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ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF VARIOUS EXTRACTS OF BROWN ALGAE PADINA TETRASTROMATICA HAUCK (DICYTOTACEAE)

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ABSTRACT:-

Padina tetrastromatica is widely distributed marine macro algae along the coast line of Sindhudurg, which contains several interesting bioactive constituents and possesses health promoting properties. In this study, phytochemical constituents and the antioxidant activity with the total phenolic content of different extracts (petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol) from thallus of Padina tetrastromatica were assessed in an effort to find out concentration of the antioxidant potential. Phytochemicals were extracted using solvents like aqueous, ethanol and HCL. Phytochemical analysis showed positive results for the presence of flavonoids, phenolic compounds, saponins, steroids, tannins, carbohydrates and protein. The antioxidant activity was determined by 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) assay and total phenolic content was estimated by using Folin-Ciocalteu's reagent. All tested extracts possessed appreciable antioxidant potential but methanol extract was significantly higher in total phenolic content and in antioxidant assay. This study validates the antioxidant potential of the Padina tetrastromatica and the positive relationship between total phenolic content and antioxidant activity

KEY WORDS: *antioxidant activity, phenolic content, brown algae, Padina tetrastromatica, Sindhudurg.*

INTRODUCTION:

Padina tetrastromatica is a brown algae widely distributed along the coastline of Sindhudurg district of Maharashtra (India). **Phaeophyta (Brown Algae);** are exclusively marine forms. They have different forms from simple, freely branched filaments to highly differentiated forms. Photosynthetic pigments of the brown algae are Chlorophyll a and c, carotene, xanthophylls and fucoxanthin (pigment responsible for brown colour) (Dhargalkar and Kavlekar, 2004).

In recent years, species of brown algae has been reported for antioxidant activity, antiviral activity, antifungal activity and antimicrobial activitiy etc. (Newman *et al.,* 2003; Bansemir *et al.*, 2006; Chew *et al.*, 2008).Brown-algal polyphenols phlorotannins worked as antioxidants, antibacterial and anti-algal compounds (Zeliha *et al.,* 2009). Bioactive properties of compound from edible brown seaweeds; polysaccharides are anti-tumor, anti-viral and anticoagulant; Diterpenes have cytotoxic, anti-viral and anti-tumor activities. Phlorotanin exhibits anti-diabetic, anti-oxidation, anti-cancer, anti-HIV and anti-allergic properties (Gupta and Abu - Ghannam, 2011).

An antioxidant is a molecule which can slower down or can prevent the oxidation of the other molecules (Sies, 1997).Phenolics exhibit several properties beneficial to humans and its antioxidant properties are important in determining their role as protecting agents against free radical-mediated disease processes (Saxena *et al.,* 2013). Phenol is used as an antiseptic and disinfectant, and in the preparation of drugs and dyes.

In seaweeds, the antioxidant activity carried out by several processes and compounds such as lipophilic scavengers (Carotenoids), enzymatic scavengers (catalase, superoxide dismutase and peroxidase), polyphenols and antioxidative molecules (ascorbic acid, tocopherols, chlorophyll related compounds, bromophenols, polysaccharides etc. They have effects such as anti-inflammatory and skin protective (Brown *et al.,* 1977).

Seaweeds have attracted considerable interest due to their easily availability, safety, nutritional and medicinal value. In recent years, the use of natural antioxidants increases in foods Epidemiological and in vitro studies strongly suggest that food containing phytochemicals with antioxidants have potentially protective effects against many diseases. Phytochemicals, such as phenolic compounds are considered highly beneficial for human health, decreasing the risk of degenerative diseases by the reduction of oxidative stress and inhibition of macro molecular oxidation. These compounds have been as well correlated with antioxidant potential. Seaweeds have attracted considerable interest due to their easily availability, safety, nutritional and medicinal value. This study was designed to investigate the total phenolic content and the DPPH scavenging activity of the *Padina tetrastromatica* from the coastline of Sindhudurg district of Maharashtra, India.

MATERIAL AND METHODS:

Collection of*Padina tetrastromatica*: *Padina tetrastromatica*was collected from different sites along coastlineof Sindhudurg district during low tide. The sample was identified by referring Deodhar (1987) and Jha *et al.,* (2009).

Preparation of Sample: Thalli of *Padina tetrastromatica*were washed in sea water and fresh water thoroughly to remove the epiphytes as well as contamination. Then the sample was immediately transferred into a polythene bag with a small hole to leak out sea water drop wise and shade dried.

