Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(8):355-362

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

In vitro antiradical and neuroprotective activity of polyphenolic extract from marine algae *Padina australis* H.

Amarchand Chordia Murugan[1], Dahineeswari Vallal[1], Md. Rezaul Karim[1]*, Natanamurugaraj Govindan[*1], Mashitah Binti Mohd Yusoff[1] and Md. Mokhlesur Rahman[2]

[1]Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, Gambang, Kuantan, Pahang, Malaysia
[2]Faculty of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Kuantan, Pahang, Malaysia

---

ABSTRACT

The neuroprotective properties and antioxidant potential of polyphenolic extracts from a brown seaweed Padina australis, were evaluated by the inhibition assay against acetylcholine esterase using indoxyl acetate as the substrate, and by the radical scavenging activity assay against 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and ferrous ion chelating (FIC) activity assay. Methanolic (80%) crude extracts was fractionated by different solvent partitioning, and the assays of each fraction were performed using the microplate reader. The highest total phenolic content (TPC) was observed in n-butanol soluble fraction with a value of 188.75±0.21 mg GAE/g. In addition, the n-butanol soluble fraction showed the highest ferric reducing power (188.34±0.15 mg GAE/g) and the highest ferrous ion chelating ability (76.52±0.12%). The highest free radical scavenging activity against DPPH was found in water soluble fraction with 77.5±1.82% inhibition. However, the β-carotene bleaching activity was found higher with the non-polar solvent, n-hexane extract (58.12±0.58%). A strong positive correlation was found between the total phenolics and antioxidant activities of the fractions. Acetylcholinesterase (AChE) inhibitory properties of all the fractions were assayed where n-butanol soluble fraction was found to have better AChE inhibitory activity (57.94±0.17%) with an IC_{50} value of 1.54±0.045 mg/ml. The results suggested that the polar fractions possess higher AChE inhibitory and antioxidant activity. It can be concluded that Padina australis possesses an appreciable amount of polyphenols with notable antioxidant and anti-AChE properties that could be promisingly used in the treatment of neurodegenerative disorders.

Keywords: Brown seaweeds; Phlorotannins; Alzheimer's disease; Antioxidants.

INTRODUCTION

Alzheimer's disease (AD) is a common form of dementia among the elderly citizens. Dementia is a loss of cognitive ability that seriously affects a person's ability to carry out simple to complex tasks in their daily life. It is not a single disease rather a non-specific syndrome. Acetylcholine (Ach) is a major excitatory neurotransmitter found in the neuromuscular junction and ganglionic synapses that is hydrolyzed by a powerful hydrolytic enzyme acetylcholinesterase (AChE) into acetate and choline. The hydrolysis of ACh by AChE causes the termination of the cholinergic neurotransmission. Therefore, the inhibition of AChE might appreciably recover the level of ACh depleted in the Alzheimer's patients [1]. Indeed, it was proved that the inhibitors of AChE improve the cognitive capability of AD patients in early stage of the disease development. The Food and Drug Administration, U.S. had currently approved five drugs for the therapeutic use that may improve the AD patient's condition. The Alzheimer's treatment horizon has never looked brighter. Due to the bioavailability problems and some side effects such as gastrointestinal disorders and hepatotoxicity, there is still a great interest in finding better ChE inhibitors from

natural resources [2]. The development of more potent disease-modifying drugs continues as to identify natural bioactive compounds, which can be used to ameliorate the disease conditions [3].

Oceans are the richest resources with half of the total global biodiversity of fauna and flora with untouched potentials. Among the marine organisms, marine macro algae or seaweeds have been acknowledged as an under-exploited plant source. The Malaysian marine algae recorded so far comprised of 6 Cyanophyta, 93 Chlorophyta, 85 Phaeophyta and 174 Rhodophyta [4, 5] of which the Johor state has a 400 km coast-line facing South China Sea with most diversified seaweeds represented by Rhodophytes (81 taxa), Chlorophyta (56 taxa), Orchrophyta (34 taxa) and Cyanophyta (26 taxa) ([6]. As seaweeds have many pharmacologic properties such as antioxidant, anti-coagulant, anti-viral, anti-allergic, anticancer, anti-obesity, anti-inflammatory, etc., they have been considered as medicinal food of 21st century and researchers are being carried out to unravel their pharmacologic functions. *Padina australis* Hauck, brown seaweed belongs to the family Dictyotaceae which is widely distributed throughout the tropical marine waters and are used by common people as food additives, animal feed and fertilizers [7]. Since cholinesterase inhibitory activities of seaweeds are still unexplored, the objective of our study was to screen the antioxidant property and cholinesterase inhibitory activity of *P. australis* to be used as therapeutic agents for the treatment of AD.

