Biochemical Characterization and Antioxidant Potential of Elodea (*Egeria densa*) Methanolic Extract

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Article

Biochemical Characterization and Antioxidant Potential of Elodea (*Egeria densa*) Methanolic Extract

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Abstract

Elodea species, including *Egeria densa*, are globally recognized as invasive aquatic macrophytes that significantly disrupt aquatic ecosystems through the formation of dense floating mats thereby inducing anoxic conditions. However, their potential as a source of high-value bioactive compounds remains mostly under-explored. This study aimed to evaluate the phytochemical profile and antioxidant capacity of Elodea methanolic extract to assess its potential for industrial application. Total soluble protein was quantified via the Bicinchoninic Acid (BCA) assay, while total phenolic content (TPC) and total flavonoid content (TFC) were determined using colorimetric methods. The antioxidant activity of the extract was assessed using the Ferric Reducing Antioxidant Power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays, supplemented by qualitative screening for secondary metabolites. Results revealed that the extract possessed a mean soluble protein content of 4.5 ± 0.19 mg BSA eq./g FW, a TPC content of 4.015 ± 0.3 mg GAE/g FW, and a 1.86 ± 0.12 mg QE/g FW. Qualitative screening of the extract revealed the presence of alkaloids and flavonoids. The extract displayed high reducing power (FRAP: 196.6 ± 0.5 µmol Fe^{2+} g^{-1} FW) and significant radical scavenging activity with an IC50 of 65.5 µg/mL, comparable to the commercial standard, ascorbic acid (IC50 of 61.98 µg/mL). These findings showed that *E. densa* is rich in pharmacologically active bioactive components; alkaloids, proteins, phenolics, and flavonoids, thereby highlighting its potential as a reservoir of natural antioxidants. It also suggests that the biochemical synergy between soluble proteins and phenolics drives the high antioxidant efficacy of the extract. The results also suggest that *E. densa* biomass, often overlooked as an invasive species may serve as a sustainable source of bioactive metabolites in the pharmaceutical or food preservative industries.

Keywords: Elodea; antioxidant activity; FRAP; phenolics; BCA assay; invasive species; bioactive compounds

1. Introduction

Human civilization has relied for ages on the use of plants and their products for the provision of food, clothing dyes, fibers, pesticides, herbicides, and medicines [1]. Many plant genera have been utilized in traditional medicine to cure certain ailments or diseases in different countries [2]. Generally, about 20% of terrestrial and aquatic plants have already been subjected to pharmaceutical or biological tests to evaluate their therapeutic or biological activities [3]. A large number of these plants are higher angiosperms, and they have the potential to produce a large quantity of phytochemicals or secondary metabolites [4]. These secondary metabolites, including phenols, tannins, terpenoids, and flavonoids, have been reported to be responsible for important physiological and medicinal properties of plants [5,6]. The phytochemicals are very important components utilized in the development of potentially safer and more environmentally-friendly drugs [6]. These

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phytochemicals abound in different parts of the plants, hence different plant parts including the flowers, leaves, stem, roots and fruits are used traditionally in treatment of diseases [7].

Free radicals are reactive species, with very short half-life, naturally synthesized in the cell for various highly essential cellular processes (such as cell signaling and defense) and metabolism in aerobic organisms. However, when in an out-of-control manner, they can generate an oxidative stress condition in cells resulting in the damage of several cellular macromolecules such as proteins, lipids and DNA [6,8]. Several human diseases and pathological conditions, including cancer, diabetes, neurological disorders, and ischemia, have been linked to oxidative stress and free radical generation [9]. Apart from endogenous production of free radicals in the cells, free radicals can also be produced exogenously by synthetic drugs and other substances. Examples of synthetic drugs capable of generating free radicals include non-steroidal anti-inflammatory drugs (NSAIDs), used in the treatment or management of inflammation, fever, pain, inflammation, and rheumatic conditions [10]. The oxidative damage caused by these free radicals and reactive oxygen/ nitrogen species can be neutralized, inhibited, or delayed by endogenously produced or exogenously obtained antioxidant molecules, including flavonoids, and other phenolic compounds [2,6,10].

