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# Phytochemical constituents, antioxidant properties and *p*-coumaric acid analysis in some seagrasses

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ABSTRACT

Seagrasses have a long history of being used for a variety of remedial purposes, such as treatment of fever, skin diseases, muscle pains, wounds and stomach problems. Hence it is essential to study their bioactive metabolites and medicinal properties when considering their food applications. In the present study, the leaves of six seagrasses *Enhalus acoroides, Thalassia hemprichii*, *Halodule pinifolia, Syringodium isoetifolium, Cymodocea serrulata* and *Cymodocea rotundata* were extracted with aqueous methanol and tested for their antioxidant capacity. Among them, *H. pinifolia* recorded high phenolic (21.64 mg $g^{-1}$), tannin (17.12 mg $g^{-1}$) and vitamin E (34.49 mg $g^{-1}$) content. Flavonoid level was high in *C. serrulata* (5.12 mg $g^{-1}$) and vitamin C in *C. rotundata* (28.43 mg $g^{-1}$). Higher total antioxidant activity of 15.75, 8.37 and 6.65 mg ascorbic acid equivalent/g was observed respectively in *H. pinifolia*, *E. acoroides* and *C. rotundata;* while *C. rotundata* (70.30%) was found to be the most potent DPPH radical scavenger. The inhibition was more than 53.74% in *C. rotundata* and 51.05% in *H. pinifolia* for scavenging OH radicals to prevent oxidative degradation of deoxyribose substance. The HPTLC analysis confirmed the presence of *p-coumaric acid* in the aqueous methanolic extracts of *H. pinifolia* and *C. rotundata* and these findings suggest the possible pharmacological application of these seagrasses.



## 1. Introduction

Antioxidants in biological systems have multiple functions which include protection from oxidative damage and in the major signaling pathways of cells. The major action of antioxidants in cells is to prevent damage caused by the action of reactive oxygen species (ROS). ROS such as superoxide radical ($O_2^{\cdot}$), hydroxyl radical ($OH^{\cdot}$), peroxide radical ($ROO^{\cdot}$) and nitric acid radical are generated in living organisms during excessive metabolism (Aruoma & Cuppette, 1997). These radicals cause extensive oxidative damage to cells that lead to age related degenerative diseases, cancer and wide range of other human diseases (Aruoma, 1999; Reaven & Witzum, 1996).

At present, there is special interest on natural antioxidants coming from plants sources. There are evidences suggesting that phytochemicals having antioxidant properties are associated with a lower risk of mortality (Dixon, Xie, & Sharma, 2005; Rice-Evans, 2004). The antioxidant activity of plants may be due to their phenolic compounds, flavonoids, alpha-tocopherol and carotenoids (Geetha, Malhotra, Chopra, & Kaur, 2005). Antioxidant activity from marine resources is not yet fully explored (De, Devasagayam, Adhikari, & Menon, 2006). More recently, reports have revealed that seagrasses are a rich source of antioxidant compounds (Athiperumalsami, Devi Rajeswari, Hastha Poorna, Kumar, & Louis Jesudass, 2010; Gokce & Haznedaroglu, 2008; Hasina, Kolenchenko, Sgrebneva, Kovalev, & Khotimchenko, 2003; Kolenchenko, Sonina, & Khotimchenko, 2005; Ragupathi Raja Kannan, Arumugam, & Anantharaman, 2010; Ragupathi Raja Kannan, Arumugam, Meenakshi, & Anantharaman, 2010; Ragupathi Raja Kannan, Arumugam, Grignon-Dubois, & Anantharaman, 2012; Sureda, Box, Terrados, Deudero, & Pons, 2008). They are used as human food especially by the coastal populations (Hemminga & Duarte, 2000). In folk medicine, seagrasses have been used for a variety of remedial purposes, e.g. for the treatment of fever and skin diseases, muscle pains, wounds and stomach problems, remedy against stings of different kinds of rays, tranquillizer for babies (de la Torre-Castro & Rönnbäck, 2004). Seeds of *Enhalus acoroides* are thought to have aphrodisiac and contraceptive properties (Aliño et al., 1991). Whereas recent reports on the phytochemical constituents of seagrasses of Gulf of Mannar, South India are limited with the exception of few studies (Athiperumalsami, Venkatraman Kumar, & Louice Jesudass, 2008; Kannan, 1992; Ragupathi Raja Kannan, Arumugam & Anantharaman, 2010; Ragupathi Raja Kannan, Arumugam, & Meenakshi et al., 2010; Ragupathi Raja Kannan, Arumugam, & Anantharaman, 2012; Raja Kannan, Arumugam, & Grignon-Dubois et al., 2012; Ragupathi Raja Kannan, Arumugam, Iyapparaj, Thangaradjou, & Anantharaman, 2013; Rajeswari, 1998).

