

Determination of antioxidant potential of *Padina gymnospora* algal extract

G Subramanian*, P Ravi

Post Graduate & Research Department of Botany, Arignar Anna Government Arts College (Affiliated to Periyar University),
Namakkal, Tamil Nadu, India

Abstract

The antioxidant activity *Padina gymnospora* extract was measured using standard radical scavenging activity and results were interpreted and compared with standard. The scavenging activity of ethanolic extract showed IC₅₀ value of 99.1189, 48.9401, 40.3518, 35.3255 and 84.3675 µg/mL respectively for DPPH, ABTS, superoxide FRAP, and hydroxyl. Where as IC₅₀ value of standard were 55.0498, 102.4735, 81.3405, 28.2886 and 44.0826 µg/mL. ABTS and superoxide of *Padina gymnospora* were comparatively have low IC₅₀ value than standard. Maximum % scavenging activity of *Padina gymnospora* was found in FRAP scavenging (62%) followed by hydroxyl (61%) at 50 µg/mL. standard showed 57 to 68% scavenging activity while samples ranged from 54 to 62%. The data reveals that the *Padina gymnospora* constituents have antioxidant with high therapeutic efficacy. Further studies are required to evaluate potential pharmacological properties.

Keywords: free radicals, brown algae, marine, ferric reducing, superoxide

1. Introduction

Plant based Antioxidant therapy also holds promise for the supportive treatment of AD, and has proven to be a successful treatment of cancer too (Bolognesi *et al.*, 2009) [3]. A wide range of studies has described the high antioxidant capacity of a range of edible seaweeds have been reported possess a unique structure and bioactive compounds. Since ancient times, algae have been used as nutritional and functional food sources in Asian countries like Japan and China have known about the algae are part of their diet (Mark *et al.*, 2017) [7]. Apart from raw material properties like agar, alginic acid, and carrageenan, but they continue to be widely consumed as food in Asian countries (Rizvi and Shameel, 2004) [11]. There is evidence in these countries that the incidence of certain kinds of cancer, such as prostate and breast, which is lowered due to regular consumption of seaweeds, the leading cause being their bioactive compounds that may prevent against those diseases. The *Padina* has the second most speciose with the 43 recognized species of dictyotalean genus after Dictyota (76 identified species). Brominated phenols, heterocyclics with nitrogen, sulfur-nitrogen and oxygen, sterols, terpenoids, peptides, proteins, halogenated ketone, alkanes, and cyclic polysulfides are generally present in the seaweed as photochemical compounds (Keyrouz *et al.*, 2011) [6]. Natural antioxidants are potential with an essential for multifunctional to prevent malfunctioning of oxidation in complex food systems than the synthetic antioxidants as interest. Aquatic plants are naturally gaining importance as a potential source of antioxidants. The ascorbic acid acts as an antioxidant with direct effects to prevent, control, and regulate cellular malfunctions. It is a substrate for the redox enzyme ascorbate peroxidase, which is particularly important in stress resistance in plants. Many synthetic antioxidants are namely butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertbutyl hydroxy quinones (TBHQ) available which are the maximum used in different food that they are highly potential health hazards,

which were strictly prevented from using in various food products in many countries (Al-Amoudi *et al.*, 2009) [1]. The marine algal extracts have also been demonstrated to have strong antioxidant properties, especially, brown seaweeds which show comparatively higher antioxidant activity than green and red. The marine brown algal seaweed *Padina* is a genus of Phaeophyceae under the order of Dictyotales in Algae, which is occurring worldwide in specifically tropical and temperate seas. Global species diversity and distribution ranges, however, remain mostly unknown (Thomas *et al.*, 2013) [14]. It was also reported that seaweeds are an excellent source of polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and carotenoids has exhibits different biological activities (Priyadharshini *et al.*, 2011) [9].

Materials and Methods

Collection of Marine Algae

Brown algae were collected from the Pamban coast of Rameswaram, Tamilnadu, India in December 2015. The seaweeds were washed well to remove impurities by gentle agitation and carefully transported to lab. After drying samples were pulverized into powder and stored at - 80 °C before extraction.

Preparation for Analysis

2.5% of ethanolic extract of *Padina gymnospora* kept under shaking overnight under cold condition and then centrifuged at 3000 rpm for 10 min. The supernatant was collected in a separate labeled bottle and frozen at - 80 °C until analysis. The freeze-dried extracts were re-dissolved in ethanol and used for the analysis.

DPPH radical scavenging activity (Karagozler *et al.*, 2008) [5]

10-50 µg / ml samples were mixed with 1ml of ethanol solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM [6]. The mixture had been shaken vigorously and kept to stand for 30 minutes and

the absorbance (OD) was taken at 517 nm. Ascorbic acid was used as a control. The percentage of DPPH decolorization of the sample was calculated as per the following equation;

$$\text{DPPH radical scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{test}}) / A_{\text{control}}] \times 100$$

IC₅₀ values (µg/ml) where the inhibitory concentration at which DPPH radicals were scavenged by 50%. Ascorbic acid was used for comparing.

