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Phytochemical screening and *in vitro* antioxidant activity of the seagrass *Cymodocea serrulata*

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The seagrass *Cymodocea serrulata* was collected from Ramanathapuram coastal region and its antioxidant potential was determined. The ethanol extract showed the highest phenolic content of 284.94 mg/ml gallic acid equivalence and the ethyl acetate extract showed the highest flavonoids content of 40.18 mg/ml quercetin equivalence. The tannin content was higher at 264.71 mg/ml tannic acid equivalence in aqueous extract. The ethanol extract exhibited the highest 2,2 diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity with IC_{50} values of 44.47 µg and 2.5 µg, respectively. The ferric reducing ability and nitric oxide scavenging activity were efficient in both ethanol and aqueous extracts. The superoxide scavenging activity was high in hexane extract. The comparative antioxidant study of the subsequent extract of *C. serrulata* showed that the ethanol extract possesses the highest free radical scavenging property compared to other extracts. This may be due to the presence of high phenolic compounds. The study brings out the medicinal value of *C. serrulata* which can be used as a nutraceutical compound in various food and pharmaceutical industries.

[**Keywords**: *Cymodocea serrulata*; Antioxidant activity; DPPH assay; Phytochemical analysis; Seagrass; ABTS assay; Phenols; Flavonoids; Tannin]

Introduction

With an increase in the lack of disease resistance and adverse effects of synthetic drugs, it has become essential to explore our traditional use of medicine and search for a natural compound that can act as a drug or supportive agent to treat various diseases. Thus, the search for a novel, potent natural compound from marine angiosperm is best suited for this purpose. The marine angiosperms are hydrophytes that grow and complete their lifecycle in a completely submerged saline environment[1]. Since their habitat is in the stressed environment, they can produce structurally diverse secondary metabolites, such as alkaloids, phenols, flavonoids, terpenoids, tannins, steroids, and so on. These secondary metabolites were proved to serve as medicine and supplement against various human diseases. Compared to algae, seagrasses remain less exploited despite the fact that they offer tremendous opportunities to find new commercially valuable phytochemicals[2]. In this study, the seagrass *Cymodocea serrulata* has been chosen and justified for traditional use as medicine as it possesses antioxidant potential.

Materials and Methods

Fresh seagrass *C. serrulata* was collected in June from the coastal waters of Thiruppalaikudi, Ramanathapuram district. *C. serrulata* was identified and authenticated by CMFRI (ICAR, Govt. of India). The whole seagrass *C. serrulata* was washed thoroughly and shade-dried at room temperature. The dried seagrass was then powdered using a mechanical grinder. About 150 g of the powder was soaked in 1:2 ratio of five different solvents successively in increasing order of polarity, namely, hexane, chloroform, ethyl acetate, ethanol, and water, for 15 days (three days for each solvent) at room temperature and shaken mildly. The extracts were collected and the solvent was evaporated by using a vacuum distillation unit. Both dried and powdered forms of the extracts were weighed and subjected to further analysis. The net weight of the subsequent extract was measured. The proximate analysis of the subsequent extracts of *C. serrulata* was carried out by the standard procedure[3,4]. The method and inference of phytochemical analysis are given in Table 1.

The total phenol content was determined by the Folin–Ciocalteu's method and the values obtained were expressed as gallic acid equivalence (GAE) (mg/ml)[5]. The total flavonoids were measured following Singh *et al.*[6] and the values obtained were expressed as quercetin equivalence (QE) (mg/ml). The tannin content was determined by the Folin–

Table 1 — Methods and inference of phytochemical analysis

Phytochemicals	Method	Inference
Flavonoids	10% lead acetate	Change of white color on adding acid
Steroids	Con. H_2SO_4	Ring formation
Terpenoids	Salkowski method	Reddish brown color formation
Tannin	0.1% ferric chloride	Brownish green or blue–blackcolor formation
Saponin	Shaking	Foam persist for 10 min
Alkaloids	Meyers test and Wagner test	White-colored precipitate Reddish brown color
Phenols	Ferric chloride test	Bluish green color formation
Quinine	Con. H_2So_4	Color formation
Carbohydrate	Fehling's test	Formation of red precipitate
Glycosides	Keller Killani's test	Lower reddish brown, upper bluish green layer