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However, little attention has been given to the study of antioxidant compounds from seagrasses. In respect to our interest in the search of bioactive components from seagrasses, the present study was undertaken to evaluate the antioxidant activities of six seagrasses and quantification of *p-coumaric acid* in selected seagrasses.

## 2. Materials and methods

### 2.1. Seagrasses

The fresh leaves of *Enhalus acoroides* (Linnaeus f.) Royle, *Thalassia hemprichii* (Ehrenberg) Ascherson, *Halodule pinifolia* (Miki) den Hartog, *Syringodium isoetifolium* (Ascherson) Dandy, *Cymodocea serrulata* (R. Brown) Ascherson & Magnus and *Cymodocea rotundata* Ehrenberg & Hemprich ex Ascherson were collected from Chinnapallam, Gulf of Mannar Biosphere Reserve, Tamilnadu, India (Latitude 8°35′–9°25′N; 78°08′ –79°30′E) during March 2010 and identified following the keys of Ramamurthy, Balakrishnan, Ravikumar, and Ganesan (1992) and herbarium specimens were maintained in CAS in Marine Biology, Annamalai University (Table 1). The collected seagrasses were immediately brought to the laboratory in plastic bags containing seawater to prevent evaporation. The seagrasses were washed thoroughly with tap water to remove all sand particles and epiphytes, shadedried at 35 ± 2°C until a constant weight was obtained and ground well using mixer grinder. The powdered seagrasses were then stored individually in airtight containers for further use.

### 2.2. Extraction of seagrasses

Crude extracts were obtained by soaking 100 g of each seagrass powder individually in 2 l of aqueous methanol (1:4) for 24 h at room temperature under dark condition (Nuissier, Rezzonico, & Grignon-Dubois, 2010). The extraction was repeated thrice, pooled and filtered through Whatmann No. 1 filter paper (11 microns). Each filtrate was concentrated to dryness under reduced pressure using a rotary flash evaporator (VC100A Lark Rotavapor®). The dry aqueous extracts were lyophilized and stored in refrigerator for further analysis.

### 2.3. Determination of total phenolic content

Total phenolic content in the extracts were determined using Folin–Ciocalteau reagent according to the method of Malick and Singh (1980) using gallic acid as a standard. Folin–Ciocalteau reagent (0.5 ml) was added to 3 ml extract and then 2 ml 20% sodium carbonate added. The contents were incubated for 5 min at room temperature and the absorbance of blue colour was read at 650 nm, using a PerkinElmer Lamda 25 UV-VIS Spectrophotometer. A calibration curve of Gallic acid (ranging from 8 to 40 μg) was prepared and the total phenolic content was standardized against gallic acid and is expressed as mg Gallic acid equivalents per gram of sample on a dry weight basis. $y = 9.6x - 6.4$, $R^2 = 0.973$.

### 2.4. Determination of flavonoid

The aluminium chloride colorimetric technique was used for flavonoid estimation (Chang, Yang, Wen, & Chern, 2002). Each extract (0.5 ml of 1:10 g $ml^{-1}$) in methanol was separately mixed with 1.5 ml methanol, 0.1 ml 10% aluminium chloride, 0.1 ml 1 M potassium acetate and 2.8 ml distilled water. It was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was plotted by preparing the quercetin solutions at concentrations 8–40 μg dissolved in methanol. $y = 9.61x - 6.41$, $R^2 = 0.976$.

