of *Caulerpa peltata*, we can see that steroids, tannins, flavonoids, terpenoids and saponins are not detected in its petroleum ether solution.Andin *Centrocerasclavulatum*,steroids, tannins and saponins are absent in its petroleum ether extract. But most of the phytochemicals are present in acetone, ethanol, and water extract of these algae. It may be due to the variations in solubility of algae in different extracts as algal powder is less soluble in petroleum ether solution. Petroleum ether is non-polarand acetone, ethanol and water is polar in nature. Hediat *et al.*,2010 reported that different solvents have been reported to have the capacity to extract different phytoconstituents depending upon their solubility or polarity in the solvent.Inthepresentbiochemicalstudy,themaximumproteincontentwa srecorded in the ethanolic extract of green alga *Caulerpa peltata* and minimum in the petroleum ether extract of red alga *Centroceras clavulatum*. This findings were in agreement with the result of Mairh *et al*.,1983 reported 22.22% of crude protein in *Ulva fasciata.* Carbohydrate is also rich in *Caulerpa peltata* thanin *Centroceras clavulatum*. The present results are supported by the findings of Anantharaman*et al*.,2013. In the present study, phenol and flavonoid content iscomparatively higher in *Centrocerasclavulatum*than *Caulerpapeltata*. Acetone extract of *Caulerpa peltata* showed higher phenolic content.Phenolic compounds are generally more soluble in

polar organic solvents (Waterman and Mole 1994). Antioxidants are those substances which have important roles such as their protective role in the development of diseases like cancer, atherosclerosis, arthritis, diabetes, Alzheimer's and agings. Marine algae have remained in use by many in a variety of ways since medieval time (Kuda *et al*.,2005).

In this study *Caulerpa peltata*, the amount of phenol and flavonoid shows inverse relationship. But these type of relationship cannot be seen in *Centroceras clavulatum*..In the present study, flavonoid content in*Caulerpapeltata* varies from 10.59 to 34.53 mg/g and in red algae *Centroceras clavulatum* it varies from 15.81 to 43.43 mg/g.

In the present study, the seaweed extracts have high DPPH scavenging capacity, which increased with increasing concentration. These findings were in agreement with the results of Sumathi and Krishnaveni, 2012.In this study, extracts of both *Caulerpa peltata* and *Centroceras clavulatum* showed significant free radical scavenging activity. Extracts of *Caulerpa* specieswere reported to be exhibiting high phenolic content and DPPH radical scavenging activity among chlorophyceae (Zubia *et al*., 2007.The higher value of DPPH radical were recorded in the non-polar petroleum ether extract of *Caulerpa peltata* than the rest of the solvent extracts. This is in conformation with the earlier report of Zeliha *et al*.,2011. In the present study, petroleum ether extract of *Caulerpa peltata* shows high free radical scavenging activity and acetone extract of *Centroceras clavulatum* shows high free radical scavenging activity than other extracts. Rhodophyta have been reportedtoexhibitweak

DPPHquenching activity when obtained using water, ethanol or methanol as solvents for the extraction (Zeliha *et al*., 2011).The current research findings were in agreement with the above result.

In the present study, an inverse relationshipwas reported between phenolic content and DPPH free radical from the extract of *Caulerpa peltata*. This activity could be due to the presence of certain other phytochemicals in addition to phenolics.This is in conformation with the studies of Dharmesh *etal*.,2014. But no correlation was observed between the phenolic content and antioxidant activity. The result of our study is supported by Kokilam*et al*.,2013.Aposition correlation has been documented between antioxidation capabilities and total flavonoid content for *Caulerpa peltata* and *Centroceras clavulatum* .Similar observations have been reported by Chai and Wong 2012 and Bouba *et al*.,2010.

CONCLUSION

The present study provides useful information that most of the phytochemicals are present in both the algae,*Caulerpapeltata* and *Centrocerasclavulatum.* It shows variations in different solvents because of its differential solubility. Most of the phytochemicals were found absent in its petroleum ether solution because they are less soluble in petroleum ether. Compared to *Centroceras clavulatum*, *Caulerpa peltata* showed high amount of protein and carbohydrate. Both these algae have antioxidant activity. Their antioxidant activity shows negative correlation with the phenolic content. Thus both these algae can be used as antioxidants in the field of medical science.

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