EXPERIMENTAL SECTION

2.1. Plant materials and chemicals

The brown algae Padina australis was collected from the east costal region of Penyabong, Mersing, Johor State, Malaysia. The alga was washed in tap water to remove soil and other adhered particles, air dried under shade, pulverized, and the powdered algae were then stored at room temperature. DPPH, Gallic acid, AChE, indoxyl acetate, Paraoxon, β-carotene, linoleic acid, and ferrozine were purchased from Sigma, Malaysia. All other chemicals used in this study were of analytical grade.

2.2 Extraction of seaweed

The methanolic extracts were obtained according to the methods of Yang et al. (2003) [8] using 80% MeOH instead of 100% MeOH. A 100 g portion of the dried and powdered *P. australis* was extracted in 500 ml aqueous in a conical flask by continuous shaking for 48 h at room temperature. The extract was filtered, and the MeOH was evaporated under vacuum. The crude extract was re-dissolved in water and was sequentially partitioned with n-hexane, chloroform, ethyl acetate, and n-butanol. These partitioned fractions were dried under vacuum, stored at -20°C for further use in the study of total phenolic content, antioxidant and acetylcholinesterase inhibitory activity.

2.3 Total phenolics content

Total phenolic content was determined by using Folin-Ciocalteu reagent as described by Zhang et al. (2007) [9] in a 96-well micro titer plate. A definite amount of the fractions were dissolved separately in DMSO and 20 µl of each fraction and the serially diluted (0-100 µg/ml) standard Gallic acid solutions were placed in different well. Then 100 µl of the diluted (1:10 v/v) Folin-Ciocalteu reagent was added to each well, mixed and incubated for 5 min at room temperature. Subsequently, 80 µl of 7.5% sodium carbonate solution was added and mixed well. The plate was covered and kept in the dark at room temperature for 30 min. Each fraction and Gallic acid standard were run in triplicate. The absorbance was measured at 750 nm with a spectrophotometric microplate reader (Infinite Pro200, TECAN, Switzerland). TPC was expressed as mg GAE (Gallic acid equivalent)/g.

2.4 Antioxidant activities (AOA)

2.4.1 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging

DPPH radical scavenging assay was performed according to the method of Brand-Williams et al. (1995) [10]. Instead of reading samples spectrophotometrically, the assay was performed with a microplate reader. Briefly, A 50 µl of each fraction and 200 µl of DPPH solution (150 µM in 80% methanol) was added in different wells of a 96-well flat-bottom micro titer plate (Corning Inc. U.S.A.). Serially diluted ascorbic acid was used as standard and each sample was run in triplicate. The plate was covered and kept in dark at room temperature (27 °C). After 30 min, the plate was read in an Infinite 200 pro (TECAN, Switzerland) plate reader using a 520 nm filter. The percent free radical scavenging activity was calculated as follows:

$$\% \text{ Antiradical activity} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

2.4.2 Ferric- reducing antioxidant power (FRAP)

The FRAP assay was done following the method of Chew et al. (2008) [11]. A 50 µl portion of 0.1 M potassium phosphate buffer (pH 6.6) and 50 µl of 1% (w/v) potassium ferricyanide were mixed with 20 µl of each fraction (1 mg/ml) in the wells of a micro titer plate and incubated at 50 °C in a water bath. After 20 min, 50 µl of 10% (w/v)

trichloroacetic acid was added. To 50 µl of the above reaction mixture, 50 µl of deionized water and 10 µl of 0.1% (w/v) ferric chloride was added. The reaction mixture was incubated at room temperature for 30 min for color development. The absorbance was measured at 700 nm and the FRAP value was expressed as mg GAE/g.

2.4.3 Ferrous ion chelating (FIC) assay

The FIC assay was performed following the method of Singh and Ranjini (2004) [12]. A 50 µl portions of each of 0.1 mM ferrous sulphate and sample fractions (1000 µg/ml) were mixed together and 50 µl of 0.25 mM ferrozine was added to the mixture in a micro titer plate and incubated for 10 min. The reactions were run in triplicate, and absorbance was measured at 562 nm. The percentage of chelating ability was calculated by using the following equation:

Chelating ability (%) = $[(A_{control} - A_{sample})/ A_{control}] \times 100$

2.4.4 Beta carotene bleaching (BCB) assay

The β-carotene bleaching assay was performed using the method of Kumazawa et al. (2002) [13]. A 3 ml portion of β-carotene (0.1 mg/ml in chloroform) was added to 40 mg of linoleic acid and 400 mg of Tween 40 in a conical flask. The chloroform was evaporated, and 100 ml of deionized ultra-pure water was added into the dried mixture and mixed well. The β-carotene/linoleic acid emulsion was then mixed well, and the initial absorbance of the emulsion was measured at 470 and 700 nm. An aliquot of 250 µl of the emulsion was added to 10-100 µl of the sample fractions, incubated at 50 °C. After 60 min absorbance of the reaction mixtures were measured at 470 and 700 nm. The absorbance at 700 nm (due to haze) was subtracted from the absorbance at 470 nm.

