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Original Article

POLYPHENOL CONTENT, PHYTOCHEMISTRY COMPOSITIONS AND ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS FROM MARINE SPONGE *AAPTOS SUBERITOIDES* GROWN IN NHATRANG BAY, VIETNAM

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ABSTRACT

Objective: To investigate the content and antioxidant activities of polyphenol, the correlation between polyphenol content and their antioxidant activities, and phytochemistry compositions of different extracts from marine sponge *Aaptos suberitoides* commonly found growing in Nhatrang bay, Vietnam orienting application into functional food and pharmacy.

Methods: Evaluating the toxicity of antioxidant polyphenol powder preparing from the initial concentrated extract was by the adjusted Behrens Karber method and a correlation between polyphenol content and antioxidant activities basing on the Pearson coefficient in Excel. Separating antioxidant polyphenol content was base on solvents polarization of n-hexane, chloroform, ethanol, ethyl acetate and n-butanol which the quantification of polyphenol content and antioxidant activities, and preliminary phytochemical compositions qualitative.

Results: Antioxidant polyphenol powder did not affect mice weight during the assay time of 28 d. Polyphenol content and antioxidant activities got the highest value at chloroform extract in comparison to other extracts, a significant difference ($p<0.05$) and strong correlation ($R2>0.9$). Polyphenol content (122.682 mg gallic acid equivalent ml-1), total antioxidant activity (368.183 mg ascorbic acid equivalent ml-1), reducing power activity (24.08 mg FeSO4 equivalent ml-1) and DPPH scavenging (72.48±1.54 %) were the highest values. Alkaloids, flavonoids, steroids, tannins and triterpenoids existed in initial methanol extract. Weakly polarized polyphenol content was 70.27% in comparison to initial methanol extract.

Conclusion: Antioxidant polyphenol of sponge *Aaptos suberitoides* has the potential for application into the field of functional food and pharmaceuticals.

Keywords: *Aaptos suberitoides*, Antioxidant, Phytochemistry, Polyphenol, Sponge

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INTRODUCTION

Marine sponges are the basalmost clade of animals commonly found growing in died coral areas. They have been considered as a drug treasure house containing great potential bioactive substance [1], for example, fatty acid, polyketides, pyrimidines [2], alkaloids [3, 4], polyphenols, tannins, flavonoids [5, 6]. Bio-substances possessed numerous various biological activities including marine culture [7], anti-inflammatory, anti-microbial, antioxidant and toxic [8], new cytotoxic [3, 4], and anti-cancer of protein [9], and biological substances belong to different sponge genera, such as *Haliclona, Discodemia, Fasciospongia, Cacospongia, Theonellas, Spongosorites, Aaptos, Smenospongia, Dysidea, Haliclona*, and *Petrosia.*

Recently reports on biological activity substances of sponge growing in Vietnam waters mainly on alkaloids, terpenoids and steroids belong to different sponge species for instance *Aaptos suberitoides* [10, 11], *Petrosia nigricans* [12], *Dysidea cinerea* [13], *Dysidea fragilis* [14, 15], *Smenospongia cerebriformis* [16, 17], *Haliclona oculata* [18], and bioactivities of them were showed including the toxic [16, 18], anti-inflammatory [17], anti-bacterial [19], and anti-cancer activity [10, 11]. There are not any report on antioxidant activity of marine sponges in Vietnam waters. *Aaptos suberitoides* species commonly grow in Vietnam waters and many seas in the world.

Polyphenol is an interest bioactive substance group finding in almost plants and some animals in the world. They possess various biological activity such as antioxidant, anticancer, antibacterial, anti-inflammatory, immunosuppressive, and neuro suppressive, etc [20]. Indeed, their antioxidant activity is most interesting, because this ability helps them limit free radicals, ageing and diseases in humans. The content, structure characteristics, and biological activities of polyphenol are depended on species, harvest time and growth condition of marine sponge, storage and extraction techniques.

Therefore, antioxidant activities, phenol content, and phytochemistry compositions of different extracts from *Aaptos suberitoides* Brøndsted (1934), and the correlation between phenol content and antioxidant activity have been focused on study.

