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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITIES OF METHANOL EXTRACT AND ITS SOLVENT PARTITIONED FRACTIONS OF SRI LANKAN MARINE RED ALGAE *Gracillaria edulis* (Gmelin) Silva

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Introduction

Among the under-explored marine flora, seaweeds are an important source of bioactive metabolites in drug development and nutraceuticals. Bioactive compounds are generated as a result of their biochemical and physiological mechanisms. Some of these bioactive compounds are used in pharmaceutical industries for various therapeutic purposes as they exhibit anti-oxidant, anti-diabetic, anti-proliferative, cytotoxic and anti-inflammatory properties. Oxidative stress plays a major role in chronic inflammation and it is tightly linked with the pathophysiological processes of numerous degenerative diseases such as diabetes, cardiovascular disorders, cancer, inflammation and aging. Therefore, in lieu with herbal drug development, combat of oxidative stress through natural antioxidants present in less utilized marine flora are very important since there are under explored in Sri Lanka (Correa-Rotter *et al.,* 2004).

Gracillaria edulis (Gmelin) Silva is a red algae belongs to the family Gracilariaceae. Polyphenols purified from red algae are considered as a rich source of antioxidants with significant health promoting properties (Correa-Rotter *et al.,* 2004). Therefore, the present study was undertaken to investigate the phytochemicals and in-vitro antioxidant potential of methanolic extract and its solvent fractions of *G.edulis.*

Materials and Methods

The permit to collect algae sample was obtained from Department of Wild life Conservation (permit number- WL/3/280/17). G. *edulis* was manually collected from Kalpitiya area during the month of February. The collected samples were cleaned and washed with fresh water to remove salt, sand, attached epiphytes and organic matter. The samples were freeze dried and ground into a fine powder and stored at -20°C until further use.

Homogenized powder (10.0g) extracted three times using 70% methanol was subjected to sonication at 25 °C for three 90 min periods. The polyphenols were separated by precipitating crude polysaccharides by adding 25 volumes of 70% ethanol (v/w% -1:25) and allowed to stand overnight. The supernatant was

separated by centrifugation (12,000 rpm). The portion of the supernatant was used to solvent-solvent partition with hexane, chloroform and ethyl acetate respectively (Lakmal *et al.,* 2014). The resulting hexane fraction (GEMH), chloroform fraction (GEMC), ethyl acetate fraction (GEME), aqueous fraction (GEMA) and crude methanol extract (GEMM) of *G.edulis* were used for qualitative and quantitative determination of phytochemicals and for evaluation of antioxidant activities.

Methanol extract of *G. edulis* was used for qualitative analysis of phytochemicals. Cardiac glycosides, saponins, terpenoids, phenols, alkaloids, steroids, tannin and phytosterols were analyzed by qualitative chemical methods (Jeyaseelan *et al.,* 2012). Total polyphenolic and total flavonoid content were determined using Folin-Ciocalteu (Singleton *et al*., 1999) and aluminum chloride method (Chang *et al.,* 2002) respectively. Total alkaloid content was determined by Sreevidya *et al*., (2003) with some modifications.

Four different in-vitro antioxidant assays were used to determine the antioxidant activity of methanolic extract and fractions of *G.edulis.* Ferric reducing antioxidant power (FRAP) and DPPH radical scavenging activity was performed according to the method described by Benzie & Szeto (1999) and Blois (1958) respectively. The ferrous iron chelating capacity (FICC) and oxygen radical absorbance capacity (ORAC) was determined using ferrozine reagent according to the method by Carter (1971) and Ou *et al.,* respectively (2001).

Statistical analysis was performed using Minitab 2017 and Excel 2013. All data values are expressed as mean±standard deviation based on three replicates. P values less than 0.05 ($P< 0.05$) was considered as significant.

Results and Discussion

The qualitative phytochemical screening revealed the presence of cardiac glycosides, saponins, terpenoids, phenols, alkaloids, steroids, tannin and phytosterols in the methanolic extract of *G. edulis.* The results of quantitative analysis of TPC, TFC and total alkaloid content is presented in table 1.