Egeria densa is a submerged perennial aquatic plant, popularly known as Brazilian waterweed or Brazilian Elodea. It is indigenous to South America, namely Brazil, Uruguay, and Argentina, but is known globally as a highly invasive species [11]. It is a fast-growing and deeply rooted plant with a dense canopy-forming habit. It is capable of extracting nutrients from both water and sediments and behaves as an ecosystem engineer by controlling the growth of phytoplankton [12]. *E. densa* is extremely invasive and forms dense, monospecific mats that significantly impact aquatic ecosystems by contributing to algal blooms, which reduce oxygen levels and kill aquatic species [12,13]. In spite their noxious nature, many submerged aquatic macrophytes have been reported to be a valuable yet underexploited source of bioactive phytochemicals. These plants synthesize a range of secondary metabolites, including polyphenols such as flavonoids [14]. These polyphenolic compounds are synthesized by the plant as defensive strategies against environmental stresses [2]. Studies have shown that Elodea contains myriads of reducing agents, such as phenolics, flavonoids, proanthocyanidins, saponins, alkaloids, tannins, and flavonols [15]. In spite of the widespread distribution and its potential for biomass accumulation, there is a dearth of published information on the secondary metabolic profile of *E. densa* and the correlation between its soluble protein fraction and antioxidant efficacy. Hence, the objective of this study was to characterize the phytochemical composition and antioxidant potential of *E. densa* methanolic extracts. The antioxidant data expected from this study is also expected to be more baseline and reliable due to the utilization of standardized lab-cultivated Elodea. This is due to the reduction in the possible environmental noise, due to differences in nutrients, sunlight, and the presence of pollutants, often encountered in wild-harvested samples.

2. Materials and Methods

Plant Material

Specimens of *Egeria densa* (Elodea) plants were procured from Carolina Biological Supply Company (Burlington, NC, USA). Upon arrival, the plants were authenticated based on morphological characteristics. The plant specimens were subsequently cultivated in the laboratory under controlled laboratory conditions in a 20 L glass tank at a temperature of 25 ± 2 °C, utilizing dechlorinated tap water supplemented with Hoagland's nutrient medium (pH 6.8 ± 0.2). The plants were subjected to a 12-hour light/12-hour dark photoperiod provided by cool-white fluorescent lamps. After an acclimatization period of three weeks, healthy twigs were harvested, rinsed with deionized water, and prepared for methanolic extraction.

Preparation of Elodea Extract

Fresh *E. densa* plant twigs were harvested after three weeks of acclimatization, rinsed with deionized water to remove epiphytic materials and surface dirt, blotted dry, and shade-dried. The dried sample was prepared for methanolic extraction by pulverizing using a mechanical grinder.

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The powdered *E. densa* leaf material (2g) was placed in an airtight Erlenmeyer flask and macerated with 20 mL of 80% analytical grade methanol, to give the plant material/ solvent ratio (1:10 w/v). The mixture was shielded from light to prevent photo-degradation of the antioxidant compounds. The extraction was carried out by continuously shaking the mixture on an orbital shaker at 150 RPM for 24 hours at room temperature. The slurry was subsequently filtered through a fine muslin cloth, followed by Whatman No. 1 filter paper. The process was repeated twice, to aid maximum metabolite recovery, and the pooled filtrates were concentrated under vacuum at 40 °C yielding 4.8% of methanol extracts.

The resulting crude methanolic extract was weighed, and the percentage yield was determined using Equation 1.

$$\textbf{\textit{Yield}}\ (\%) = \frac{\textbf{\textit{Weight of Dry Extract}}}{\textbf{\textit{Weight of Dry Plant Material}}} \textbf{\textit{x}}\ \mathbf{100} \quad (1)$$

The crude extract was stored in sterile amber vials at 4°C until further biochemical characterization and antioxidant analysis.