MATERIALS AND METHODS

Chemicals and reagents

Ferric chloride ($FeCl_3$), ammonia, chloroform, n-hexane, ascorbic acid, Folin-Ciocalteu reagent, sodium bicarbonate ($NaHCO_3$), sulphuric acid (H_2SO_4), hydrochloric acid (HCl), acetic anhydride, chloroform ($CHCl_3$), $FeSO_4$, methanol, n-butanol, ethyl acetate, sodium phosphate, ammonium molybdate, phloroglucinol, phosphate buffer pH 7.2, $K_3[Fe(CN)_6]$, CCl_3COOH, 2, 2 diphenyl 1 picrylhydrazyl (DPPH). All chemicals using in the analysis were purchased from *Sigma*-Aldrich (St. Louis, MO).

Sample collection

Collecting sponge species *A. suberitoides* (fig. 1) was on July 2016 in Hon Mot Island, Nha Trang Bay, Khanh Hoa, Vietnam. The species was classified and saved at the Institution of Oceanography, Vietnam.

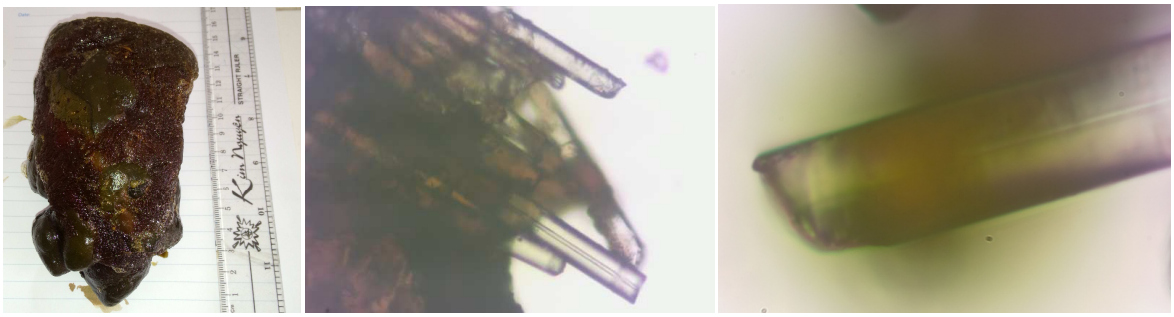

Fig. 1: Morphology and bone framework of sponge species *A. suberitoides* (n=3)

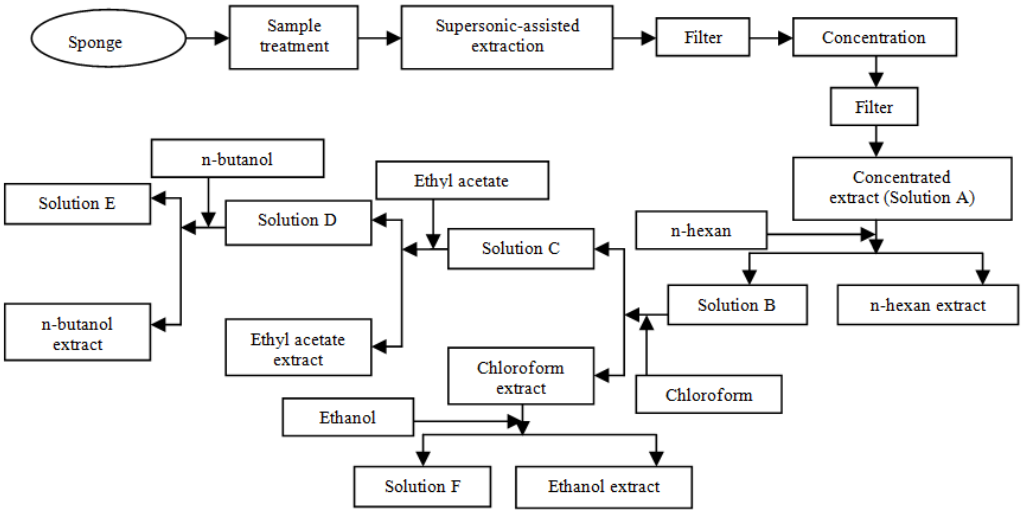

Fig. 2: Monography of extract fractions from sponge species *A. suberitoides* (n=3)