Table 2 — Phytochemicals present in C. serrulata extracts

Compounds	S1 Hexane	S2 Chloroform	S3 Ethyl acetate	S4 Ethanol	S5 Aqueous
Alkaloids	+	+	+	Nil	Nil
Flavonoids	+	+	+	+	+
Phenols	+	+	+	+	Nil
Quinone	+	+	+	+	+
Tannin	Nil	+	+	+	Nil
Carbohydrates	Nil	Nil	Nil	+	+
Steroids	Nil	+	+	+	Nil
Glycosides	Nil	+	+	Nil	+
Terpenoids	+	Nil	Nil	+	+
Saponin	Nil	Nil	Nil	Nil	+

Denis method[7] and the values obtained were expressed as tannic acid equivalence (TAE) (mg/ml). The protocol of Mensor *et al.* was followed for the DPPH (2,2 diphenyl-1-picrylhydrazyl) radical scavenging assay[8]. A solution of 0.135 mM DPPH was prepared in methanol. The 0.5 ml of radical solution was mixed with different concentrations of extracts and also with the standard (5–200 g/ml) and left in the dark at room temperature for 30 min. The absorbance was measured at 518 nm. The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity was determined by the method of Re *et al.*[9] About 1.9 ml of ABTS solution (7 mM) was added both to the extracts and to the standard of different concentrations (5–200 µg) and mixed well. Then the mixture was allowed to stand at room temperature for 20 min. The absorbance was measured at 734 nm by using a spectrophotometer. Nitric oxide (NO) scavenging activity was determined according to the method reported by Tsai *et al.*[10] The scavenging activity of *C. serrulata* extracts toward superoxide (SO) anion radicals was measured by the method of Liu *et al.*[11] The absorbance was measured by a spectrophotometer at 560 nm.

The free radical scavenging activity of the assays was calculated according to the following equation:

$$\%\,Inhibition = \left[\frac{A0 - A1}{A0} \times 100\right]$$

where A_0 is the absorbance of the control (blank, without extract) and A_1 is the absorbance in the presence of the extracts. The ascorbic acid was kept as standard.

The ferric-reducing ability of the extract was determined by the procedure followed by Benzie and Strain[12]. The absorbance measured at 593 nm spectrophotometrically was proportional to the combined ferric-reducing ability and antioxidant property of the extract. The relative activity of the sample was compared with the standard ascorbic acid.

Results and Discussion

The preliminary phytochemical analysis of *C. serrulata* extracts showed the presence of phytocompounds, such as alkaloids, flavonoids, phenol, quinone, terpenoids, steroids, and glycosides. The results obtained were similar to the report published by Zapata *et al.*[13] Table 2 presents the qualitative analysis of the phytocompounds present in the extracts of *C. serrulata.* These phytochemicals, which are the integral part of the defense system, may be responsible for the survival of *C. serrulata* in the sea. Phenolic compounds are very important

to plants and have multiple functions. They play a major role in the plant defense system by acting against pathogens and also as an antioxidant thereby preventing cells from damage[14].

The total phenol content was higher in the ethanol extracts (284.94 mg/ml) followed by aqueous (1878.36 mg/ml) and ethyl acetate extracts (83.89 mg/ml). The lowest phenol content was reported in chloroform (47.58 mg/ml) and hexane extracts (35.21 mg/ml) (Fig. 1).