Phytochemical Analysis

The crude extract (0.1g) was reconstituted in 10 mL of methanol, sonicated at 40 kHz for 10 mins (Branson Ultrasonics, USA), and the suspension was centrifuged at 4,000 RPM for 15 minutes at 4 °C using an Avanti J-15R refrigerated centrifuge (Beckman Coulter, USA) to ensure complete homogenization and removal of undissolved particles. The resulting supernatant *E. densa* methanolic extract (ELE) was collected and utilized for phytochemical screening.

Preliminary phytochemical screening of ELE was conducted to identify the presence of secondary metabolites. Standard tests were employed: Mayer's reagent for alkaloids, Alkaline reagent test for flavonoids, Ferric Chloride test for tannins, and Froth test for saponins according to Sofowora [16] and modified by Solomon *et al.* [17].

Briefly, for Alkaloids, 2 mL of ELE was treated with 4 drops of Wagner's reagent (0.64g of iodine and 1g of potassium iodide dissolved in 50 mL of distilled water) and observed for the presence of reddish-brown precipitate.

For flavonoids (alkaline reagent test), 2 mL of ELE was treated with a few drops of 20% NaOH solution, and the formation of an intense yellow color, which becomes colorless, on addition of dilute HCl acid, indicates the presence of flavonoids.

For tannin (ferric chloride test), 2 mL of ELE was treated with 10% alcoholic ferric chloride solution (prepared by dissolving 2g of anhydrous ferric chloride in 20mL of 95% ethanol). The formation of a bluish or greenish color solution indicates the presence of tannin.

Quantitative evaluation of the phytochemical constituents was also conducted. The total phenolic content (TPC) of the ELE was done according to the Folin-Ciocalteu colorimetric method described by [18] with slight modification. 500 µL of the extract was mixed with 2.5 mL of 10% (v/v) Folin-Ciocalteu reagent. The reaction was stopped after 5 minutes at room temperature (25 ± 2 °C) by adding 2 mL of a 7.5% (w/v) sodium carbonate solution. The solutions were mixed and incubated in the dark at room temperature for 30 minutes. The absorbance of the resulting solution was measured at 765 nm against a reagent blank on a Biospec-1601 UV–vis spectrophotometer. Gallic acid (0, 20, 40, 60, 80, and 100 µg/mL) was utilized to construct the calibration curve (y = 0.0045x + 0.0101; R2 = 0.9981). The results were presented as mg gallic acid equivalents (GAE) per gram of fresh weight.

The total flavonoid content (TFC) of the ELE was evaluated, using standard Aluminum Chloride (AlCl3) spectrophotometric method, according to the modified method of Agbo et al., [19]. Briefly, 100 µL of the diluted methanolic extract was mixed with 150 µL of distilled water, and 15 µL of 5% sodium nitrite (NaNO2) solution. The mixture was allowed to stand for 5 minutes, and 15 µL of 10% aluminum chloride (AlCl3) was added, mixed, and allowed to stand for 6 minutes. This was followed by the addition of 100 µL of 1M NaOH solution, and the absorbance of the yellow-colored complex was measured immediately at 510 nm using a BioTek Synergy H1MF Microplate Reader. Quercetin (0, 25, 50, 100, 200, and 400 µg/mL) was utilized to construct the calibration curve (y = 0.0005x + 0.0093;

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R2 = 0.999). The results were presented as mg Quercetin Equivalents per gram of fresh plant weight (mg QE/g FW).

Protein Extraction and Quantification

Fresh *E. densa* leaves and twigs (1.2g) was extracted using 12 mL of ice-cold extraction buffer and the homogenate was filtered through Whatman No. 1 filter paper. The resultant aqueous extract was centrifuged at 12000g for 15 minutes at 4°C, and the supernatant was collected and placed on ice for quick biochemical analysis. The total soluble protein content (TSP) of the supernatant was evaluated spectrophotometrically, using the Pierce™ BCA Protein Assay Kit (Thermo Scientific, USA), according to the method described by [20]. The assay is based on the reduction of Cu^{2+} to Cu^{+} by peptide bonds in an alkaline environment (biuret reaction), followed by chelation of Cu^{+} by bicinchoninic acid (BCA), resulting in a purple complex. Briefly, 25µL of the plant extract or standard was combined with 200 µL of BCA working reagent and incubated at 37°C for 30 minutes. The absorbance was measured at 562 nm. Bovine Serum Albumin (31.25, 62.5, 125, 250, 500, 1000, and 2000 µg/mL) was utilized to construct the calibration curve (y = 0.0016x + 0.122; R2 = 0.9939). The final TSP content was expressed as BSA Equivalent in milligrams per gram of fresh weight (BSA Equiv. mg/g FW).