Preparation of extract

Cleaning the sponge sample *A. suberitoides* was by seawater after sampling and transported to the laboratory under the condition at 10 °C. In the laboratory, storing the sponge was in-80 °C. When liquid nitrogen fully degraded the sponge cells, bringing the mixture assimilated at 5.000 rpm, then stored at-80 °C. Collecting the initial extract was by supersonic-assisted maceration method and methanol solvent. Segmenting concentrated methanol extract was by liquid-liquid extraction in the following solvent order: n-hexane, chloroform, ethanol, ethyl acetate, n-butanol, respectively (fig. 2). All extract fractions were filtered through Whatman filter paper no. 4, and then concentrated at 40 °C. After concentration, all residues dissolve were into 20 ml of 96 % ethanol, and the storage of them at 4 °C.

Evaluation of the effect of antioxidant polyphenol powder on mice weighing

The experiments animal farm of the Vietnam Institute of Vaccines and Medical Biologicals approved Swiss mice (7 w old, 17 to 22g bodyweight). Doing the experiments on mice were according to the Ethics Committee on Animal Experimentation of the Vietnam Institute of Vaccines and Medical Biologicals based on the guidelines of the Vietnam Ministry of Health on Animal Experimentation. During the period of the isolation and the experiment, mice were cultured under the standard laboratory conditions of temperature (22-25 °C), relative humidity (53–67 %) and light-dark cycle (12h x 12h): the closed and controlled environment. The food processing plants of the Vietnam Institute of Vaccines and Medical Biologicals provided the dry food recipe which registered for Swiss mice. Each batch of food was inspected for quality and certified. Drinking water samples are monitored periodically for quality (microbiology, chemistry), twice a week. Distributing mice were randomly into cages (10 mice/cage) and marked on a specified location on the coal to identify. Three mice groups took part in an experiment, and each plot consisted of five male mice and five female mice with time triplicate. Mice drunk distilling water and named as a control plot (plot A). A plot of mice (plot B) drunk a solution containing maltodextrin and distilled water. A plot of mice (plot C) drunk a solution containing distilled water and antioxidant polyphenol powder. Mice drunk twice times per day. Swiss mice were cultured according to the standard condition of Institute of Vaccines and Biological Medical, Vietnam. Mice were weighed a week one time for 28 d according to the adjusted Behrens Karber method.

Where in

Preparing the antioxidant powder was by using a spray drying technical as follows: 100 g maltodextrin aids and 5 ml tween 80 added into 01 L initial concentrated polyphenols extract and assimilated for 30 min. Then, the mixture put into the spray drying machine LD 50 had been running at 70 °C with disk speed of 20.000 rpm and pump speed of 18 rpm for collecting the antioxidant polyphenol powder. Antioxidant polyphenol powder fully dissolves in the water (fig. 3).

Fig. 3: Antioxidant polyphenol powder from sponge extract (n=3)

Quantification of polyphenol content

Quantification of polyphenol content (PC) was according to the method of Swanson and Druehl [21].

Qualitative of phytochemistry composition

Alkaloids, flavonoids, steroids, tannins, triterpenoids were qualitative according to the description of Jamuna *et al.* [22]

Evaluation of antioxidant activity

Determination of total antioxidant activity

Total antioxidant activity was evaluated based on molybdenum metabolism [23].

Determination of reducing power activity

Reducing power was evaluated based on the reduction reaction of Fe^{3+}into Fe^{2+}[23].

DPPH free radical scavenging activity

Free radical scavenging activity on DPPH was determined to base on the colour reaction between the extract and DPPH [23].

Data analysis

The analysis of statistic, ANOVA and regression were by software Microsoft Excel 2013. Statistically, the p-value is less than 0.05 ($p<0.05$) indicating a significant level. Unnormal values were removed by using Duncan method. All experiments performed in triplication (n = 3).

RESULTS AND DISCUSSION

Mice weighing

Assay results on mice exhibited in table 2. The difference in mice weighing between various mice groups was significant when the evaluation of mice weighing was ago 14 d or more. Minimum powder content causing mice death did not show in the study.

