

Nutraceutical properties of the marine macroalga *Gayralia oxysperma*

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Abstract

We analyzed *Gayralia oxysperma* (Monostromataceae, Ulvales) from coastal waters in India for nutrients and biochemicals including nutritionally important fatty acids and active radical scavenging properties. This seaweed had moderately high lipid and other biochemical components, which were comparable to levels in other nutritionally rich seaweeds. The species is an important source of Ca (2.74 mg g⁻¹) and other essential elements with sufficiently low (below permissible limits) contaminant trace metals. There were four major fatty acids, palmitic acid (PA, C16; 34.6%), linoleic acid (LA, C18:2n-6; 15.8%), gamma-linolenic acid (GLA, C18:3n-6; 15%), and stearic acid (SA, C18; 9.6%). Totally saturated fatty acids predominated (TSFA, 57%), followed by polyunsaturated acids (PUFA, 39%); the monounsaturated fraction (MUFA) was smallest (3.8%). Antioxidant potential assessed through phenolic content, di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH), hydrogen peroxide (H₂O₂), reducing potential and metal chelating ability indicated a dose-dependent relationship with increasing activity with increased concentration. Thus *G. oxysperma* has essential properties of nutritional, antioxidant and medicinal value for its use in dietary and pharmaceutical applications.

Key words: Fatty acids, seaweeds, nutritional value, mineral content, antioxidant properties

Introduction

Seaweeds have traditionally formed a human dietary component in Asia mainly Japan, China, Philippines, Malaysia and Sri Lanka, but are now consumed in North America, South America and Europe (McHugh, 2003). These macroalgae are rich sources of protein, lipids, minerals and vitamins (Norziah and Ching 2002; Sanchez-Machado et al, 2002 and Wong and Cheung, 2000) and find wide use in the pharmaceutical, cosmetic and food industry. However the major commercial application of these seaweeds is for the production of hydrocolloids such as agar, carrageenan and alginates (Barbara & Cremades, 1993). These polysaccharides are used as food additives for their thickening properties (McHugh, 2003). While the species belonging to *Ulva*, *Gayralia*, *Caulerpa*, *Undaria*, *Durvillaea*, *Sargassum*, *Turbinaria*, *Porphyra*, *Chondrus*, *Hypnea*, *Gigartina* and *Gracilaria* have been commonly utilized (Zemke-White and Ohno, 1999), there are several others whose nutritive value is yet to be established.

Lipid content in marine algae varies from 1-5% of dry matter; of this, polyunsaturated fatty acids (particularly ω_3 and ω_6) are of vital clinical importance (Mishra et al., 1993). Fatty acids (FAs) in marine flora are usually reported to be rich in eicosapentanoic acid (C20:5 ω_3) and arachidonic acid (C20:4 ω_6). The green algae are rich in linolenic acid (C18:3), a precursor for prostaglandins and thromboxans which are involved in the modulation of immunological, inflammatory and cardio-vascular responses (Renaud et al., 1999). Similarly, palmitic acid, steric acid and gamma linolenic acid are used in the pharmaceutical, nutraceutical and cosmetic industries (WHO, 2003).

Besides being a prominent source of essential fatty acids, seaweeds have important capacities in the scavenging of oxyradicals. Phloroglucinol and phenolics in marine algae serve as metal chelators and enzyme modulators protecting the cell from reactive oxygen species damage and lipid peroxidation (Rodrigo and Bosco, 2006). Since seaweeds represent a valuable natural resource with potential nutritional and economic value, a number of species have been explored for antioxidant properties in different countries (Nagai and Yukimoto, 2003), but there is limited information from India (Pise et al., 2010). Nutritional potential of seaweeds vary by species, geographic area, season and environmental conditions (Ito & Hori, 1989). *Gayralia oxysperma* (Kützing) K.L.Vinogradova ex R.F. Scagel, P.W. Gabrielson, D.J. Garbary, L. Golden, M.W. Hawkes, S.C. Lindstrom, J.C. Oliveira et T.B. Widdowson is a floating green alga that is very abundant in the Acharya estuarine region on the Maharashtra coast,

India. Its nutritional properties have not been fully examined. The focus of the present study was to evaluate the potential nutritive properties and free radical scavenging capacity of this seaweed species in order to determine its economic value for wider application in food products.