In vitro antioxidant activities

The antioxidant activities of the E. densa methanolic extract (ELE) was evaluated using the 1,1-diphenyl-2- picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assay.

The DPPH and FRAP assays are single electron transfer (SET) assays. The DPPH assay is based on the combination of a plant extract with a stable, intensely purple-hued DPPH radical solution. The antioxidant constituents in the extract then donate an electron or a hydrogen atom, thereby reducing the DPPH to its non-radical, yellow form, DPPH-H, which is quantified spectrophotometrically by measuring the decrease in absorbance [21]. The FRAP assay, however, quantifies the extract's capacity to reduce a ferric iron-ligand complex (Fe3+-TPTZ) to the vividly blue ferrous form (Fe2+-TPTZ) at low pH, resulting in colorimetric change [22].

The DPPH assay was utilized to determine the percentage inhibition/ percentage radical scavenging activity (%RSA) of E. densa plant extracts, according to the procedure reported by [23], according to equation 2.

$$\boldsymbol{Inhibition}\ (\%) = \frac{\boldsymbol{Absorbance_{control} - Absorbance_{sample}}}{\boldsymbol{Absorbance_{control}}} \boldsymbol{x\ 100} \quad (2)$$

Ascorbic Acid (0, 10, 20, 40, 60, 80, and 100 µg/mL) was utilized to construct the calibration curve (y = 0.8123x + 7.9119; R2 = 0.9724). The extract concentration exhibiting 50% radical scavenging activity (IC50) was calculated from the graph of % free radical scavenging activity (%RSA) versus extract concentration.

FRAP activity of *E. densa* plant extract was evaluated spectrophotometrically using the FRAP assay kit (QuantiChrom™ FRAP Assay Kit (DFRAP-250), according to the modified method of [22]. Briefly, 50 µL of the methanolic Elodea extract and standards (180 µM, 108 µM, 54 µM, and 0 µM) were separately mixed with 200 µL of working reagent (prepared by mixing Reagent A, Reagent B, and Reagent C in a 20:1:1 v/v/v ratio respectively). The mixture was then allowed to equilibrate to room temperature (37 °C) for 40 min, and the absorbance was measured at 590 nm using a BioTek Synergy H1MF Microplate Reader. A plot of the optical density (OD) against standard reduced iron concentrations was utilized to prepare a calibration curve (y = 0.0027x + 0.0106; R2 = 0.996). The slope of the plot was determined using linear regression fitting. The FRAP of the sample was calculated as (Equation 3);

$$[\boldsymbol{FRAP}] = \frac{\boldsymbol{OD_{sample} - OD_{blank}}}{\boldsymbol{Slope}\left(\mu\mathbf{M}^{-1}\right)} \boldsymbol{x\ n}(\mu\boldsymbol{M}\mathbf{Fe}^{3+}\boldsymbol{reduction\ potential}) \quad (3)$$

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where OD_{sample} and OD_{blank} represents the OD of the sample and water blank respectively. Slope is the slope of the standard curve and n is the dilution factor. (1 µM Fe3+ reduction potential = 0.5 µM Trolox equivalents).

Statistical analysis

All experiments were conducted in triplicates. Statistical analysis was conducted using SPSS v.24 and the results were expressed as mean ± SD. The relationships between the phytochemical constituents and antioxidant activities were evaluated using Pearson's correlation coefficients (R2).