Toxicity of sponge extract on mice depends on the species of sponges, extracted biological substances, grown local of sponges. Marine sponges produce potent cytotoxic compounds [24]. The toxicity of marine sponges depends on latitude and increase when latitude decrease [25]. Bioactive compounds decide the toxicity of marine sponges [26]. In the current study, antioxidant polyphenol powder from the initially concentrated extract was non-toxic, because they did not impact on mice weighing for 28 d. Antioxidant polyphenol powder can be full fishes applied into in life. Antioxidant polyphenol powder should be continuously studied, for example, storage time, thermal properties, size and surface characteristics of particles.

Polyphenol content

Polyphenol content was strongly affected by different solvents ($p<0.05$), and varied from 3.163 mg gallic acid equivalent ml-1 to 122.682 mg gallic acid equivalent ml-1 (fig. 4). Polyphenol content was decreased in the order: chloroform/solution E/n-butanol/ethanol/n-hexane/ethyl acetate (fig. 4). Highest polyphenol content had been detected in chloroform extract and corresponded to 70.27% in comparison to the polyphenol content of the initially concentrated extract. Polyphenol content was 74.630 mg gallic acid equivalent ml-1 when the ratio of chloroform and solution B was equal (v v-1).

Table 2: Effect of antioxidant polyphenol powder on mice weighing in 28 d

Sample	The average weight of mice groups was assayed according to 28 d			
	7 d	14 d	21 d	28 d
Control	26.360±1.050 g mouse^{-1}	28.010±1.024 g mouse^{-1}	29.142±1.529 g mouse^{-1}	30.572±1.328 g mouse^{-1}
Plot B	24.570±1.660 g mouse^{-1}	26.723±1.231 g mouse^{-1}	28.463±1.723 g mouse^{-1}	30.855±1.477 g mouse^{-1}
Plot C	25.320±1.900 g mouse^{-1}	27.518±1.302 g mouse^{-1}	28.775±1.192 g mouse^{-1}	31.216±1.723 g mouse^{-1}

*Data expressed under mean value±SD and (n=3)

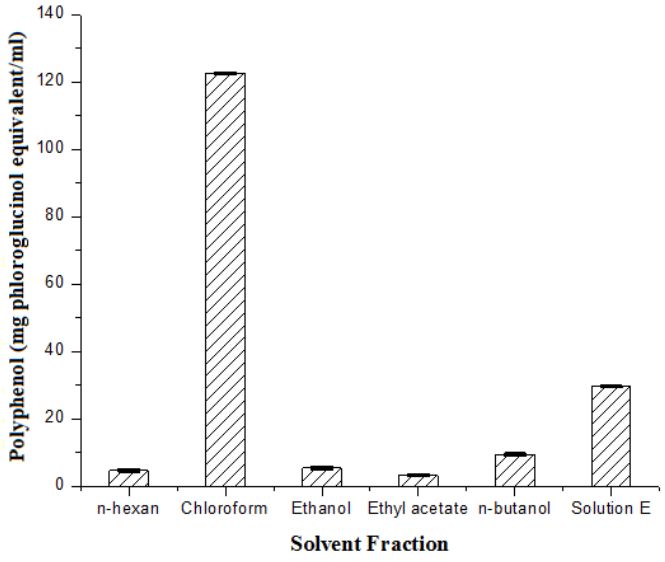

Fig. 4: Polyphenol content of different extracts, *data expressed under mean value±SD and (n=3)

Polyphenol content corresponded to 2.63%, 1.81%, 5.35%, 6.94%, and 3.01% for the extract of n-hexane, ethyl acetate, n-butanol, solution E, and ethanol, respectively, compared to the polyphenol content of the initially concentrated extract. Non-polar polyphenol content was 3.03 times total polyphenol content of polar aprotic and polar protic. The total volume of ethyl acetate, n-butanol, and chloroform were three, three and eight times that of solution C, solution D and initially concentrated extract, respectively. Ethyl acetate extract had the lowest polyphenol content, compared to other extracts.