The percentage of AOA, calculated as follows:

Degradation rate (DR) of β-carotene = $- \ln (A_{initial}/ A_{sample})/ 60$

Antioxidant activity (% AOA) = $[(DR_{control} - DR_{sample})/ DR_{control}] \times 100$

2.5 Acetylcholinesterase inhibitory activity

The cholinesterase activity was measured by the method of Pohanka et al. (2011) [14] using indoxyl acetate as the chromogenic substrate. A 25 µl portion of each fraction (in DMSO) was mixed with 50 µl of PBS and 5 µlof AChE solution added and mixed gently. The reaction was started by the addition of 20 µl of indoxyl acetate (in 5% ethanol). Paraoxon (a para-sympathomimetic drug) was used as standard. Absorbance was measured at 670 nm after 30 min. The percent inhibition of cholinesterase activity was calculated as follows:

% Anti-AChE activity = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

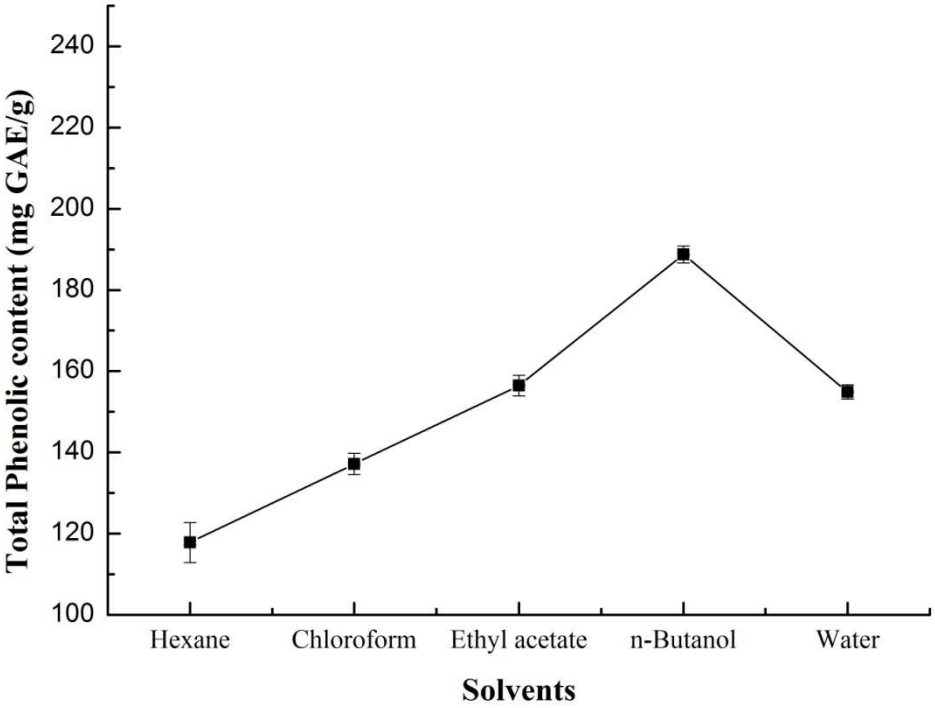
2.6 Statistical Analysis

All the experiments were conducted in triplicates (n=3) and Pearson's correlation coefficient (using OriginPro v.8.6 statistical software) was calculated to compare the mean values of each experiment. Significant differences between the means of parameters were determined by using the 2-tailed test of significance ($P < 0.05$).

RESULTS AND DISCUSSION

3.1. Phenolic composition

Fig. 1 demonstrates the total phenolic content of different fractions of *P. australis* extract. It is evident in the figure that n-butanol fraction possesses the highest total polyphenol. Polyphenols are highly soluble in high polar solvents which can be evidenced from the results obtained. The difference in TPC among different factions of *P. australis* was found statistically significant. Li et al. (2009) [15], in a similar study, isolated and structurally characterized seven phlorotannins and three sterols from *E. cava* methanolic extract. Wang et al. (2009) [16] found significant differences in the total phenolic content among different seaweed species extracted with aqueous methanol, ethanol and acetone where brown algae demonstrated higher amounts of polyphenols than green and red algae. Furthermore, a positive correlation between the thallus age and TPC was reported [17]. Chew et al. (2008) [11] reported that aqueous methanolic extracts of *K. alvarezzi*, *P. antillarum* and *C. racemosa* showed high TPC than absolute organic solvents. The seaweed polyphenols known as phlorotannins (polymers of phloroglucinol) are hydrophilic in nature and abundantly found only in brown seaweeds [18].