3. Results and Discussions

Phytochemical screening

The qualitative phytochemical screening of *E. densa*'s aqueous extract revealed the presence of alkaloids and flavonoids (Table 1).

Table 1. Qualitative Phytochemical Screening of *E. densa* Aqueous Extract.

Secondary Metabolite	Test Performed	Observation	Result
Alkaloids	*Mayer's Reagent Test*	*Formation of creamy-white precipitate*	+
Flavonoids	*Alkaline Reagent Test*	*Appearance of an intense-yellow color, which disappeared upon the addition of dilute acid.*	+
Tannins	*Ferric Chloride Test*	*No dark coloration observed*	-
Saponins	*Foam Test*	*Stable foam layer not formed*	-

The aqueous extract of *E. densa* tested positive for alkaloids, as evidenced by the development of a creamy-white precipitate with Mayer's reagent. Furthermore, the flavonoid test was positive, as evidenced by the formation of a bright yellow color upon treatment with NaOH solution, which subsequently turned colorless upon acidification. The positive result observed for Alkaloids and Flavonoids is similar to that observed by [15] in aqueous extract of *Egeria densa*. However, the negative result observed for Saponins and Tannins contrasts with the result of [15]. This difference could be as a result of environmental differences, and biological variability in plant samples, extraction conditions, or plant chemovars. The presence of these compounds implies that the biological activity observed in the extract can be attributable, at least in part, to the presence of these bioactive metabolites.

The results of quantitative analysis of *E. densa* plant extract (Table 2) revealed that the extract had a total soluble protein (TSP) concentration of 4.5 ± 0.19 mg BSA Equiv./g FW of Elodea. This represents a moderate-to-high soluble protein content when compared to similar aquatic macrophyte. The result also indicates that there is substantial amount of reducing and capping/ stabilizing agent in the plant extract. The 4.5 mg BSA Equiv/g FW obtained in this study was higher than the 2.5 mg/ g FW obtained by [24] in *Elodea canadensis*.

The result of total flavonoid content evaluation showed that the Elodea extract had a TFC of 1.86 ± 0.12 mg QE/g FW. This demonstrated that *E. densa* plant extract has a significant and effective reduction capacity. Studies have shown that flavonoids possess significant reducing capabilities, capable of donating electron to convert metal ions into zero-valent nanoparticles [25]. The TFC obtained in this study is much lower than that obtained by [26 & 27].

Table 2. Phytochemical and Antioxidant Properties of *E. densa* Methanolic Extract.

Parameter	Value (Mean ± SD)	Unit
Total Phenolic Content (TPC)	*4.015 ± 0.3*	*mg GAE/g FW*
Total Flavonoid Content (TFC)	*1.86 ± 0.12*	*mg QE/g FW*
Total Soluble Protein (TSP)	*4.5 ± 0.19*	*mg BSA Equiv./g FW*

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Ferric Reducing Antioxidant Power (FRAP)	*196.6 ± 0.5*	*µmol Fe^{2+} g^{-1} FW*

The total phenolic content evaluation showed that Elodea plant extract had a TPC of 4.015 ± 0.3 mg GAE/g FW, which equates to a TPC value of 42.42 - 59.26 mg GAE/g DW. The observed TPC indicates that Elodea contains a relatively high quantity of polyphenolic constituents such as flavonoids, tannins, and phenolic acids. Phenolic compounds are recognized for their capacity to donate hydrogen atoms or electrons, thereby neutralizing free radicals and preventing oxidative damage [28]. They also act as reducing, capping and stabilizing agents in the green-synthesis of nanoparticles [29]. This result is similar to the 4.94-67.7 mg/g FW TPC content obtained by [30] in 15 aquatic macrophytes. However, it contrasts with the elevated TPC content ranging from 72.66-292.56 mg GAE/g DW obtained by [31] in methanolic extracts of wild vegetables from Nepal. The result is also much higher than the 31.63 – 70.01 mg GAE/g FW obtained by [26] in ethanolic extract of Lotus (*Nelumbo nucifera*). The slight variation in TPC content observed in this study and that of others may result from varying concentrations of sugars, carotenoids, or ascorbic acid, as well as differences in duration, geographical factors, or extraction processes [32].