Material and methods

Chemicals

Fatty acid methyl ester (FAME) obtained from Sigma-Aldrich, (Deisenhofen, Germany) was used as the GC (gas chromatography) standard. Toluene and potassium carbonate (anhydrous) were also obtained from Sigma while methanol, chloroform, hydrochloric acid, sodium sulfate and sodium hydroxide were of analytical grade from Merck, (Mumbai, India).

Detailed methodology of sample preparation

Fresh algal material were collected from 3 different stations within a distance of 800 meters from the mouth towards inland at an Achara estuarine location (16°11'57.71"N, 73°26'38.99"E) located on the central west coast of India in April 2011. Immediately after collection the algae were thoroughly washed in seawater to remove adhering debris and epiphytes. In the laboratory the material was further rinsed with distilled water and subjected to oven drying at a constant temperature below 60°C. The dried tissue from 3 different sites were separately ground to a fine powder, sieved through a 0.5mm mesh and used as replicates to carry out all tests including antioxidant and biochemical analysis.

Methanol extract preparation:

Five grams of dry algal powder from each of three sites were extracted separately with 500ml methanol for 24 h at 180 rpm on a shaker. The extract was concentrated to 20 ml in a vacuum evaporator (BUCHI Rotavapor R-200, Mumbai, India) at 40°C and was stored at -20°C for use in antioxidant assays.

Biochemical analysis

Total protein was determined by the Lowry et al. (1951) method using bovine serum albumin (BSA) as a standard, and was expressed as percent (%) dry weight. Carbohydrate was estimated by the Anthrone method (Nelson 1944), utilizing glucose as a standard and expressed as percentage (%); lipids were measured following the Folch method described by Christie (2003). The standard method of the AOAC (2005) was applied for the estimation of ash contents. Water content was determined by drying the

seaweed samples in an oven at 105°C until a constant weight was obtained. The ash content was obtained by calcinations in a muffle furnace at 550°C for 4 h.

Elemental analysis

Metal contents were analyzed by digesting 0.5 g of dry algal powder in 20 ml of concentrated HNO₃ in a beaker covered with a watch glass. After complete digestion, the samples were diluted to 100 ml and filtered through Whatman No1 filter paper (Merck, Mumbai, India). The acid digest was analyzed for trace metals such as Ca, Fe, Mn, Cr, Zn, Cu, Cd, Pb, As and Hg using atomic absorption spectrophotometry (Model: Analyst 300 Perkin-Elmer, Massachusetts, USA; AOAC 2005). Iodine was determined using a spectrophotometric kinetic assay (Mahesh et al. 1992) based on measurement of iodide-catalyzed reduction of Ce(IV) to Ce(III) by As(III). The reaction was continuously monitored at 370 nm and the initial velocity of decreasing absorbance was plotted against iodine concentration. Samples from three different sites were subjected to triplicate analyses.

Phenolic contents

Total phenolics (TP) were determined according to Slinkard and Singleton, (1977). The reaction mixture containing 1ml of sample extract and 1ml of Folin-Ciocalteu reagent swirl mixed with distilled water at 1:5 dilution and incubated for 1 to 8 min at room temperature (28°C). Three milliliters of 2% Na₂CO₃ solution were added to the mixture, which was allowed to stand for 2 h, with intermittent shaking. Absorbance was measured spectrophotometrically at 760 nm, and compared with a standard curve of gallic acid.

DPPH assay

Free radical scavenging potential in methanol extract was measured (Blois, 1958) using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The reaction mixture consisted of 2.5 ml DPPH solution (0.1mM in methanol) and plant extract (0.1-0.3 ml) adjusted to a total volume of 3 ml by adding methanol. The absorbance was measured at 0 min and after 30 min at 517 nm. Butylated hydroxytoluene (BHT) was used as the standard. The scavenging effect was expressed as percent as shown below:

$$\text{Percent inhibition} = \left[\frac{A_0 - A_1}{A_0} \right] \times 100$$

Where, A₀ is absorbance at 0 minute and A₁ is absorbance at 30 minutes.