Results showed the significant difference of TPC in four different fractions ($P<0.05$). The highest TPC and TFC were reported in GEME fraction (2414.51±50.34 µg Gallic acid equivalent/g of fraction and 1461.49±75.22 µg quercetin equivalents/g of fraction) when compared to crude methanolic extract (1007.81±54.21 µg Gallic acid equivalent/g of extract and 541.01±51.84 µg quercetin equivalents/g of fraction). The highest alkaloid content was observed in crude methanolic extract (173.41±4.03 mg alkaloids/g of extract) of *G.edulis* compared to four fractions. Ganesan *et al*., (2008) reported that the total phenolic content of ethyl acetate fraction of *G.edulis* as 7.81± 0.76 mg gallic acid equivalents/g extract. The present study showed comparatively lower content of TPC than the previous study compared. This might be due to the environmental

variations which they are grown.

Table 01. phytochemical analysis of methanolic extract and fractions of *G.edulis*

	TPC (µg gallic acid equivalents/g of extract)	TFC (µg quercetin equivalents/g of extract)	Alkaloids mg of alkaloids/ g of extract
Methanol extract (GEMM)	1007.81±54.21	541.02±51.84	173.41±4.03
Hexane fraction(GEMH)	760.85±37.75	688.60±9.55	60.96±5.45
Chloroform fraction(GEMC)	560.85±55.08	289.39±9.55	58.40±5.26
Ethyl acetate fraction (GEME)	2414.51±50.34	1461.49±75.22	65.01±5.78
Aqueous fraction (GEMA)	1704.69±43.16	786.95±62.04	141.84±19.27

Results represent means ± standard deviation of triplicate determinations.

The antioxidant potential of *G. edulis* was measured by its ability to scavenge the stable DPPH radical (Table 02). The highest DPPH radical scavenging activity was reported in GEME fraction of *G.edulis* (2732.81±36.49 µg Trolox equivalents/g of extract) with IC_{50} of 3.17±0.04 mg/ml compared to standard trolox (IC_{50}:8.68±0.06 µg/ml). Present study revealed that the reduction of DPPH occurred in a concentration-dependent manner as observed with the high reduction of DPPH (higher radical activity) in high concentrations (table 03).

According to study conducted by Ganesan *et al.,* (2008) in India using methanol extract and fractions of *G. edulis*, ethyl acetate fraction has shown 4.73% DPPH radical scavenging activity at 1000 µg/ml and methanol extract has shown 5.20% DPPH scavenging activity at 1000 µg/ml. When compared to present study, 21.06% and 19.52% DPPH radical scavenging activity was observed in GEME fraction and methanol extract of *G.edulis* at 937.5 µg/ml respectively.

Francavilla *et al.*, (2013) reported the biochemical composition of *G.edulis* vary with seasonal factors. According to the study of Francavilla *et al*, EA fraction of the sample collected in July showed highest DPPH activity (EC_{50}:0.82 mg/ml) than the EA fraction of another sample collected in October (EC_{50}:2.55 mg/ml). Present study reported 3.17mg/ml IC_{50} for EA fraction of *G.edulis* which was collected in February. Differences in activity may be due to the seasonal factors.

Table 02. Antioxidant activity of methanolic extract and fractions of *G. edulis*

	Antioxidant activity			
	DPPH (µg Trolox equivalents/g of extract)	**FRAP (µg Trolox equivalents/g of extract)**	**FICA (µg EDTA equivalents/g of extract)**	**ORAC (µg Trolox equivalents/g of extract)**
GEMM	2720.36±22.18	268.95±34.97	2093.32±36.75	1446.36±56.19
GEMH	1394.76±2.19	1661.75±60.81	7552.42±95.89	531.43±25.18
GEMC	2634.82±18.61	2405.35±14.41	8022.24±48.60	770.84±48.04
GEME	2732.81±36.49	8505.50±44.27	8750.27±33.21	1462.88±16.39
GEMA	2220.64±14.36	1417.10±41.38	7186.33±43.41	363.93±27.52