***In vitro* antioxidant studies**

The results of in vitro antioxidant studies presented in Figure 1 showed that both the extract and the standard exhibited a dose-dependent increase in Radical Scavenging Activity (% RSA). The RSA of the plant extract increased from 16.50% at 5 µg/mL to a peak of 52.68% at 100 µg/mL. Ascorbic acid, the reference molecule, demonstrated a significant enhancement in activity, beginning at 17.24% at 5 µg/mL and achieving an impressive 90.94% inhibition at the maximum dose of 100 µg/mL. ELE exhibited a swift initial rise in %RSA, reaching 46.34% at 40 µg/mL, but its activity subsequently plateaued at elevated concentrations, only marginally increasing from 49.40% at 60 µg/mL to 52.68% at 100 µg/mL. This plateau indicates that the quantity of the extract's most active, rapidly responding components was likely exhausted, or that the remaining active chemicals exhibit slower reaction kinetics with the DPPH radical. In contrast, ascorbic acid exhibited a consistent and very effective scavenging capability across the concentration spectrum, with a notable increase in activity at elevated concentrations, reflecting its potent and swift hydrogen-donating capacity.

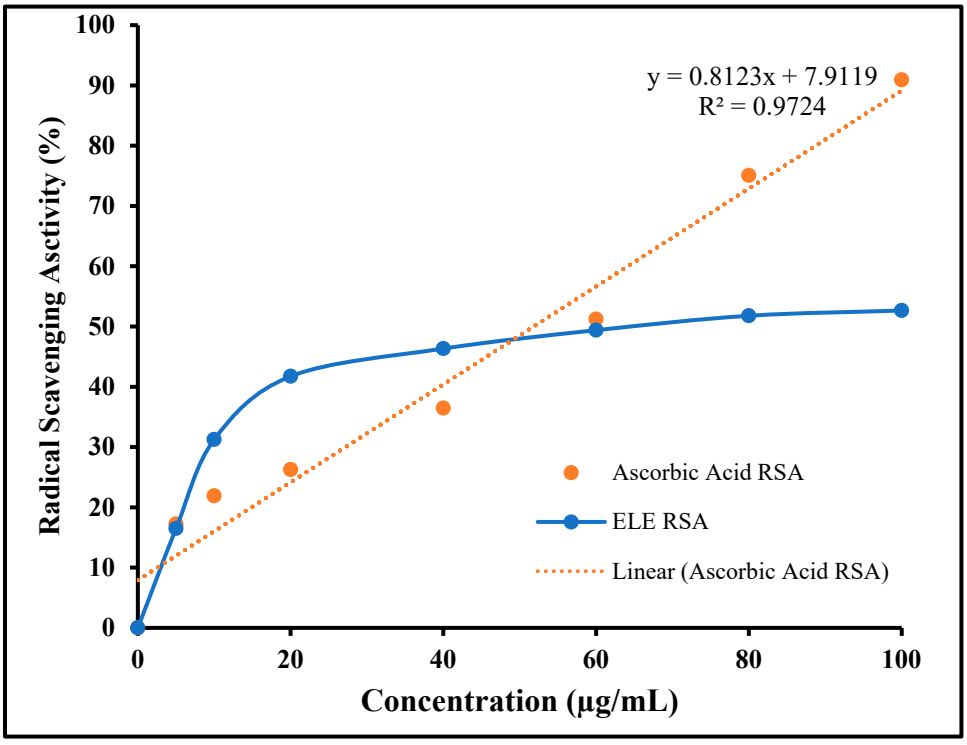

Figure 1. Dose-response curve for the radical scavenging activity (%RSA) of E. densa extract and ascorbic acid.