Flavonoids are naturally occurring biological compounds that can act as potent antioxidants and can prevent cardiovascular disease by preventing the oxidation of LDL. They also reduce the risk of cancer by acting as a natural scavenger of free radicals[15]. The aqueous extract shows the highest (45.89 mg/ml) flavonoids content followed by the ethyl acetate extract consisting of 40.18 mg/ml of flavonoids content (Fig. 2). Ethanol extract consists of considerable flavonoids content (34.56 mg/ml). The high content of phenols and flavonoids estimated in ethanol extracts of *C. serrulata* implies the application of this extract not only as an anticancer and anti-inflammatory agent but also as an agent to treat various cardiac ailments[16]. The amount of phenolic compounds extracted depends on the solvent used. *C. serrulata* is a plant living in salt water, and it produces unique phenolic compounds as a phytoprotectant to act against stress. These phenols have the antioxidant property that can scavenge free radicals which are responsible for various dreadful diseases. Kannan *et al.* observed that the phenols and flavonoids contributed significantly to the antioxidant property of the seagrass[17]. Nanthakumar *et al.* reported that *C. serrulata* methanolic extract exhibits antioxidant property and cytotoxicity activity against the HeLa cells with growth inhibition of 40.47% at 100 µg/ml[18].

Tannins are high molecular weight compounds. They usually bind to proteins, lipids, or carbohydrates. In Ayurveda, tannin-rich plant is used to treat various diseases because of its excellent healing property[19].[19] The determination of tannin shows that the total tannin content was higher in the aqueous extract (264.71 mg/ml), followed by ethyl acetate extract (115.24 mg/ml), whereas the ethanol extract consists of low amount of tannin (32.55 mg/ml) (Fig. 3). The presence of high amount of tannin shows that *C. serrulata* can be used both as an antiseptic and as a healing agent.

DPPH is a stable free radical that can donate a hydrogen ion to the molecule with its delocalization property and results in a deep violet color[20]. It is stable at ambient temperature owing to its dimerization capacity. The ethanol extract showed the highest DPPH scavenging activity compared to other extracts. Its activity is almost near to the scavenging activity of standard ascorbic acid (Fig. 4). An IC_{50} value of ethanol extract (44.47 µg/ml) was near to the