Reducing power

We determined reducing power using methanol extract following a modified method from Oyaizu (1986). The reaction mixture contained 2.5 ml of phosphate buffer (0.2 M, pH 6.6), 2.5 ml potassium ferricyanide (1%) and varying volumes of extract (0.1 to 0.3 ml); the volume was adjusted to 6 ml with distilled water. This mixture was incubated at 50°C in a water bath for 30 min and allowed to cool at room temperature (28°C). To each reaction mixture, 2.5 ml of 10% TCA (trichloroacetic acid) were added, followed by centrifugation at 2000 rpm for 10 min. Two and a half milliliters of distilled water and 0.5 ml of FeCl₃ (1.0%) were added to the supernatant (2.5 ml); the mixture was allowed to react for 10 min at room temperature (28°C), and absorbance was measured at 700 nm. Ascorbic acid (ASA) solution was used as the standard, and the blank contained the same reaction mixture, but without the sample extract.

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging capacity of the extract was determined using the method described by Ruch et al. (1989). A solution of H₂O₂ (10 mM) was prepared in phosphate buffer (pH 7.4). Reaction mixtures containing 2.5 ml H₂O₂ and varying concentrations of algal extract (0.1 to 0.3 ml) were adjusted to 3 ml by adding phosphate buffer (0.1 M, pH 7.4). Absorbance was measured at 0 min and 60 min at 240 nm. Ascorbic acid was used as the standard. The scavenging effect was calculated in % using the formula:

$$[A_0 - A_1 / A_0] \times 100$$

Where, A₀ is initial absorbance (0 min) and A₁ is final absorbance (60 min)

Metal chelating activity

Metal chelating activity was determined according to the method of Dinis et al, 1994. Ferrous chloride (FeCl₂, 2 mM) and ferrozine (5 mM) were prepared. Briefly, different volumes of the extract (0.5 to 1.5 ml) were added to 0.2 ml of 2 mM FeCl₂. The reaction was initiated by addition of 0.2 ml of ferrozine. The mixture was shaken vigorously and allowed to stand for 10 min at room temperature (28°C). The absorbance was measured (at 562 nm) with a spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan). The ability of the extract to chelate ferrous ions was compared with the standard Na₂EDTA (0.1mg ml⁻¹), calculated as below:

$$\text{Chelating effect (\%)} = [1 - (A_1/A_0)] \times 100$$

Where, A_0 is absorbance of the control, and A_1 is absorbance with the test compound

Analysis of fatty acid composition

Extraction of oil

Wet algal thalli were washed thoroughly as described above and oven dried (below 60°C), and then powdered. This powder was extracted in petroleum ether using a Soxhlet apparatus. To analyze FAs, the oil was subjected to esterification of lipids by the addition of freshly prepared acetylchloride–methanol (1:20), after which, the reaction was left to take place at 95°C for 1 h in a heating module (Block-Therm, MTA KUTESZ, Budapest, Hungary). The fatty acid methyl ester fraction was eluted with petroleum ether:diethyl ether = 50:50 (v/v). The fractions were re-dissolved in hexane and subjected to gas chromatographic analysis.

GC-FID analysis

Fatty acid methyl esters (FAMES) were analysed by GC-FID (gas chromatography flame ionization detector; SHIMADZU GC-17A, Kyoto, Japan). FAMES were separated on a CHROMPACK (Bristol, PA, USA) WCOT 25×0.25 mm ID, 0.2 µm film thickness capillary column using a temperature program from 150°C^{5 min}, 40°C^{min} until 235°C and 50 min at 235°C with the following conditions: injector temperature 260°C, FID temperature 260°C and helium as the carrier gas. Identification of fatty acids in the samples was performed by comparison with chromatograms of fatty acids standard (C₄-C₂₄ fatty acids) from Sigma Chemicals. Usually, 80-90% of the peaks in the chromatograms were identified. Fatty acids composition was calculated from the total identified fatty acids area and the values were always the average of at least two to three injections of each triplicate extract.