Data represented as mean ± SD of triplicate determinations. GEEMM- *Gracillaria edulis* 70% methanol extract; GEMH- *Gracillaria edulis* 70% methanol extract hexane fraction; GEMC- *Gracillaria edulis* 70% methanol extract chloroform fraction; *GEME- Gracillaria edulis* 70% methanol extract ethyl acetate fraction; GEMA- *Gracillaria edulis* 70% methanol extract aqueous fraction

Table 3. Dose response relationship for DPPH radical scavenging activity

	(% Inhibition)						
	Assay Concentration (mg/ml)						
	3.75	**1.875**	**0.938**	**0.469**	**0.235**	**0.117**	**IC_{50}**
GEMM	55.38±0.5	35.83±0.4	21.05±0.2	13.41±0.4	11.01±0.7	6.98±0.4	3.19±0.02
GEMH	56.43±0.2	36.66± 0.6	23.70± 0.3	14.85±0.9	11.89±0.2	9.57±0.5	6.22±0.01
GEME	57.95±0.5	31.06±0.3	19.52±2.4	16.07±0.5	11.41±0.1	3.74±0.7	3.17±0.04
GEMC	54.68±0.4	32.85± 1.2	20.95± 0.4	15.29±0.3	9.81±0.7	4.79±0.5	3.29±0.02
GEMA	46.92±0.3	29.45±0.5	18.25±0.7	13.46±0.2	9.45±0.4	4.96±0.2	3.90±0.02

Data represented as mean ± SD of triplicate determinations. GEEMM- *Gracillaria edulis* 70% methanol extract; GEMH- *Gracillaria edulis* 70% methanol extract hexane fraction; GEMC- *Gracillaria edulis* 70% methanol extract chloroform fraction; *GEME- Gracillaria edulis* 70% methanol extract ethyl acetate fraction; GEMA- *Gracillaria edulis 70%* methanol extract aqueous fraction.

The ferric reducing antioxidant power (FRAP) assay uses antioxidants as reductants. Hence, the antioxidants present in the plant extract can reduce the Fe3+/ferricyanide complex to the ferrous form (Sampath *et al.,* 2015). In the present study, highest FRAP was observed in GEME fraction (8505.50±44.27 µg Trolox equivalents/g of extract). In contrast, Francavilla *et al.*, (2013) had reported highest reducing power in ethyl acetate fraction (808.90 µmol Trolox/ g of extract) of *G.edulis* collected seasonally in the Lesina Lagoon at Italy during the period of July. The present study result shows higher reducing power in ethyl acetate fraction which was collected in February. Therefore differences in reducing power might be due to the seasonal variation.

The ferrous iron chelating capacity of *G.edulis* was determined using ferrozine reagent. Ferrozine can chelate with Fe^{2+} and form ferrozine-fe^{2+} red coloured complex (Ranasinghe *et al.,* 2012). The highest iron chelating activity was observed in GEME fraction (8750.27±33.21 µg EDTA equivalents/g of extract) with IC_{50} value of 2.22± 0.01 mg/ml compared to the standard EDTA (IC_{50}:19.34± 0.07 µg/ml). The % chelating activity was observed as 92.99±0.29% to the highest sample concentration (15mg/ml) and 13.97±0.37% for the lowest sample concentration (0.468mg/ml) respectively.

Oxygen radical absorbance capacity of methanolic extract and fractions of *G. edulis* is given in Table 02. The ORAC assay measures the oxidative degradation of fluorescein molecule. During ORAC Assay, peroxyl radicals are generated from the thermal decomposition of AAPH. In the presence of peroxyl radicals, fluorescein start to decay. Addition of samples rich in antioxidants reduce the decay of fluorescein molecule (Ganske, 2010). In the present study, highest Oxygen radical absorbance capacity was reported in methanolic extract (1462.88±16.39 µg Trolox equivalents/g of extract) of *G.edulis* compared to its solvent fraction.

Conclusions

It is concluded that methanol extract and its fractions of *Gracillaria edulis* contains phytochemicals and antioxidant activities with varying degrees of potential. However, EA fraction of *G.edulis* showed significantly high levels of bioactive phytochemicals and possess marked antioxidant activity. Hence the isolation of active compounds in ethyl acetate fraction is warranted.

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