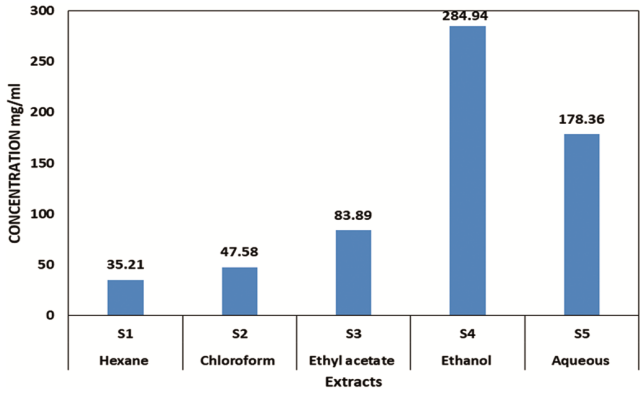

Fig. 1 — Total phenolic content of C. serrulata extracts

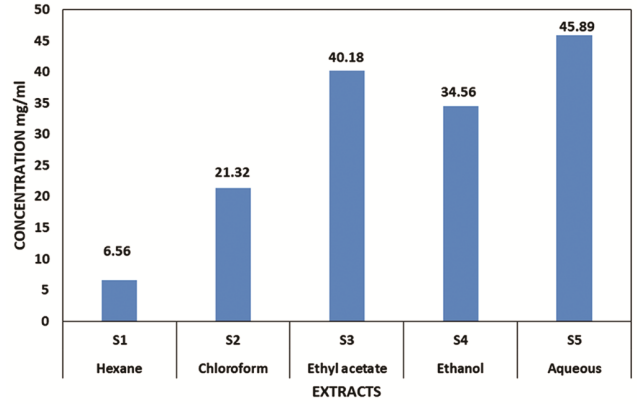

Fig. 2 — Total flavonoids content of C. serrulata extracts

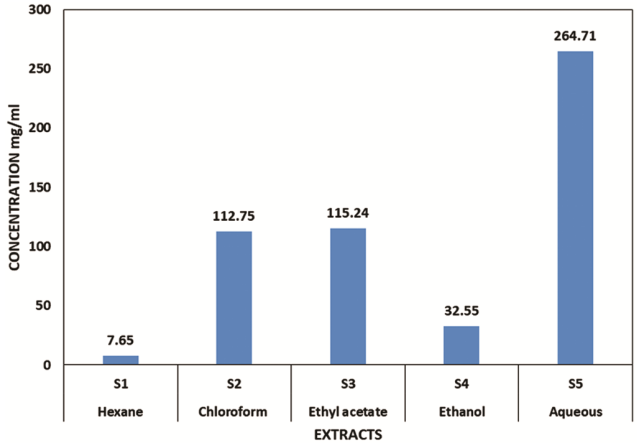

Fig. 3 —Total tannin content of C. serrulata extracts

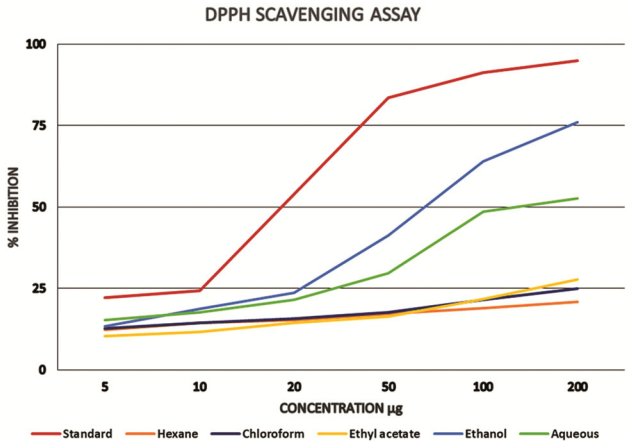

Fig. 4 — DPPH scavenging activity of C. serrulata extracts

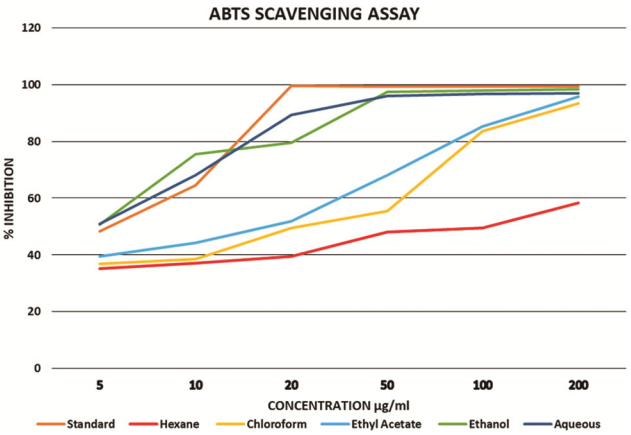

Fig. 5 — ABTS scavenging activity of C. serrulata extracts

IC_{50} value of standard ascorbic acid (13.57 µg/ml). This study reports that *C. serrulata* has a compound that has the ability to donate electrons or hydrogen ions to scavenge DPPH free radicals, thus they can act as a potent antioxidant. When compared to the phytochemicals present in the ethanol extract, they showed a high content of phenol compounds, showing that DPPH scavenging activity is due to the presence of phenol in the ethanol extract of *C. serrulata.*

ABTS is a radical cation (toxic) reactive oxygen species that can disrupt various cell signaling pathways and lead to cell death[21]. Antioxidant acts on these radicals and prevents the formation of cation. The maximum ABTS scavenging activity was reported in the ethanol extract which was equivalent to standard ascorbic acid (Fig. 5). This reveals the efficiency of *C. serrulata* to treat radical cation-related diseases.

NO is a potent pleiotropic mediator of various physiological processes, such as smooth muscle