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ELE exhibited an IC50 value of 65 µg/mL, while ascorbic acid has an IC50 value of 61.98 µg/mL thereby revealing that ascorbic acid demonstrates more DPPH radical scavenging activity than the evaluated plant extract. This result thereby showed that ELE exhibits moderate antioxidant activity, which can be attributed to the presence of phenolic chemicals, flavonoids, or other phytoconstituents functioning as hydrogen or electron donors. The IC50 value conforms with that observed by [33] in some Egyptian wild plant extracts but contrasts with that observed by [34] in Blueberries (*V. corymbosum*).

The results of FRAP assay showed that ELE had a FRAP value of 196.6 µmol Fe^{2+} g^{-1} FW, showing significant reducing power on a fresh-weight basis. This equates to an approximate amount of ~1,966 µmol Fe^{2+} g^{-1} DW when adjusted for an assumed 90% water content, indicating significant antioxidant/ ferric ion reducing capacity relative to terrestrial and aquatic species. This high reducing power reflects the abundance of phytochemical constituents capable of donating electrons to convert Fe^{3+} to Fe^{2+}, thus confirming the extract's potent antioxidant potential. Such redox-active compounds not only confer protection against oxidative stress but also facilitate green synthesis of nanoparticles by acting as natural reducing and antioxidant agents. The observed FRAP activity contrasts with the 151 µmol Fe^{2+} g^{-1} observed by [35] in hydroethanolic extract of Eichhornia crassipes, as well as the 71.01 µmol Fe^{2+} g^{-1} observed by [36] in methanolic leaf extract of T. cordifolia. The higher FRAP value observed in laboratory-grown Elodea may be due to the presence of naturally richer content of reducing compounds in the species, the choice of solvent used for the extraction, excellent food availability and the absence of environmental stresses that decrease phenolic content.

Furthermore, the high FRAP value (196.6 µmol Fe^{2+} g^{-1} FW) is likely attributed to the synergistic effects between the quantified phenolic compounds and the total soluble protein content. Studies has shown that plant proteins are capable of acting as secondary antioxidants by chelating transition metals or by working synergistically to enhance the overall radical scavenging activity of plant extracts [37,38].

Pearson Correlation Analysis

The result of correlation analysis (Table 3) revealed the presence of a perfect linear relationship across all variables. This high correlation is indicative of a system where an increase in secondary metabolites (phenolics and flavonoids) directly and proportionally results in an increase in antioxidant capacity.

Table 3. Phytochemical and Antioxidant Properties of *E. densa* Methanolic Extract.

Parameter	TPC	TFC	TSP	FRAP	IC_{50}
TPC	1	1.000**	1.000**	1.000**	1.000**
TFC		1	1.000**	0.999**	1.000**
TSP			1	0.999**	1.000**
FRAP				1	1.000**
IC_{50}					1

The perfect correlation observed between TPC and FRAP ($R^2 = 1.000$, $p < 0.01$) indicates that the antioxidant activity of ELE is almost entirely governed by its phenolic constituents. The correlation of TPC and TFC with IC_{50} indicates that the increase in concentration of these bioactive compounds resulted in a corresponding increase in the radical scavenging potential of the extract, and showing its functional bioactivity and potency. The strong correlation observed between TPC and TFC with FRAP value indicates that they both contribute to an increase in FRAP value and the antioxidant activity of the plant extract. This result aligns with that observed by [39] in six indigenous plants used in traditional Thai medicine. However, the positive correlation observed between TPC and DPPH indicates that the radical scavenging activity of the extract is plateauing, while the TPC is increasing, as observed in Figure 1, or that it is being influenced by increasing TSP.

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4. Conclusions

E. densa is rich in pharmacologically active bioactive components; alkaloids, proteins, phenolics and flavonoids. This highlights its potential as a reservoir of natural antioxidants. The quantitative data obtained through BCA and FRAP assays demonstrates a significant correlation between soluble protein concentration and ferric-reducing capacity. The presence of critical secondary metabolites- specifically alkaloids and flavonoids- indicates that *E. densa* biomass, often overlooked as an invasive species may serve as a sustainable source of bioactive metabolites in the pharmaceutical or food preservative industries. These findings lay the foundation for future research into the isolation of bioactive components from *E. densa.*

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