δP polar, δD dispersion and δH hydrogen bonding are different for each solvent, for example, 0.0, 3.1, 5.3, 5.7, 8.8, 12.3 and 16.0 for δP polar of n-hexane, chloroform, ethyl acetate, n-butanol, methanol, ethanol and water, respectively. Inside, n-hexane and chloroform solvent are in the non-polar solvent group; ethyl acetate is a polar aprotic solvent; n-butanol, methanol, ethanol, and water belong to the group of polar protic solvent. Therefore, the polyphenol content of each extract is significantly different, and their structural characteristics are diversity. The non-polar polyphenol content of sponge species *A. suberitoides* is the highest, comparative to the polyphenol content of polar aprotic and polar protic. The non-polar polyphenol storage of *A. suberitoides* is in oil applying into value-added products processing, for example, antioxidant polyphenol-rich fish oil. Non-polar polyphenol is suitable for antioxidant powder production containing maltodextrin, tween 80, and non-polar polyphenol. Without these three compositions, the resulting powder will have low stability. Many studies show that the content and structure characteristic of polyphenol is different for each sponge species. These differences might be due to the inhibit condition and species of sponge.

Total antioxidant activity

Total antioxidant activity varied from 3.516 mg ascorbic acid equivalent ml^{-1} to 368.183 mg ascorbic acid equivalent ml^{-1}. Total antioxidant activity of various extracts increased in order to: n-butanol/ethyl acetate/n-hexane/ethanol/solution E/chloroform (fig. 5). The difference in total antioxidant activity was significant for different solvents ($p<0.05$). Chloroform extract had the highest total antioxidant activity, compared to other extracts, except for initial concentrated extract. Total antioxidant activity of chloroform extract corresponded to 75.36% of that of all fractions, and 3.138 times that of initial concentration extract. The strong correlation between total antioxidant activity and polyphenol content was found ($R^2>0.9$) and changed according to non-linear function (Eq. 1).

($y = 0.006x^2+2.186x-0.019$ $R^2 = 0.996$) (Eq. 1.)

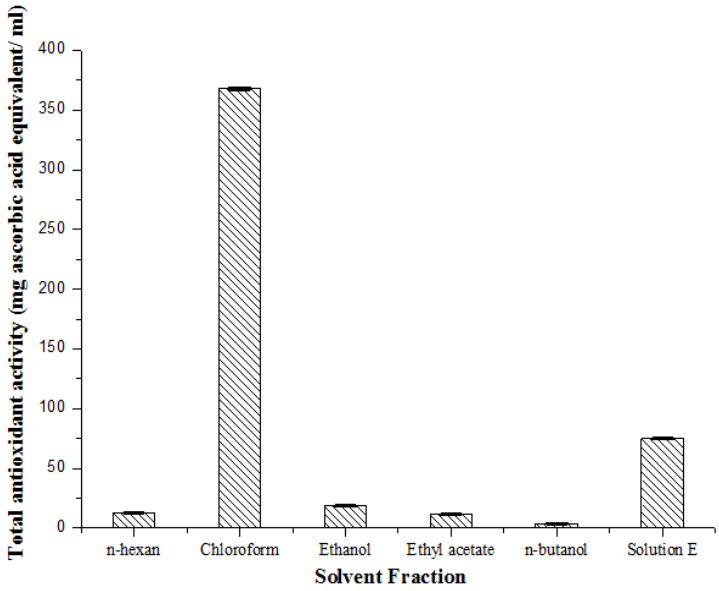

Fig. 5: Total antioxidant activity of different extracts, *data expressed under mean value±SD and (n=3)