### 2.5. Determination of tannin

Tannin content in leaves of six seagrasses was determined by Folin–Denis reagent according to the method of Schanderl (1970) using gallic acid as a standard. The extracts (0.5 g) were taken in a conical flask containing 25 ml distilled water and boiled for 30 min. The solution was centrifuged at 1000 ×*g* for 20 min and supernatant was collected. To this supernatant (1 ml), 1 ml Folin–Denis reagent and 2 ml sodium carbonate solution were added. The solution was made up to 5 ml with distilled water, incubated for 30 min at room temperature and the absorbance was read at 700 nm. The values were expressed in gallic acid equivalents per gram fresh weight. $y = 9.6x - 6.4$, $R^2 = 0.973$.

### 2.6. Determination of vitamin C

The vitamin C concentration was evaluated following the method described by Prieto, Pineda, and Aguilar (1999). Briefly, 0.3 ml sample was mixed with 3.0 ml reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95 °C for 90 min in a water bath. Absorbance of all the sample mixtures was measured at 695 nm. The total antioxidant activity was standardized against ascorbic acid and is expressed as mg ascorbic acid equivalents per gram of sample on a dry weight basis. $y = 0.002.4x - 0.0058$, $R^2 = 0.9919$.

### 2.7. Determination of vitamin E

Vitamin E was measured by the method of Oyaizu (1986). To the sample, a drop of thiourea (10%) and 0.25 ml 2% Dinitro phenyl hydrazine (in 9N $H_2SO_4$) were added and incubated at 37 °C for 3 h. After incubation, 1.25 ml of 85% $H_2SO_4$ was added under ice-cold condition and kept at room temperature for 30 min. The absorbance was measured at 540 nm against a blank. $y = 0.0578x + 0.0042$, $R^2 = 0.9792$.

### 2.8. Quantitative analysis of coumaric acid by HPTLC

Chromatography was performed on pre-activated (100 °C) silica gel $60F_{254}$ HPTLC plates (10 × 10 cm; 0.25 mm layer thickness; Merck). The

**Table 1**
Level of phytoconstituents.

| Seagrasses | Voucher Number | Yield (%) | Phenol[1] | Flavonoid[2] | Tannin[1] | Vitamin C[3] | Vitamin E[4] |
|---|---|---|---|---|---|---|---|
| *Enhalus acoroides* | CASPA1 | 1.3 | $1.62 \pm 0.080^{a}$ | $0.51 \pm 0.070^{a}$ | $1.13 \pm 0.091^{a}$ | BDL | BDL |
| *Thalassia hemprichii* | CASPA2 | 1.2 | $2.76 \pm 0.170^{b}$ | $1.17 \pm 0.111^{b}$ | $2.72 \pm 0.188^{b}$ | BDL | $0.65 \pm 0.080^{a}$ |
| *Halodule pinifolia* | CASPA3 | 0.96 | $21.64 \pm 1.845^{c}$ | $0.22 \pm 0.082^{c}$ | $17.12 \pm 0.315^{c}$ | $16.09 \pm 1.747^{b}$ | $34.49 \pm 2.710^{b}$ |
| *Syringodium isoetifolium* | CASPA4 | 2.36 | $3.94 \pm 0.265^{b}$ | $0.84 \pm 0.050^{d}$ | $2.45 \pm 0.150^{b}$ | BDL | $6.64 \pm 0.043^{c}$ |
| *Cymodocea serrulata* | CASPA5 | 0.91 | $13.27 \pm 0.152^{d}$ | $5.12 \pm 0.061^{e}$ | $8.45 \pm 0.115^{d}$ | $10.96 \pm 0.166^{c}$ | $26.86 \pm 1.74^{d}$ |
| *Cymodocea rotundata* | CASPA6 | 1.04 | $12.64 \pm 0.275^{d}$ | $4.56 \pm 0.136^{f}$ | $6.98 \pm 0.115^{e}$ | $28.43 \pm 1.455^{d}$ | $16.44 \pm 0.248^{e}$ |

BDL — Below Detectable Limit.
Column wise value sharing same superscript letters are not significant ($P<0.05$).
[1] mg gallic acid $g^{-1}$.
[2] mg quercetin $g^{-1}$.
[3] mg ascorbic acid $g^{-1}$.
[4] mg tocopherol $g^{-1}$ BDL — Below Detectable Limit.