Qualitative analysis of phytochemicals: For the screening of phytochemicals fresh sample was used. Five grams of the fresh sample was weighed and homogenized with 50 ml of water, HCL (1%) and ethanol separately. The extracts was boiled for 1 hour, cooled and filtered. The filtrate was used to screen for the presence of phytochemicals using standard procedure (Harborne, 1973).

Preparation of the organic extract of sample for antioxidant analysis: The shade dried sample of *Padina tetrastromatica* ground to coarse powder. Twenty gram of powder weighed and wrapped in Whatmann No.1 filter paper and successively extracted with 200ml of different solvents such as petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol with their increasing order of polarity by soxhlation for 12-24 hours. The Extracts were analysed for the antioxidant activity using standard procedure (Thoudam *et al*., 2011).

DPPH Radicle scavenging activity:

The radical scavenging effect of algal extracts was determine by using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) method. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of the solution mixed with 1.0 ml of seaweed extracts in methanol with different concentrations (0.5-2.5 mg/ml). The reaction mixture was mixed thoroughly and kept in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Blank is methanol, control is DPPH without incubation. BHT and BHA were used as references (Liyana-Pathiranan and Shahidi, 2005). The percentage of antioxidant activity was calculated by the following formula, **% scavenging= (OD $_{Blank}$- OD $_{sample}$)/OD $_{Blank}$*100**

Determination of total phenolic content: Total phenolic content in algal extracts were determined by using modified Folin-Ciocalteu reagent method. According to (Zahin *et al*., 2009), gallic acid is a standard phenolic compound. The reaction mixture contained various concentrations of the extracts and Folin- Ciocalteu reagent. To 500 µL (10 mg/ml) of seaweed extracts in methanol, 2.5 ml of 1:10 dilution of Folin-Ciocalteu's reagent and 2 ml of Na_2CO_3 (7.5% w/v) were added and mixed thoroughly and incubated at 45^{o}C for 15 minutes. Same procedure is followed seaweeds extracts with petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol respectively. The absorbance measured at 765 nm. Blank is all without the extract. The concentration of total phenolic content in the seaweed extracts was determined as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/g DW) (Zahin *et al*., 2009).

Total phenolic content = O.D/Slope.

RESULT AND DISCUSSION:

Qualitative analysis for the presence of phytochemicals: *Padina tetrastromatica* showed the presence of flavonoids, phenolic compounds, saponins, steroids, tannins, and proteins in water extracts. In ethanolic extract illustrated the presence of flavonoids, phenolic compounds, steroids, tannins, and proteins. Aqueous extracts, ethanolic extract and HCL extracts were showed the presence of phenolic compounds, saponins, and tannins. Domettila *et al*., (2013), showed the presence of flavonoids, phenolic compounds, saponins, steroids, tannins, and carbohydrates in different solvent extracts.

Padina tetrastromatica showed the presence of steroids in ethanol extracts. Saponins possess numerous biological properties which include antimicrobial, anti-inflammatory, antifeedent and hemolytic effects (Melpha *et. al*., 2014).

Table 1: Qualitative phytochemical analysis of aqueous, ethanolic and HCL extract of *Padina tetrastromatica*.

Sr. no.	Algal extracts	Phytochemical constituents									
		1	2	3	4	5	6	7	8	9	10
1.	Aqueous extract	-	+	-	+	+	+	+	-	+	-
2.	Ethanol extract	-	+	-	+	-	+	+	+	-	-
3.	Hcl extract	-	-	-	+	+	-	+	+	-	-

+ Presence; - Absent

1- alkaloids, 2- flavonoids, 3- glycosides, 4- phenolic compounds, 5- saponins, 6- steroids, 7- tannins, 8- carbohydrates, 9- proteins, 10- fats

DPPH Radical Scavenging Activity:

Methanolic extract of Padina tetrastromatica, showed highest scavenging activity (75%); followed by chloroform extracts (37.549%), ethyl acetate extract (25.494%), petroleum ether extracts (23.611%), ethanol extract (22.023 %) and benzene extracts showed lowest (55.334 %). DPPH radical scavenging activity was highest at methanolic extract and benzene extract showed lowest DPPH radical scavenging activity from Padina tetrastromatica.