Statistical analysis

Analytical data was tested statically and expressed as means ± standard deviation. In addition, data concerning the biochemical parameters i.e. carbohydrates, proteins, lipids, water, ash and inorganic elements were normalized and tested with **Kolmogorov-Smirnov & Lilliefors test** and the assumption of homogeneity of variances was tested using **Cochran's test**. Significant results were investigated using a one-way ANOVA for identification of significant statistical differences if any along the studied

stations. The F-ratio and its respective p-value are also presented. Post hoc tests (Newman-Keuls) was used to separate mean values and significant effects were accepted at $p < 0.05$ for antioxidant assays. Correlations (r-value) were calculated between DPPH and reducing power.

Results and discussion

The genus *Gayralia* has a wide distribution along Indian coast; *G. oxysperma* is especially abundant in estuarine waters at Achara on the west coast. Fronds of this species reach maturity with light-green tissues along the thallus margin in February and March, attaining a largest size of 14 cm in length, 10 cm in width, and 2–5 g wet weight. The blades are one cell thick. The cells in surface view are unevenly positioned or are clustered in groups of 2-4; they are generally 7-18 μm in diameter, uncommonly up to 26 μm . The thallus is often sac-shaped, folded and irregularly undulate. Each cell has one cup-shaped chloroplast with a single pyrenoid. The cells are usually rounded or oval.

Biochemical composition

Gayralia oxysperma contained 23.27% carbohydrate, 11.97% protein and 4.94% lipid (Table 1). The composition of this seaweed matches well with lipid rich *Ulva clathrata* (Roth) C. Ag. [as *Enteromorpha clathrata* (Roth) Greville] from among the 12 reported species from Mandapam coast (Manivannan et al 2009) containing adequate proteins and carbohydrates. These components are also closer to the some other tropical green seaweed species in Petchburi province, Thailand. (Ratnan-arporn and Chirapart, 2006).

This seaweed rather resembles soyabean, meat, milk and bakers yeast in terms of carbohydrate, wheat, rice and corn in protein content and baker's yeast in lipid content (Table 2; Becker, 2004). Presence of carbohydrates such as starch, cellulose, sugars and other polysaccharides effectively serves to improve the metabolizable energy content of this alga (Becker, 2004). Similarly the high ash content of 26.70% in *G. oxysperma* probably indicates an elevated mineral concentration (Ratnan-arporn and Chirapart, 2006), which would enhance the nutritive status of this algae.

Inorganic elements

There were adequate nutritional quantities of Fe, Mn, Ca, Cu and Zn (ranging between 0.0035 to 2.74 mg g^{-1} dry wt; Table 1). These minerals, which are required for the activation of cellular enzyme and

hormone systems (Norziah and Ching 2000), occurred in the following rank order of abundance Ca>Fe>Mn>Zn>Cu.

A system of continuous surveillance of contaminant content in food is crucial for consumer protection and facilitates international trade (Kuhnlein and Chan, 2000). Metal concentrations in algae are strongly dependent on the physico-chemical parameters in the environment (Zbikowski et al., 2006), structural difference among the algae (Favero et al., 1996), the bioavailability of metals in the vicinity and the uptake capacities of the individual forms (Sanchez-Rodriguez et al., 2001). Yaich et al (2011) showed that discharge of treated wastewater is responsible for the wide range of variations in metal content in *Ulva lactuca* L. along the Tunisian coast. The trace metal content in *Gayralia oxysperma* within the permissible limits for human consumption ranging from 0.0024 to 0.0102 mg g⁻¹ dry wt and occurred in the following rank order of abundance Cd<Cr<As<Hg<Pb (Table 1). *Ulva lactuca* and *U. intestinalis* L. [as *Enteromorpha intestinalis* (L.) Nees] from India have similar concentrations of these elements (Manivannan et al., 2009). Concentrations of these minerals, especially the toxic trace metals, are far below recommended dietary intake standards, making this seaweed an ideal human dietary supplement (Munoz et al 1999a, b and IOM 2004; Table 3).

Iodine is an essential element of human nutrition, and marine organisms are usually rich in this element as they concentrate it from the surrounding waters. *G. oxysperma* contains sufficient iodine fraction for human nutrition up to 4820 mg g⁻¹ dry wt, comparable to *Porphyra tenera* Kjell. and *Laminaria digitata* (Hudson) J.V. Lamour. (Teas et al., 2004).