Fig, 1. Total phenolic content of different fractions of P. australis extract

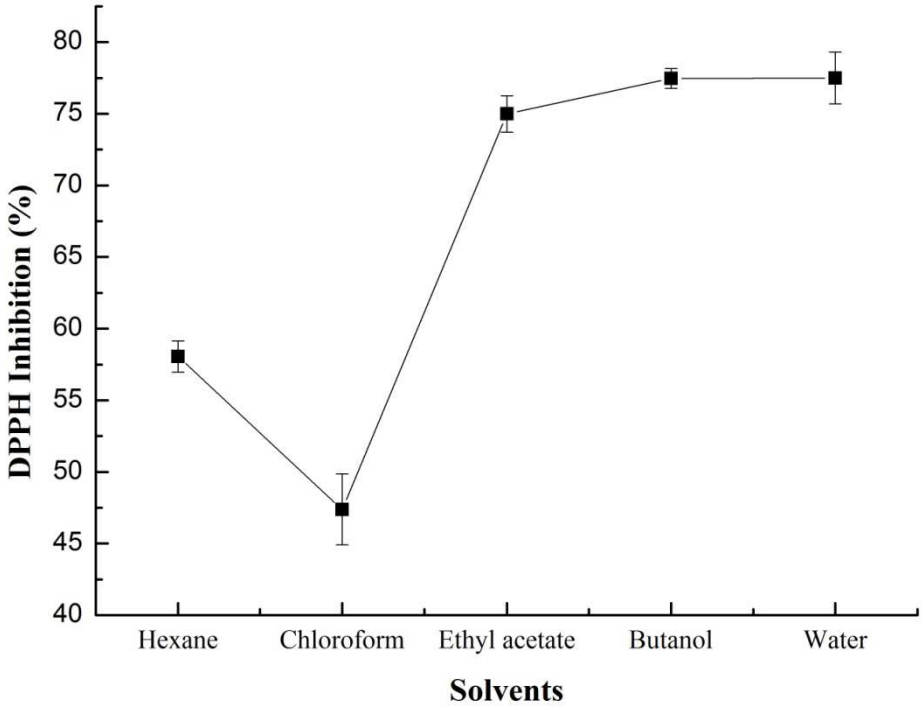


Fig. 2. DPPH free radical scavenging activity of different fractions of P. australis extract

3.2. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The free-radical scavenging activities of different fractions of *P. australis* are shown in Fig. 2. All the fractions showed significant DPPH quenching activity at a final concentration of 1.0 mg/ml. The IC_{50} values of the fractions ranged from 1.63 – 130.14 µg/ml. the scavenging capacity of the fractions increased in the increasing order of polarity of the solvents, although chloroform fraction demonstrated the lowest value. There was a strong correlation between the phenolic content in all the fractions and the DPPH free radical scavenging activity. Wang et al. (2009) [16] also found high correlation between the total phenolic content of Icelandic seaweed extracts and their DPPH radical scavenging capacity. This may be due to the presence of phloroglucinol polymers in high concentrations in these fractions [19, 20]. The methanolic and aqueous extracts of 10 species of Chlorophyta and 25 species of Phaeophyta were screened for their antioxidant activity by Heo et al. (2005) [21]. Both the extracts showed a

significant amount of activity against the reactive oxygen species (ROS). Similarly, the methanolic extract of *E. cava* significantly reduced DPPH radical in a dose-dependent manner [22]. The scavenging activity of DPPH free radicals had been used extensively to determine the antioxidant power of bioactive natural products. Antioxidants interrupt free radical chain oxidation by donating the hydrogen from hydroxyl groups to form a stable end-product, which does not initiate further oxidation of lipids [23].

3.3. Ferric reducing antioxidant power (FRAP)

Fig. 3 illustrates the total antioxidant power of the *P. australis* fractions (1 mg/ml) and the values are expressed as mg gallic acid equivalent per g. The results showed that n-butanol fraction possessed the highest antioxidant power followed by ethyl acetate and water fractions. Alike DPPH scavenging power, chloroform demonstrated the lowest FRAP value. The results were statistically significant ($P < 0.05$). The reducing power of the fractions was found strongly correlated with their total phenolics [11, 24]. The FRAP assay offers an index of antioxidant or reducing capability of foods, beverages and food supplements. The antioxidant activity was based on the ability of the antioxidant components present in the samples that involve in the ionic reduction of ferric (III) to ferrous (II) in a colorimetric reaction [25], mediated by single electron transfer.