Many report have been recently described the ability to scavenging DPPH free radical on seaweeds. Heo *et al*., (2006) also showed that each sea organic extract have positive effect in DPPH free radical scavenging. Ragan and Glombitza (1986) reported the radical scavenging activity of seaweeds to be mostly related to their phenolic contents.

Total Phenolic Content:

In determination of total phenolic content of Padina tetrastromatica methanolic extracts showed highest phenolic content (69.940) followed by ethyl acetate extracts (48.208), ethanolic extracts (31.164),

chloroform extracts (27.970), benzene extracts (15.731) and petroleum ether extracts showed lowest phenolic contents (4.358) (fig. 2).

A number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical-scavengers (Kumar *et al*., 2008). Total phenolic compounds found in plants, have several biological effects including antioxidant, antiapoptosis, anti-aging, anti-carcinogen and considered for their important dietary roles as antioxidant and chemoprotective agents (Han *et al*., 2011).

Fig. 1: DPPH-Radical Scavenging Activity (%) of *Padina tetrastromatica* at 517nm.

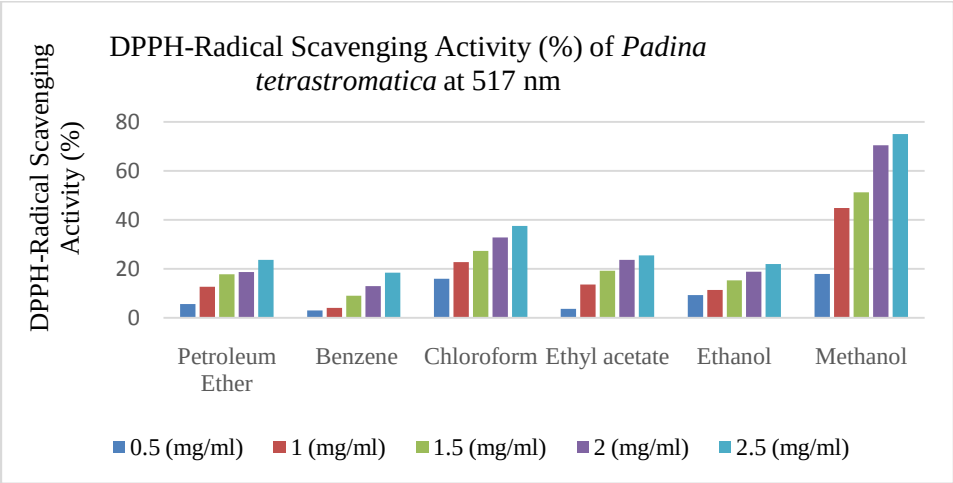
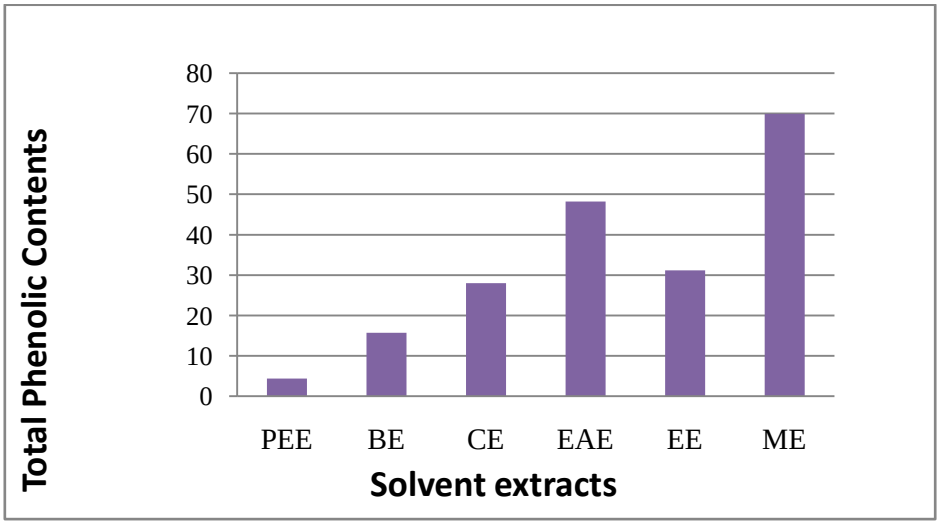


Fig. 2: Total phenolic contents (mg gallic acid/100 g dried seaweed).