Fatty acid

The FAMES of dried samples of the algae contains saturated fraction (SFA) of 57%, followed by polyunsaturated acids (PUFAs) at 39%, and monounsaturated fatty acid (MUFA) at 3.8% (Table 4). Among the saturated portion, palmitic acid (C16:0) was the most abundant with concentration of 34.60%. This value is close to *Porphyra* sp. containing 37% and much higher than that in *Undaria pinnatifida* (Harvey) Suringar, which has 14% of palmitic acid (Christine et al. 2007). In *Caulerpa* species, the contribution of this acid is much higher, ranging from 42.98–57.07% (Kumar et al 2011). The other major contributor to the saturated fraction was stearic acid (C18:0) with a concentration of 9.6%. *Ulva lactuca* is also reported to contain a similar stearic acid content (Ortiz et al 2006). This acid is an important ingredient in the production of candles, shampoos, soaps, plastics and other cosmetic

products (WHO, 2003). Pentadecanoic acid (C15:0) contributed to a minor extent of 1.3%, and myristoleic acid (C14:0) was present in trace quantities 0.6%, similar to *Laminaria* sp. (Fleurence et al., 1994; Takagi et al., 1985). An appreciable quantity of longer chain fatty acids (C20-24) varying from 1.2 to 3.8% are also present in (*G. oxysperma*); these are higher proportions than in the *Caulerpa* species (Kumar et al 2011)

Predominant among the monounsaturated class was eicosenoic acid at a concentration of 1.5% (Table 4). The oleic acid content of *Gayralia oxysperma* was very low (0.5%) in comparison with *Porphyra*, *Undaria* and *Hizikia* (2.6–9.3%), while the eicosenoic acid (C20:1; n-9; 1.5%) content was closely similar to those in *Laminaria*, *Undaria* and *Porphyra* (Christine et al., 2007). The essential component of the fatty acids for human nutrition is the polyunsaturated type, such as linoleic acid (15.8%), gamma linolenic acid (15%), cis-eicosadienoic acid (2.4%) and arachidonic acid (3.7%; Table 4). These formed the principal components in *Gayralia oxysperma* as reported for other algal species (Fleurence et al., 1994; Takagi et al., 1985).

These unsaturated fatty acids play vital roles in the prevention of cardio-vascular complications, osteoarthritis, diabetes, hypertension and autoimmune diseases. Normal human development and growth requires balanced concentration of PUFAs as their increased concentrations can alter physiological functions in the body. High concentration of alpha linolenic acid (C18:3 n-3), gamma linolenic acid (C18:3 n-6) and stearic acid (C18:0) in *G. oxysperma* could be of great value in pharmaceutical and food industry. Red and brown algae on the other hand are particularly rich (Fleurence et al., 1994) in eicosapentanoic acid (EPA, C20:5 n-3) and arachidonic acid (AA, C20:4, n-6).

Total phenolic compound

Phenolic compounds in plants are responsible for multiple biological effects, including antioxidant properties. The total phenolic content in *Gayralia oxysperma* was estimated to be 33.91 mg g⁻¹. This value is close to some of the *Caulerpa* species reported from Indian waters (Kumar et al 2011). The non-enzymatic antioxidants in the form of phenolic compounds play crucial roles in neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). The presence of a high phenolic content in *G. oxysperma* is sufficient to enhance its value as important dietary supplement for control of diseases related to mutagenesis and carcinogenesis (Silvio De Flora and Claes Ramel, 1988).

Hydrogen peroxide scavenging assay

The ability of *Gayralia oxysperma* extracts to scavenge H₂O₂ increased with elevated concentrations (Fig 1B). Though H₂O₂ itself is not very reactive, it generates highly reactive molecules such as OH• by interacting with metals (Fe²⁺ or Cu²⁺), and superoxide anions in the Haber-Weiss reaction. Therefore, removal of H₂O₂ is very essential for the cell function which may be valuable property of this seaweed species

DPPH radical scavenging activity

Methanol extracts of *Gayralia oxysperma* significantly reduced DPPH radicals in a dose-dependent manner. The scavenging action in *G. oxysperma* (Figure 1A) was similar to that of *Porphyra vietnamensis* T.Tanaka -Pham-Hoàng Ho (Pise et al., 2010). More recently, some of *Caulerpa* species have also been reported to possess the potential to reduce free radicals (Kumat et al., 2011). The reducing action of *G. oxysperma* may be mediated through the donation of hydrogen to a free radical, which is reduced to a nonreactive species (Wang et al., 2008).