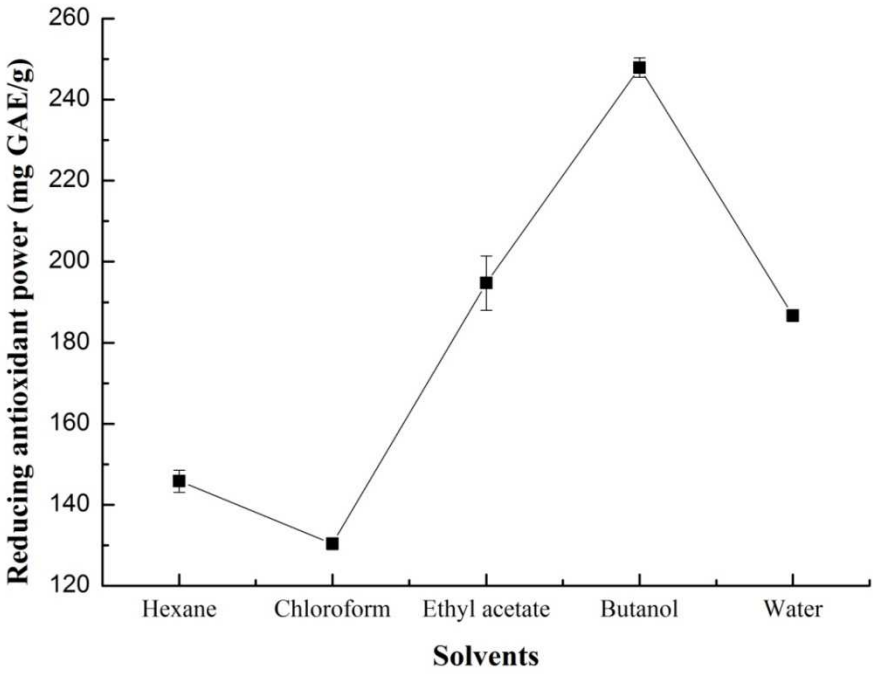


Fig. 3. Ferric reducing antioxidant power (FRAP) of different fractions of P. australis extract

3.4. Ferrous ion chelating (FIC) assay

The metal chelating ability of the antioxidant components present in the *P. australis* fractions was evaluated using FIC assay at a concentration of 1mg/ml. Fig. 4 shows the ferrous ion chelating ability of all fractions. The reducing capacity of the fractions increased in the order of increasing polarity. Although water is the solvent of highest polarity used in this study, it contained FIC value less than n-butanol fraction. This may be due to distribution of fewer polar constituents in other solvents, including butanol, water being the last solvent of choice. Studies have demonstrated that phloroglucinol polymers derived from brown seaweeds are potent ferrous ion chelator [11, 26] and its potency depends upon their unique phenolic structures and number of hydroxyl groups present [27].

3.5. Beta carotene bleaching (BCB) assay

The β-carotene bleaching (BCB) of different fractions of the *P. australis* extract is demonstrated in Fig. 5. The results showed that the BCB antioxidant activity is inversely proportional to the polarity of the solvents indicating that the BCB values of the fractions are independent of TPC. The presence of more lipophilic components in the non-polar fractions contributed to the BCB activity. The difference in BCB activity among the fractions was statistically significant ($P<0.005$).

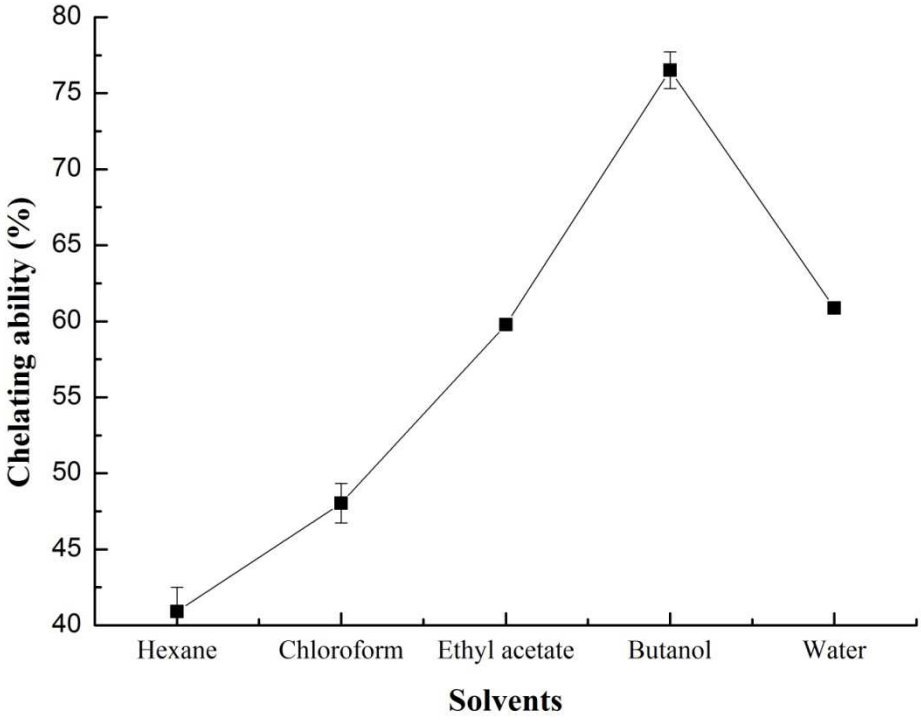


Fig. 4. Ferrous ion chelating ability of different fractions of P. australis extract