PEE- petroleum ether extract, BE- benzene extract, CE- chloroform extract, EAE- ethyl acetate extract, EE- ethanol extract, ME- methanol extract

CONCLUSION:

In the present study, obtained adequate data on the phytochemical constituent, antioxidant activity and phenolic contents, it is concluded that *Padina tetrastromatica* is a good source of phytochemicals.

Different extracts of *Padina tetrastromatica* can be used for the synthesis novel drugs, thus these seaweeds could be collected and utilized effectively in the product preparation for the beneficial of mankind.

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REFERENCES:

Bansemir, A., Blume, M, Schroder, S., and Lindequist, U. (2006).Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria.Aquqculture, **252**:79-84.

Brown, V. B., Ducker, S. C. and Rowan. (1977). The effect of Orthophosphate concentration on growth of articulated Coralline Algae (Rhodophyta). Phycologia, **16**:125-131.

Chew, Y. L.; Lim, Y. Y., Omar, M. and Khoo, K. S. (2008).Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT, **41**:1067-1072.

Deodhar, H. D. (1987). The Biology of marine algae of Bombay, Ph.D Thesis, Savtribai Phule University of Pune.Pune.

Dhargalkar, V. K. and Kavlekar, D. (2004). Seaweeds - a field manual. National Institute of Oceanography, Dona Paula, Goa.1-36.

Domettila, C., Joselin, J. and Jeeva, S. (2013). Phytochemical analysis on some south Indian seaweeds. Journal of Chemical and Pharmaceutical Research, **5(4)**:275-278.

Gupta, S. and Abu-Ghannam, N. (2011). Bioactive potential and possible health effects of edible brown seaweeds. Trends in Food Sciences & Technology, **22**:315-326.

Han, X., Shen, T. and Lou, H. (20011).Dietary polyphenols and their biological significance. Int. J. Mol. Sci., **8**:950 - 88.

Harborne J. B. (1973). Phytochemical methods: A guide to modern technique of plant analysis, Chapman and Hall Ltd. London. 49-188.

Heo, S. J., Cha, S. H., Lee, K. W. and Jeon, Y. J. (2006).Antioxidant activities of red algae from Jeju Island. Algae, **21**:149-156.

Jha, B., Reddy, C.R.K., Thakur, M. C. and Rao, M. U. (2009).Seaweeds of India, The diversity and distribution of seaweeds of Gujarat coast.CSMCRI, Springer publication, New York.

Kumar, S. K., Ganesan, K. and SubbaRao, P.V. (2008). Antioxidant potential of solvent extracts of Kappaphycusalvarezii (Doty) Doty – An edible seaweed. Food Chemistry, **107**:289–295.

Liyana-Pathiranan, C. M. and Shahidi, F. (2005).Antioxidant activity of commercial soft and hard wheat (Triticumaestivum L) as affected by gastric pH conditions. J Agric. food Chem., **53**:2433-2440.

Melpha, Y., Manchu, N. and James, J. E. (2014) Phytochemical evaluation of two brown seaweeds from Muttom and Rasthacaud coasts of Tamil Nadu, India. Journal of Chemical and Pharmaceutical Research, **6(10):**566-569.

Newman, D. J., Cragg, G. M. and Snader, K. M. (2003).Natural products as sources of new drugs over the period 1981-2002. Journal of Natural Products, **66**:1022-1037.

Ragan, M. A., and Glombitza, K. W. (1986).Phlorotannins, brown algal polyphenols. Progress in Phycological Research**, 4**:129−241.

Saxena, M., Saxena, J., Nema, R., Singh, D. and Gupta, A. (2013).Phytochemistry of medicinal plants. Journal of Pharmacognosy and Phytochemistry, **1(6)**:168-182.

Sies, H. (1997). Oxidative stress: oxidants and antioxidants. Exp Physiol., **82(2)**:291-295.

Thoudam, B., Kirithika, T., Kamala, S., Usha, K. (2011). Phytochemical screening and antioxidant activity of various extracts of Sargassum muticum. International Journal of Pharmaceutical Research and Development, **3(10)**:25-30.

Zahin, M., Farukh, A. and Iqbal, A. (2009).The in vitro antioxidant activity and total phenolic content of four Indian medicinal plants. Int. J. of Pharm. and Pharm.Sci.,**1**:88-95.

Zeliha, D., Ferda, F. Y., Ulkan, N. K., Guven, O. and Atakan, S. (2009). Antimicrobial and antioxidant activity of brown algae from the Aegean Sea, Journal of Serbian chemical society, **74**(**6**):619-628.