ABTS⁺ radical scavenging activity (Indu and Seenivasan, 2013) [4]

ABTS [2, 2'-azino-bis (3-thylbenzothiazoline-6-sulphonic acid)] was obtained by reacting to 7 mM. ABTS stock solution with 2.45 mM potassium persulfate and the mixture were left to stand in the dark at room temperature for 12 - 16 h before use. The samples were reduced to the concentration ranges from 10 to 50µg/ml. The reaction was subjected by the addition of 1ml of diluted ABTS⁺ to the different concentrations of *P. gymnospora* as a sample and 10 ml ethanol as control. The absorbance was measured after 10 min incubation at 730 nm. The ABTS radical scavenging activity was expressed as

$$\% \text{ of ABTS radical cation inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

Superoxide anion radical scavenging activity (Naskar *et al.*, 2010) [8]

This assay was based on the reduction of nitro blue tetrazolium (NBT) in the presence of nicotinamide adenine dinucleotide (NADH) and phenazine methosulfate (PMS) under aerobic condition. The 3 ml reaction mixture contained 50 ml of 1M NBT, 150 ml of 1M NADH with or without sample, and Tris buffer (0.02M, pH 8.0). The reaction was started by adding 15 ml of 1M PMS to the mixture and the absorbance change was recorded at 560 nm after 2 minutes. The inhibition percentage was calculated against a control without the extract.

Hydroxyl radical scavenging activity (Saowapa *et al.*, 2015) [13]

The reaction mixture 3.0 ml contained 1.0 ml of 1.5 mM FeSO₄, 0.7 ml of 6 mM hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate, and varying concentrations of the sample. After incubation at 37 °C for 60 minutes, the absence of the hydroxylated salicylate complex was measured at 562 nm. The percentage scavenging effect was calculated as the scavenging activity.

$$\text{Hydroxyl radical scavenging activity (\%)} = [1 - (A_1 - A_2) / A_0] \times 100$$

Where A₀ was the observance of the control (without extract), A₁ was the observance in the presence of the extract, and A₂ was the observance without sodium salicylate.

Ferric-reducing antioxidant power (FRAP) assay

The reaction mixture FRAP 3 ml and 1 ml sample mixed then incubated at 37 °C for 30 minutes and the absorbance was recorded at 595 nm. An intense blue color complex was formed when ferric tripyridyltriazine (Fe₃⁺-TPTZ) complex was reduced to ferrous (Fe₂⁺) form. The absorption at 540 nm was recorded.

Statistical analysis

All the assays were carried out in triplicate. Experimental results are expressed as mean ± standard deviation (SD). IC₅₀ value were calculated.

Results and Discussion

The scavenging activity of ethanol extracts at 10, 20, 30, 40 and 50 µg/mL was evaluated and given in table 1 and IC₅₀ of sample is given in figure 1. An organic chemical compound 2, 2-diphenyl-1-picrylhydrazyl a stable free radical molecule with a dark-colored crystalline powder was changed into yellow immediately at 40-50 µg/mL and reaction was slow between 10-30 µg/mL. The DPPH showed minimum of 35% and maximum of 55% with IC₅₀ 99 µg Assay of ABTS gave minimum 30% and maximum 59% respectively at 10 and 50 with IC₅₀ 48 µg. the antioxidant activity of superoxide is 24% at 10 µg and 54% at 50 µg where as FRAP showed 33 and 62% respectively at 10 and 50 µg/ml. The IC₅₀ of superoxide is 40 and FRAP is 35%. Hydroxyl scavenging showed minimum 35% at 10 µg and 61% at higher concentration of 50 µg with IC₅₀ 84 µg. The seaweed extracts consisted of many primary and secondary metabolites like phenols, flavonoids; steroids, etc., which are responsible for helping to prevent many diseases due to their free radical scavenging activities (Ruberto *et al.*, 2001) [12]. The seaweed *P. gymnospora* exhibited significant dose-dependent inhibition of DPPH, ABTS, FRAP activity. Table 2 reveals the data of standard free radical scavenging activity and figure 2 corresponding to IC₅₀ of standard. The antioxidant capacity, assessed through the DPPH, ABTS, Superoxide, FRAP and Hydroxyl assays, showed significant variation among samples and standard at 40-50µg/mL. Minimum and maximum scavenging (%) of standard for DPPH with IC₅₀ (µg) was estimated as 38 and 58 with 55 µg and ABTS have had 33 and 63 with 102 IC₅₀. Superoxide activity was minimum of 27% and maximum of 57% with 81 µg IC₅₀ where FRAP have showed 36 and 68% with 28 µg IC₅₀. Hydroxyl scavenging was 33/67 with 44 µg IC₅₀. It was found that algal flavonoids have gained much attention due to its potent ROS scavengers properties (Porat *et al.*, 2006). Water-soluble vitamins such as vitamins B1, B2, B3 and B6 were found in *P. gymnospora* also take part in scavenging activity by breaking the free radical chain or by donating a hydrogen atom (Umamaheswari and Chatterjee, 2008). The extracts of *P. gymnospora* showed reducing properties in a concentration-dependent manner also reported by Balakrishnan and Pandima Devi during 2016 [2]