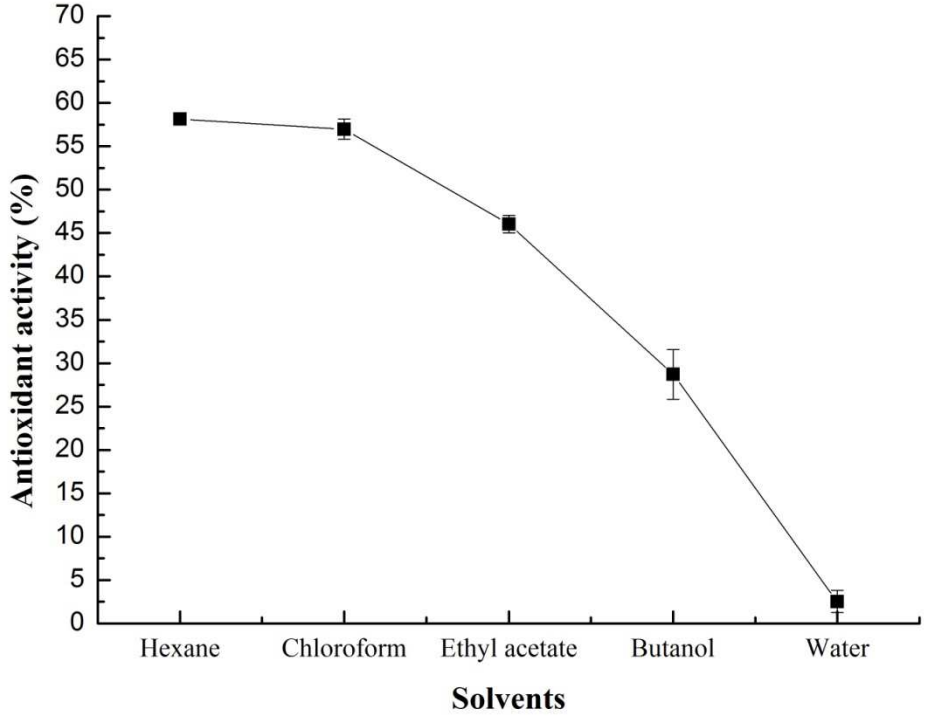


Fig. 5. Antioxidant activity of P. australis as determined by β-carotene bleaching assay

The β-carotene bleaching is mediated by free radicals resulting in the formation of peroxyl from linoleic acid due to the abstraction of diallylic methylene groups from linoleic acid [28]. This free radical will then attack the highly unsaturated β-carotene molecules which can be visualized spectrophotometrically as the orange color chromophore is degraded. However, the antioxidant will neutralize the linoleate-free radicals and prevent the bleaching of β-carotene. Good number studies showed that there was no correlation or negative correlation between the total phenolics and β-carotene bleaching [11, 29-33] Total phenolics indicate the presence of both lipophilic and hydrophilic compounds in a sample whereas, BCB indicates the levels of lipophilic compounds present in a sample.

3.6. Correlation between the total phenolic content and different antioxidant activity

Correlation analysis was carried out to investigate the relationship between the TPC and different antioxidant activity assays. Table 1 show the correlation between the TPC, DPPH, FRAP and FIC activity. A strong and statistically significant correlation of TPC with FRAP, and FIC was demonstrated by Pearson's correlation analysis (FRAP: r^2 = 0.927; FIC: r^2 = 0.994). Similarly, a high correlation was also obtained between TPC and DPPH values (DPPH: r^2 = 0.72). A negative correlation was observed between the TPC and BCB values (BCB: r^2 = -0.56), indicating that the BCB activity mainly based on the lipophilic content of the sample. Previous studies showed that the phenolic compounds are the main contributors to the antioxidant activity of various seaweeds. A positive correlation between TPC and different antioxidant activity of different seaweed extracts was observed by other researchers [16, 34-36]. Based on the results obtained, it can be postulated that the key function of brown algal extracts as an effective free-radical scavenger and pro-oxidants are based on their polyphenol content. It should also be noted that the reaction conditions, structural diversity of polyphenol, as well as their synergistic or antagonistic effects could also have a big influence on different antioxidant assays [37].