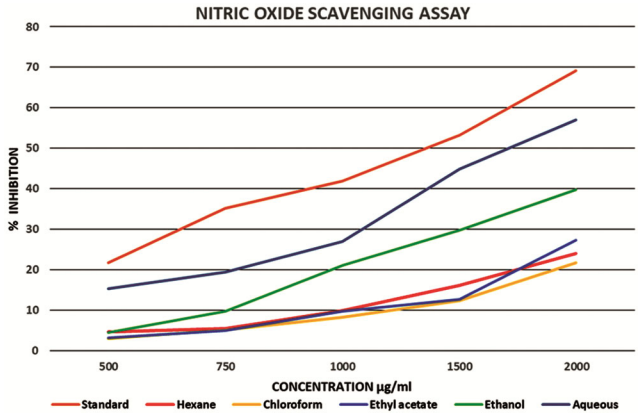

Fig. 6 — Nitric oxide scavenging activity of C. serrulata extracts

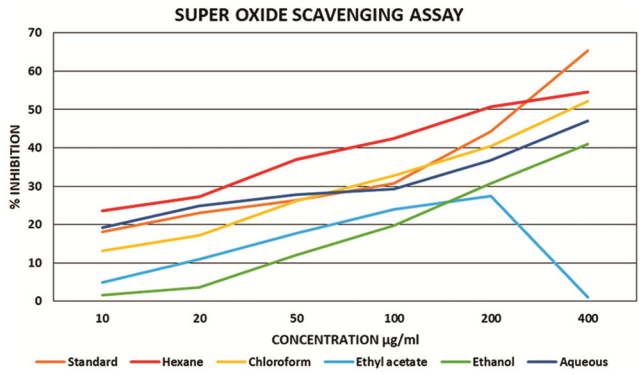

Fig. 7 — Super oxide scavenging activity of C. serrulata extracts

relaxant, neuron signaling, inhibition of platelet aggregation, and regulation of cell-mediated toxicity[22]. The NO radical scavenging activity of the extracts showed that the aqueous extract of *C. serrulata* has higher percentage of inhibition (near to the standard ascorbic acid) (Fig. 6). The ethanol extract has less inhibition activity than the aqueous extract, but higher than hexane, chloroform, and ethyl acetate extracts. *C. serrulata* extract can act against the inflammation caused by NO species during the action of phagocytosis of the pathogen by leukocytes.

SO anion radical is one of the strongest reactive oxygen species among the free radicals that are generated after oxygen is taken into living cells. There was a high SO scavenging activity of hexane extract when compared to the standard (Fig. 7). Other extracts do not show considerable SO scavenging activity and when compared to the phytochemical analysis of the extracts, hexane shows the higher content of tannins, which shows that the tannins present in the hexane extract may be responsible for SO scavenging activity[23].

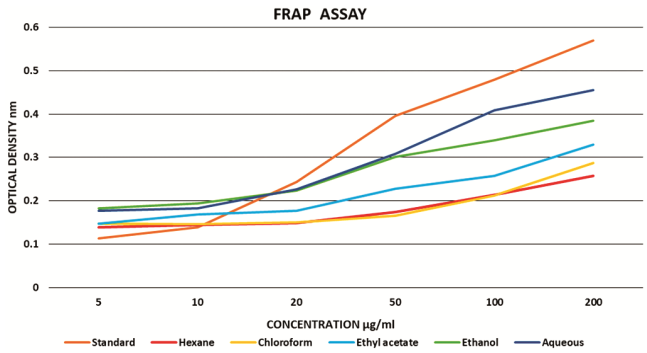

Fig. 8 — Percentage curve of FRAP assay by C. serrulata extracts

Ferrous ion can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals. FRAP (Ferric-Reducing Ability of Plasm) assay is based on reducing the colorless ferric–tripyridyl triazine complex to blue color ferrous (II). The color formation is due to the reducing power of the electron-donating antioxidants present in the test solution which can be measured[24]. In this study, the aqueous extract and ethanol extract showed a better reducing ability (Fig. 8) followed by ethyl acetate and chloroform extracts. The hexane extract showed the lowest reducing ability. The ferrous reducing ability of the subsequent extracts was concentration dependant.

Conclusion

This study not only demonstrates the phytochemicals present in *C. serrulata* but also their antioxidant activity by extracting successively, using solvents based on polaritys. When compared with other extracts, the ethanol extract of *C. serrulata* shows the maximum scavenging activity of free radicals and it has a high content of total phenols and flavonoids. The efficient free radicals scavenging property and reducing property of the ethanol extract of *C. serrulata* proves that it possesses excellent antioxidant properties, and hence it can be used to treat various free radical-mediated diseases. The antioxidant properties exhibited by *C. serrulata* justify its traditional medicinal use. Thus, a lead compound from *C. serrulata* has a great promise for providing potent, cheaper, and safer anticancer drugs, which further needs to be confirmed through extensive investigation

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