Conclusion

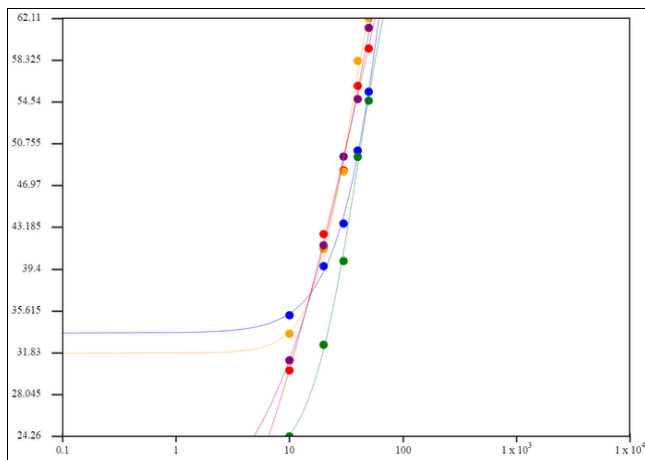
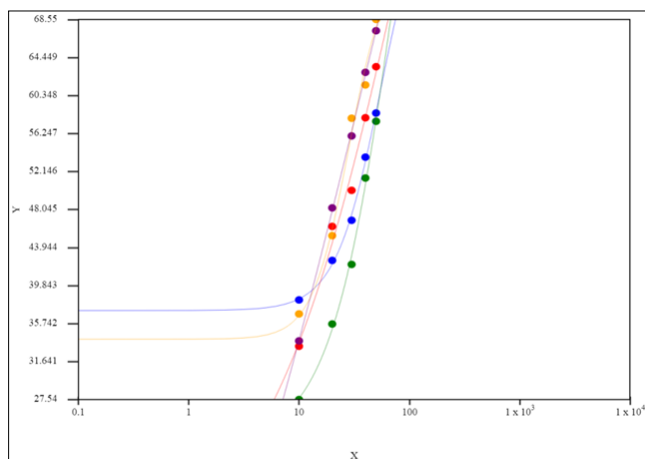
In the present study, the *Padina gymnospora* ethanolic extractions were showing significant free radical scavenging activities. The experimental findings proved that this alga extract is an excellent source pharmaceutical valuable compounds.

Table 1: Antioxidant potential of *Padina gymnospora* extract

| Concentration | DPPH | ABTS | Superoxide | FRAP | Hydroxyl |
|------------------|--------------|--------------|--------------|--------------|--------------|
| 10 | 35.22 ± 0.01 | 30.22 ± 0.02 | 24.26 ± 1.02 | 33.55 ± 0.12 | 31.14 ± 1.00 |
| 20 | 39.66 ± 0.12 | 42.56 ± 1.21 | 32.55 ± 1.22 | 41.22 ± 1.09 | 41.55 ± 1.07 |
| 30 | 43.52 ± 0.14 | 48.33 ± 2.12 | 40.12 ± 1.34 | 48.23 ± 1.46 | 49.58 ± 1.21 |
| 40 | 50.12 ± 1.01 | 55.98 ± 3.05 | 49.55 ± 2.55 | 58.24 ± 1.63 | 54.78 ± 1.34 |
| 50 | 55.45 ± 1.25 | 59.36 ± 3.15 | 54.64 ± 2.97 | 62.11 ± 1.85 | 61.23 ± 1.39 |
| IC ₅₀ | 99.1189 | 48.9401 | 40.3518 | 35.3255 | 84.3675 |

Table 2: Antioxidant potential of standard

| Concentration | DPPH | ABTS | Superoxide | FRAP | Hydroxyl |
|------------------|--------------|--------------|--------------|--------------|--------------|
| 10 | 38.29 ± 1.12 | 33.29 ± 1.25 | 27.54 ± 1.35 | 36.78 ± 1.08 | 33.87 ± 1.06 |
| 20 | 42.55 ± 1.32 | 46.22 ± 1.45 | 35.69 ± 1.52 | 45.22 ± 1.21 | 48.22 ± 2.02 |
| 30 | 46.88 ± 1.45 | 50.11 ± 1.68 | 42.12 ± 1.87 | 57.89 ± 1.37 | 55.98 ± 2.22 |
| 40 | 53.69 ± 2.22 | 57.94 ± 2.11 | 51.44 ± 2.44 | 61.48 ± 2.01 | 62.84 ± 2.76 |
| 50 | 58.45 ± 2.34 | 63.45 ± 2.88 | 57.56 ± 2.67 | 68.55 ± 2.55 | 67.33 ± 3.11 |
| IC ₅₀ | 55.0498 | 102.4735 | 81.3405 | 28.2886 | 44.0826 |

**Fig 1:** IC₅₀ value of *Padina gymnospora* extract**Fig 2:** IC₅₀ value of standards

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