Reducing power

The reducing power of *Gayralia oxysperma* in methanolic extract was high in comparison with the known antioxidant ascorbic acid (ASA; Figure 1C). Similar findings have been reported in the *Arctium lappa* by Duh (1998). Reductones in algae are responsible for reducing capacity and are involved in the prevention of chain initiation, binding of metal ions, decomposition of peroxides and radical scavenging (Yildirm et al., 2001). A significant correlation (P<0.05) between DPPH scavenging potential and reducing power (Figure 1E) indicates the antioxidant potential of *G. oxysperma*.

Metal chelating activity

Fig 1D indicates that *Gayralia oxysperma* extract obstructed ferrous and ferrozine complex formation, at higher doses, it performed well in comparison with EDTA, which is a known metal ion chelator. The metal binding capacities of dietary fibers are well known and inhibitory effects on ferrous absorption of algal dietary fibers, such as carrageenan, agar and alginate, have also been reported (Harmuth-Hoene and Schelenz, 1980).

The extract of *Gayralia oxysperma* had an ability to decrease ferrous ion in the assay, which is a measure of the reducing capacity of the extract. This indicates the presence of potential antioxidant activity in this alga as measured of transformation of Fe³⁺ to Fe²⁺ (Meir et al. 1995). Compounds with

reducing power are electron donors; they reduce oxidized intermediates of the lipid peroxidation process, thus acting as primary and secondary antioxidants (Yen and Chen, 1995).

Our observations indicate that *Gayralia oxysperma* has sufficient nutrient value in terms of essential elements, unsaturated fatty acids ($\omega 3$ and $\omega 6$), antioxidant activity and low toxic metal content, all of which are essential requirements for reducing serious cell disorders, such as mutagenesis and carcinogenesis. It could therefore be recommended as efficient food supplement for healthy living.

Conclusion

Biochemical properties of the seaweed species *Gayralia oxysperma*, which is commonly available in estuarine waters, were tested for determination of its nutritive value. The species has sufficiently high lipid content in addition to carbohydrates and proteins. The presence of elevated concentrations of Ca and polyunsaturated fatty acids such as $\omega 3$ and $\omega 6$ confirms its potential importance as a valuable nutrient source. Similarly, the free radical scavenging and metal chelating properties indicate potential usefulness in controlling serious cell disorders. We conclude that this seaweed could safely be utilized in pharmaceutical and cosmetic preparations or for direct consumption.

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Legends of the figures

Figure 1: *Gayralia oxysperma*: **A.** DPPH radical scavenging activity, **B.** Hydrogen peroxide scavenging assay, **C.** Reducing power activity, **D.** Metal chelating ability. Values are mean \pm SD (n=3). Superscripts of different letters are significantly different from each other at $P < 0.05$. “Samples” refers to volume of test solution. **E.** Correlations between DPPH scavenging potential and reducing power. ($P < 0.05$).

Table 1: *Gayralia oxysperma*: thallus composition. Values are presented on a dry weight basis (other than % wet weight)

Parameters	Mean± SD	ANOVA <i>F</i> -ratio	Probability	Cochran C test
Carbohydrate (%)	23.27±0.008	10.71	<0.01	0.68; n.s.
Protein (%)	11.97±1.1	0.369	0.70	0.38; n.s.
Lipid (%)	4.94±0.08	363.2	<0.01	0.34; n.s.
Ash (%)	26.70±1.9	0.001	0.99	0.49; n.s.
*Water (%)	93.85±0.09	1.33	0.32	0.88; n.s.
Fe (mg g ⁻¹)	1.67±4.9	0.535	0.67	0.78; n.s.
Mn (mg g ⁻¹)	0.56±1.2	1.623	0.25	0.67; n.s.
Ca (mg g ⁻¹)	2.74±3.8	24.20	<0.01	0.66; n.s.
Cu (mg g ⁻¹)	0.0035±0.002	8.257	<0.01	0.34; n.s.
Zn (mg g ⁻¹)	0.0148±0.6	2.204	0.16	0.66; n.s.
Cr (mg g ⁻¹)	0.0026±0.07	10.77	<0.01	0.51; n.s.
Cd (mg g ⁻¹)	0.0024±0.04	1.214	0.36	0.77; n.s.
Pb (mg g ⁻¹)	0.0102±0.34	0.687	0.58	0.34; n.s.
Hg (mg g ⁻¹)	0.0046±0.12	2.609	0.12	0.46; n.s.
As (mg g ⁻¹)	0.0027±0.03	9.528	<0.01	0.51; n.s.
I (mg g ⁻¹)	4820 ± 0.19	0.090	0.96	0.42; n.s.