Table 1: Pearson's correlation coefficients between the total phenolics content and different antioxidant activity assays

AOA assays	R^2 value
DPPH	0.724
FRAP	0.927
FIC	0.994
BCB	-0.56

3.7. Acetylcholinesterase inhibitory activity assay

The ChEs inhibitory properties of n-hexane, $CHCl_3$, EtOAc, n-BuOH and H_2O fractions of *P. australis* were observed at concentration of 1 mg/ml and the results are demonstrated in Table 2. Among the fractions, n-BuOH, H_2O and EtOAc fraction exhibited significantly higher AChE inhibitory activity. The n-hexane and $CHCl_3$ fractions exhibited less inhibition, suggesting that lipophilic compounds of *P. australis* are poor acetylcholinesterase inhibitors. The IC_{50} values of the different fractions ranged from 0.09 – 1.53 mg/ml. It was reported [38-40] that phlorotannins are good AChE inhibitors. However, the possible relation between the phlorotannins and AChE inhibition is still not clear. It is suggested that phloroglucinol polymers with bulky structures, are able to mask the ChE and prevent the binding of the substrates [41]. It is also suggested that the degree of polymerization, and the closed-ring structure of phlorotannins are key players in the inhibition of AChE [42]. Yoon et al., (2008) [41] studied the bioactivity of 27 Korean seaweeds and among them *E. stolonifera* exhibited potent inhibitory properties against both AChE and BChE. Moreover, some compounds derived from marine macro algae also have mixed type ChE (acetyl- and butyl cholinesterase) inhibitory activity [43] which is considered more effective in treating dementia. Overall, the brown macro algae demonstrated the potential to be used as functional neuro protective agents through their ChE inhibitory power; further studies are warranted to explore the exact candidates contributing to this action and their structure-activity relationship.

Table 2: % AChE inhibition and IC50 value of different fractions of *P. australis* extract

Solvent Extracts	% Inhibition	IC_{50} value (mg/ml)
n- Hexane	43.25 ± 0.19	0.455 ± 0.08
Chloroform	46.51 ± 0.15	0.741 ± 0.14
Ethyl acetate	53.22 ± 0.07	1.282 ± 0.02
n-Butanol	57.94 ± 0.17	1.539 ± 0.04
Water	55.71 ± 0.16	0.09 ± 0.22
Paraoxon	82.40 ± 0.47	ND

Data are expressed as mean ± SD (n=3). ND- not determined.

CONCLUSION

Our data indicates that the *P. australis* fractions contain an appreciable amount of polyphenol. All the fractions exhibited high antioxidant and pro-oxidant activities. Polyphenol-rich fractions also exhibited significant ChE inhibitory activity. Hence, the results suggested *P. australis* to be a considerable source of secondary metabolites that could be used as promising functional food additives in the pharmaceutical industry. Further work is in progress to identify the compounds accountable for the inhibitory property of cholinesterase from *P. australis* extracts.

Acknowledgments

This research work was carried out under the funding (ERGS grant #RDU 120603) of the Ministry of Higher Education, Malaysia. The authors gratefully acknowledge the Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang for providing the facility to carry out this research.