Antioxidant activity of sponge species depended on species and extract solvent. For example, the antioxidant activity of methanol extract corresponded to 52.5±0.04 ascorbic acid equivalent g^{-1} and 85±23.3 mg ascorbic acid equivalent g^{-1}, respectively, for *Zygomycale parishii* and *Callyspongia diffusa*. Total antioxidant activity of methanol extract gave 40.433±0.722 (mg ascorbic acid equivalent g^{-1}) and 63.33±3.383 (mg ascorbic acid equivalent g^{-1}), respectively, for sponge *Spongia officinalis var. ceylonensis* and *Sigmadocia carnosa*. Dichloromethane extract of *Zygomycale parishii* and *Callyspongia diffusa* possessed 42.5±0.76 mg ascorbic acid equivalent g^{-1} and 52.5±1.44 mg ascorbic acid equivalent g^{-1}, respectively [24]. Rivera *et al.* reported that ethyl acetate extract exhibited the highest total antioxidant capacity of 286 µg ascorbic acid equivalent at 500 µg ml^{-1} concentrated extract for sponge species *A. suberitoides* [8]. Concentrated methanol and chloroform extract from species *A. suberitoides* grown in Nhatrang bay was higher total antioxidant activity than the extract of methanol and dichloromethane from species *Zygomycale parishii, Callyspongia diffusa, Spongia officinalis var. ceylonensis, Sigmadocia carnosa* growing in India and *A. suberitoides* growing in the Philippines. These things exhibited that total antioxidant activity depends on sponge species, growth local of sponge, biological substances and polarization of extract. The more refined the non-polar polyphenol is, the higher their antioxidant activity is. The correlation between polyphenol and total antioxidant activity was close. Therefore, non-polar polyphenol possesses the highest total antioxidant activity in comparison to different extracts. The polyphenol of sponge *A. suberitoides* growing in Nhatrang acts as primary and secondary antioxidants. It is suitable because polyphenol usually possesses antioxidant activity. Sponge extracts containing antioxidants corresponding vitamin C that protect cells and against neutralizing free radicals [27]. Thus, different extracts from sponge may prevent damage relating to cancer, heart disease, and other related human diseases reported on *S. cumini* leaves in [28].

Reducing power activity

The difference in reducing power activity was significant in different extracts ($p<0.05$). Chloroform extract exhibited the highest reducing power activity, compared to another extract. Reducing power activity of different extracts was decreased in order to chloroform/solution E/ethyl acetate/n-butanol/ethanol/n-hexane, corresponded to 24.08 mg $FeSO_4$ equivalent ml^{-1}, 8.79 mg $FeSO_4$ equivalent ml^{-1}, 3.41 mg $FeSO_4$ equivalent ml^{-1}, 1.71 mg $FeSO_4$ equivalent ml^{-1}, 1.20 mg $FeSO_4$ equivalent ml^{-1}, 1.12 mg $FeSO_4$ equivalent ml^{-1}, respectively (fig. 6). Reducing power activity of chloroform extract was 21.56 times stronger than that of n-hexane extract. Reducing power activity of n-hexane extract was 0.597 times that of all extracts, 0.89 times that of an initial concentrated extract. Strongly interaction between polyphenol content and reducing power activity has happened ($y = 0.189x+1.213$, $R^2 = 0.974$).

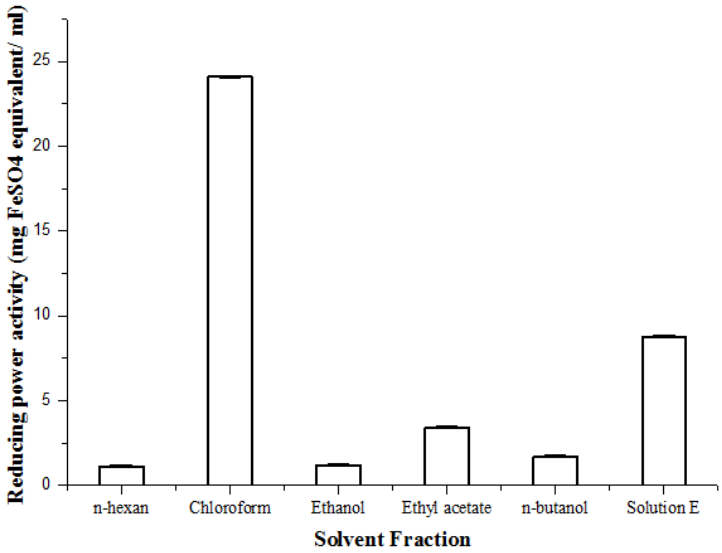

Fig. 6: Reducing power activity of different extracts, *data were expressed under mean value±SD and (n=3)