*- fresh weight, Degree of Freedom (df = 3), *n.s.* non-significant

Table 2: *Gayralia oxysperma*: Human nutritional value in comparison with staple food items.

(Beacker 2004).

Source	Carbohydrate	Protein	Lipid
<i>G. oxysperma</i>	23.27	11.97	4.94
Soybean	30	37	20
Meat	01	43	34
Milk	38	26	28
Baker's yeast	38	39	01
Rice	77	08	02
Corn	85	10	04
Wheat	84	14	04

Table 3: *Gayralia oxysperma*: elemental composition in relation to permissible limits in human nutrition.

Elements	<i>G. oxysperma</i> (mg g ⁻¹ dry wt)	Permissible limit
^s Fe	1.67±4.9	8 - 18
^s Mn	0.56±1.2	1.8 - 2.3
^s Ca	2.74±3.8	1000
^s Cr	0.0026±0.07	0.035 - 17.5
^s Cu	0.0035±0.002	2.5 - 125
^s Zn	0.0148±0.6	6 - 11
*Cd	0.0024±0.04	0.274
*Pb	0.0102±0.34	13.48
*Hg	0.0046±0.12	0.041
*As	0.0027±0.03	3.09

All values presented in means± SD

^s (IOM, 2004; recommended dietary allowances in mg/day) and

* (Munoz et al 1999a, b; permissible daily intake value (mg kg⁻¹))

Table 4: *Gayralia oxysperma*: Percentage fatty acid composition. Values are means \pm SD (n = 3)

Fatty acids	Formula	Percentage of FAME
<i>Tridecanoic acid</i>	C ₁₃ H ₂₆ O ₂	2.00 \pm 0.25
<i>Pentadecanoic acid</i>	C ₁₅ H ₃₀ O ₂	1.30 \pm 0.58
<i>Palmitic acid</i>	C ₁₆ H ₃₂ O ₂	34.60 \pm 0.40
<i>Stearic acid</i>	C ₁₈ H ₃₆ O ₂	9.60 \pm 0.05
<i>Arachidic acid</i>	C ₂₀ H ₄₀ O ₂	2.10 \pm 0.01
<i>Behenic acid</i>	C ₂₂ H ₄₄ O ₂	2.40 \pm 0.03
<i>Tricosanic acid</i>	C ₂₃ H ₄₆ O ₂	1.20 \pm 0.02
<i>Ligniceric acid</i>	C ₂₄ H ₄₈ O ₂	3.80 \pm 0.25
<i>Myristoleic acid</i>	C ₁₄ H ₂₆ O ₂	0.60 \pm 0.13
<i>Oleic acid</i>	C ₁₈ H ₃₄ O ₂	0.50 \pm 0.09
<i>Cis-11-Eicosenoic acid</i>	C ₂₀ H ₃₈ O ₂	1.50 \pm 0.06
<i>Erucic acid</i>	C ₂₂ H ₄₂ O ₂	1.20 \pm 0.33
<i>Linolelaidic acid</i>	C ₁₈ H ₃₂ O ₂	1.20 \pm 0.98
<i>Linoleic acid</i>	C ₁₈ H ₃₂ O ₂	15.80 \pm 0.11
<i>Gamma-linolenic acid</i>	C ₁₈ H ₃₀ O ₂	15.0 \pm 0.36
<i>Alpha linolenic acid</i>	C ₁₈ H ₃₀ O ₂	0.90 \pm 0.86
<i>Cis-eicosadienoic acid</i>	C ₂₀ H ₃₆ O ₂	2.40 \pm 0.85
<i>Arachidonic acid</i>	C ₂₀ H ₃₂ O ₂	3.70 \pm 0.08
Total saturated fatty acids		57.00 \pm 0.88
Total mono-unsaturated fatty acids		3.80 \pm 0.78
Total poly-unsaturated fatty acids		39.00 \pm 0.95