REFERENCES

[1] I Orhan; B Sener; MI Choudhary; A Khalid. *J. Ethnopharmacol.,* **2004**, 91, 57–60.
[2] CH Park; SH Kim; W Choi; YJ Lee; JS Kim; SS Kang; YH Suh. *Planta Med.,* **1996**, 62, 405-409.
[3] Alzheimer's Association. Alzheimer's disease facts and figures. *Alzheimer's & Dementia*, **2012**, 8, 131–168.
[4] SM Phang. *Aquatic Ecosys. Health Manage.,* **2006**, 9 (2), 185-202.
[5] SM Phang, CL Wong, PE Lim, JLS Ooi, SY Gan, I Melor, HY Yeong, M Emienor Muzalina. Seaweed diversity in Malaysia. *In*: Status of biological diversity in Malaysia and threat assessment of plant species in Malaysia. 1st Edition, Forest Research Institute of Malaysia, Kuala Lumpur, Malaysia, **2007;** 185-210.
[6] MH Gan; A Siti Aishah; CA Orisco; A Nur Wahidah; K Amyra Suryatie; MP Noraien. Diversity of seaweeds in the vicinity of Johor: With emphasis on the East coast peninsular Malaysia expedition II 2006. *UMTAS*, **2011**, LSP43: 429-433.
[7] K Pandima Devi; N Suganthy; P Kesika; SK Pandian. *BMC Complement. Altern. Med.,* **2008**, 8-38.
[8] H Yang; P Protiva; B Cui; C Ma; S Baggett; V Hequet; S Mori; BI Weinstein; JE Kennelly. *J. Nat. Prod.,* **2003**, 66, 1501-1504.
[9] J Zhang; C Tiller; J Shen; C Wang; GS Giroraud; D Dennis. *Can. J. Physiol. Pharmacol.,* **2007**, 85: 1115–1123.
[10] W Brand-Williams; ME Cuvelier; C Berset. *LWT.*, **1995**, 28, 25-30.
[11] YL Chew; YY Lim; M Omar; KS Khoo. *LWT.*, **2008**, 41, 1067-1072.
[12] N Singh; PS Rajini. *Food Chem.,* **2004**, 85, 611-616.
[13] S Kumazawa; M Taniguchi; Y Suzuki; M Shimura; M Kwon; T Nakayama. *J. Agric. Food Chem.,* **2002**, 50, 373-377.
[14] M Pohanka; M Hrabinova; K Kuca; JP Simonato. *Int. J. Mol. Sci.,* **2011**, 12, 2631-2640.
[15] Y Li; ZJ Qian; BM Ryu; SH Lee; MM Kim; SK Kim. *Ecklonia cava*. *Bioorg. Med. Chem.,* **2009**, 17, 1963-1973.
[16] T Wang; R Jonsdottir; G Olafsdottir. *Food Chem.,* **2009**, 116, 240-248.
[17] S Connan; F Delisle; E Deslandes; E Ar Gall. *Bot. Mar.,* **2000**, 49, 39-46.
[18] NM Targett; TM Arnold. *J. Phycol.,* **1998**, 34, 195-205.
[19] CW Chen; CT Hu. *J Foods Lipids*, **1995**, 2, 35-46.
[20] K Zhou; L Yu. *LWT.*, **2004**, 37, 717-721.
[21] SJ Heo; EJ Park; KW Lee; YJ Jeon. *Bioresource Technol.,* **2005**, 96, 1613-1623.
[22] C Kang; YB Jin; H Lee; M Cha; ET Sohn; J Moon; C Park; S Chun; ES Jung; JS Hong; SB Kim; JS Kim; E Kim. *Food Chem. Toxicol.,* **2010**, 48, 509-516.
[23] ER Sherwin. *J. Am. Oil Soc.,* **1978**, 55, 809-914.
[24] FF Benzie; YT Szeto. *J Agric. Food Chem.,* **1999**, 47, 633-636.
[25] YF Li; CJ Guo; JJ Yang; JY Wei; J Xu; S Cheng. *Food Chem.,* **2006**, 96, 254-260.
[26] M Senevirathne; SH Kim; N Siriwardhana; JH Ha; KW Lee; YJ Jeon. *Food Sci. Technol. Int.,* **2006**, 12, 27-38.
[27] J Santoso; Y Yoshie-Stark; T Suzuki. *Fisheries Sci.,* **2004**, 70, 183-188.
[28] A Kumaran; R Joel Karunakaran. *Food Chem.,* **2006**, 97, 109-114.
[29] R Amarowicz; U Wanasundara; J Wanasundara; F Shahidi. *J Food Lipids*, **1993**, 1, 111-117.
[30] T Tsuda; Y Makino; H Kato; T Osawa; S Kawakishi. *Biosci. Biotechnol. Biochem.,* **1993**, 57, 1606-1608.
[31] A Isamil; TS Hong. *Malays. J. Nutr.,* **2002**, 8, 167-177.
[32] T Sun; CT Ho. *Food Chem.,* **2005**, 90, 743-749.
[33] A Othman; A Ismail; N Abdul Ghani; I Adenan. *Food Chem.,* **2007**, 100, 1523-1530.
[34] A Jimenez-Escrig; I Jimenez-Jimenez; R Pulido; F Saura-Calixto. *J. Sci. Food Agric.,* **2001**, 81, 530-534.
[35] R Karawita; N Siriwardhana; KW Lee; MS Heo; IK Yeo; YD Lee. *Eur. Food Res. Technol.,* **2005**, 220, 363-371.
[36] Y Athukorala; KN Kim; YJ Jeon. *Food Chem. Toxicol.,* **2006** 44, 1065-1074.
[37] N Niciforovic; V Mihailovic; P Maskovic; S Solujic; A Stojkovic; D Pavlovic Muratspahic. *Food Chem. Toxicol.,* **2010**, 48, 3125-3130.
[38] M Moyo; AR Ndhlala; JF Finnie; JV Staden. *Food Chem.,* **2010**, 123, 69-76.
[39] H Nguyen; SM Kim. *Afr. J. Biotechnol.,* **2012**, 11, 9457-9467.
[40] RR Kannan; MA Aderogba; AR Ndhlala; WA Strik; JV Staden. *Food Res. Int.,* **2013**, 54, 1250-1254.
[41] NY Yoon; HY Chung; HR Kim; JS Choi. *Fisheries Sci.,* **2008**, 74, 200-207.
[42] NY Yoon; SH Lee; Y Li; SK Kim. *J. Funct. Foods,* **2009**, 1, 331-335.
[43] N Suganthy; SK Pandian; K Pandima Devi. *Neurosci. Lett.,* **2010**, 468, 216-219.