The more polyphenol content is, the more reducing power activity is. Polyphenol content contributes mainly to the antioxidant activity of the extract. In the method, Fe^{3+}react to polyphenol and transfer to Fe^{2+}. The results show polyphenol are electron donors. Reducing power activity is an indicator of the antioxidant activity which belongs to a compound or an extract [29]. Reducing power activity of polyphenol extract in the study is better than that of polyphenol extract from sponge species *Palythoa caribaeorum* grown in Atlantic marine, compared to the results of Alencar *et al.* [30]. The correlation between reducing power and polyphenol content of different extracts from sponge *Palythoa caribaeorum* ($r = 0.454$, $p<0.05$) [30] were lower than that of various extracts from sponge *A. suberitoides* ($R^2 = 0.974$). The thing proved that polyphenol of *A. suberitoides* plays positive for reducing power activity of the extract, and polyphenol of *Palythoa caribaeorum* play a negative role in reducing power activity of the extract. It exhibited that the polyphenol of *A. suberitoides* growing in Nha Trang bay possesses reducing power activity. Velho-Pereira *et al.* noticed that reducing power activity is a significant indicator and an immense value for determining the antioxidant activity of polyphenol [31]. The methanol: water extract of 50 g sponge *Geodia perarmata* had reduction power activity which oscillated from 0.14 mg ascorbic acid equivalent to 0.75±0.15 ascorbic acid equivalent [32]. Thus, reducing power activity of *A. suberitoides* (grown in Nhatrang) was better than that of *Geodia perarmata*. Reducing power activity of chloroform extract was also better than that of brown algae *Sargassum aquifolium* (19.72 ± 0.03 mg $FeSO_4 g^{-1}$ DW) [23]. Reducing power activity of different extracts from the sponge is better than that from *Morinda citrifolia* fruit, which used as a medicinal plant for over 2000 y by Polynesians [33]. All things showed that sponge species *A. suberitoides* is an antioxidant activity-rich marine source. The chloroform segment is a potential antioxidant segment which can be a suite in functional food production. Antioxidant activity depends on structure characteristic and molecular weight of polyphenol in the extract.

DPPH radical scavenging activity

The solvent effect on radical scavenging activity was similar to that on the polyphenol content and the activity of total antioxidant and reducing power. DPPH radical scavenging activity decreased as follow: chloroform, n-hexane, solution E, ethyl acetate, ethanol and n-butanol, correspond to 72.48±1.54 %, 69.28±1.41 %, 68.27±1.66 %, 67.54±1.37 %, 62.13±1.62 %, 61.19±1.58 %, respectively (fig. 7). The difference of radical scavenging activity revealed significant for various extracts ($p<0.05$).

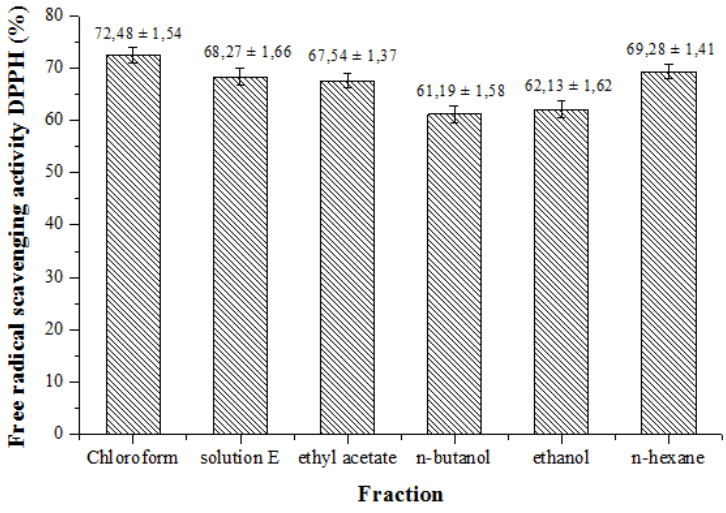

Fig. 7: Free radical scavenging activity DPPH of various solvent fraction, *Data expressed under mean value±SD and (n=3)

The results indicated that all different extracts of *A. suberitoides* were less DPPH scavenging profile than the extract of *Sigmadocia carnosa, C. gorgonoides, Callyspongia sp.* (86±1.0 %, 93.7±1.48 %, 94.8±0.83 %, respectively) [34].