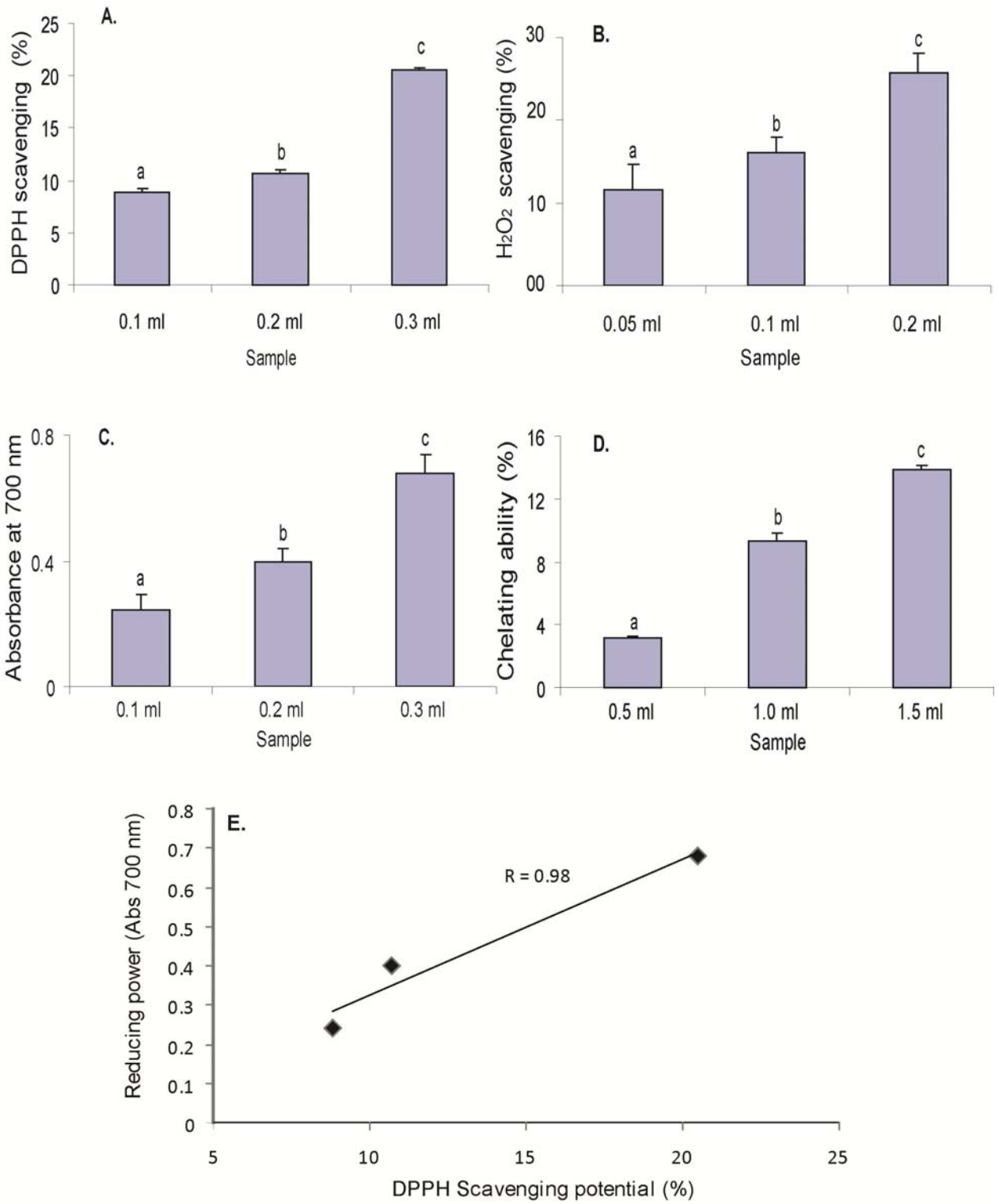


Figure: 1