Many sponge species possess DPPH free radical scavenging activity, for example, *Xestospongia sp., Fascaplysinopsis reticulata, Callyspongia sp., Petrosia contignata, A. suberitoides* [35], *Spongia officinalis var. ceylonensis* and *Sigmadocia carnosa* [24]. DPPH radical scavenging activity of different extracts in the study is at an average level, compared to the results of previous publications. DPPH radical scavenging activity of sponge extract relates closely to phenolic metabolites from marine sponges, especially polyphenol [36]. Thus, polyphenol of sponge *A. suberitoides* in the study possesses different antioxidant activities, specifically, DPPH radical scavenging activity, total antioxidant activity, and reducing power activity. DPPH radical scavenging activity of various extracts from the sponge was higher than DPPH radical scavenging activity of *Triticum aestivum* (wheatgrass), compared to [37], and it will be useful to develop new drug candidates for antioxidant therapy for various medicinal uses.

Phytochemistry composition

Finding phytochemistry compositions of different extracts were in table 1. Alkaloids, flavonoids, steroids, tannins and triterpenoids appeared in the initial extract of sponge species *A. suberitoides*, similar to the report on leaf extracts of *Vitex leucoxylon* in [38]. Triterpenoids only existed in solution E in comparison to other fractions. Both extracts of chloroform and solution E contained tannins. Flavonoids were absent in almost extracts, except for n-hexane extract.

Table 1: Phytochemical compositions of various extracts from sponge species *A. suberitoides* commonly growing in Nha Trang Bay, Vietnam

Extract	Alkaloids	Flavonoids	Steroids	Tannins	Triterpenoids
n-hexane	++	-	++	-	-
Chloroform	++	++	+	+	-
Ethanol	-	+	+	-	-
Ethyl acetate	-	++	-	-	-
n-butanol	-	+	-	-	-
Solution E	-	+	-	+	+

*Experiments were in triplication (n=3), "-" no detection; "+" Present in trace amount; "++" Present in moderate amount

The sponge extracts contained tannins, flavonoids, steroids and alkaloids [5, 24] and these phytochemical compositions have diversity bioactive, for example, antioxidant, anticancer, anti-cardiovascular diseases, dementia, cataract, macular degeneration, and cell protection [5]. The marine sponge is a resource of novel biologically active substances which is useful in other fields, for example, functional food, medicine. In the current study, all various extracts owned tannins, flavonoids, steroids and alkaloids. Therefore, sponge *A. suberitoides* growing in Nha Trang bay can possess the biological activities similar to other sponge in the world which noticed in before publication. Alkaloids, steroids and tannins owned the characteristics of non-polar substances and weak polar substances because of their dissolve ability in the non-polar solvent and the weak polar solvent. Triterpenoids existed in methanol extract and solution E showing strongly polar triterpenoids. The flavonoids and the tannins are structure diversity, but flavonoids structure is more diversity. Alkaloids existing in n-hexane fraction led the difference in comparison to the results in [39]. The thing can be a sign to recognize the difference between *Garcinia bancana* Miq. leaves and sponge.

CONCLUSION

Overall, polyphenol content and antioxidant activities existed in all various extracts of sponge species *A. suberitoides* Brøndsted (1934) grown in Nha Trang Bay. Chloroform extract had a high homology for polyphenol content, phytochemical composition and antioxidant activities. The highest polyphenol content, total antioxidant activity, reducing power activity, and DPPH free radical scavenging activity got 122.682 mg gallic acid equivalent ml^{-1}, 368.183 mg ascorbic acid equivalent, 24.082 mg $FeSO_4$ equivalent ml^{-1}, and 72.48%, respectively, compared to other extracts. Polyphenol in sponge *A. suberitoides* mainly exhibited in the form of weakly polarized compounds. Chloroform extract contained the most phytochemical compositions, except for triterpenoids. Mice weight was not affected by antioxidant polyphenol powder for 28 d. Sponge species *A. suberitoides* Brøndsted (1934) grown in Nha Trang bay is a potential resource for functional food and pharmaceutical products. These would be opportunities for rehabilitation and restoration of the marine material resources.

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AUTHORS CONTRIBUTIONS

All authors have equally contributed to the research work, except for Dang Xuan Cuong who contributed the most to Methodology, Formal Analysis, Data Curation, Writing–Original Draft, and Writing–Review and Editing.

CONFLICT OF INTERESTS

We declare that there were no conflicts of interest

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