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Extracts from egg strings of sea hare (*Dolabella auricularia*): Yield, antioxidant activity, zoochemical profile, and toxicity

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ABSTRACT

This study aimed to evaluate the crude extract yield, secondary metabolites, antioxidant and cytotoxic activities of the different crude extracts of egg strings from sea hare (*Dolabella auricularia*) taken from Guang-guang, Pujada Bay, Davao Oriental. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method showed that the antioxidant activity of the crude extract of ethanol was greater than ethyl acetate and hexane in a dose-dependent manner. Ethanolic extract with a yield of 28.96 mg/g showed the highest percentage inhibition of 24.86% at 1000 µg/mL. The antioxidant activity may be attributed to known antioxidant metabolites, especially the phenolic substances, and some forms of synergism. The result also revealed the presence of alkaloids, saponins, steroids, tannins, and terpeneoids. On the other hand, the brine shrimp assay for all extracts gave LC_{50} > 1,000 µg/mL which indicated the absence of potential cytotoxic substances. The result of this study increases the nutritional potential values and provide baseline information for another possible source of future novel antioxidants that can be used in food and pharmaceutical industries. However, further investigations must be conducted to explore other biological activities of the extracts.

1. Introduction

Marine environment has always been a source of marine natural products that have a wide range of biological activities. The secondary metabolites from marine organisms play a vital role in the development of a new drug to cure various human diseases. There are already 25,000 new compounds isolated from marine invertebrates such as mollusks, sponges and echinoderms. About 116 genera of mollusks which include 36 species of sea hare have contributed to the thousands of compounds already discovered since 1963 (Pereira et al., 2009).

Sea hare (*Dolabella auricularia*) is a shell-less mollusk that belongs to the class gastropoda and family Aplysiidae. Sea hare (Fig. 1A) can lay millions of eggs in an intertwined strand (Fig. 1B). The egg strings of sea hare are found to contain primary metabolites that are needed by the body. It is a good source of proteins and other minerals and found to be ideal for human consumption (Pepito et al., 2015). Researches show that it also contains secondary metabolites that are reported to have multiple biological effects including antioxidant activity that neutralizes the toxic effects of free radicals (Simmons et al., 2005).

Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) possess unpaired electrons that can cause lipid peroxidation not only in food but also in lipid membrane. Its major and secondary products can react with other biomolecules. Excess production of ROS and RNS beyond the antioxidant capacity of the organism can lead to various types of pathological diseases (Adedapo et al., 2008). This includes acute and chronic disorders such as diabetes, atherosclerosis, immunosuppression, aging and even cancer (Saeed et al., 2012; Pallab et al., 2013).

The secondary metabolites that have antioxidant activities produce by sea hare are greatly affected by various factors. It is dependent on the algal diet they are feed in, stages of growth and development, environmental factors as well as geographic

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locations (Pennings et al., 1993; Lozada et al., 2005). Further, the quantity and extent of antioxidant activity are totally dependent on the type of material extract and the solvent used for extracting such compounds (Anokwuru et al., 2011; Chojnacka et al., 2012).

In the continuing quest for natural antioxidants and cytotoxic agents, this study is perhaps the first attempt in Davao region, Philippines to establish such biological activities of egg strings from sea hare using different extracting solvents (ethanol, ethyl acetate, and hexane). The antioxidant activities of crude extracts were evaluated by measuring their ability to scavenge the radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Zoochemical analyses were done for determining the presence or absence of selected secondary metabolites and brine shrimp lethality assay for its cytotoxic activity.

Fig. 1: Sea hare (A) and its egg strings (B) found in Guang-guang, Pujada Bay, Davao Oriental

2. Materials and methods

2.1. Sample collection and preparation

About 6 kilograms of egg strings were randomly picked by hand along the coast of Guang-guang, Pujada Bay, City of Mati, Davao Oriental, Philippines. Pujada Bay is located in Southern part of Mindanao between the coordinates of 6^{0}48'04" and 6^{o}54'25" N latitude and between 126^{0}9'08" and 126^{0}19'33" E longitude. Fresh egg strings were washed, cleaned with distilled water, drained and dried through nitrogen blanketing at 60^{0}C.

2.2. Determination of crude extract yield

Twenty grams of dried samples was immersed with 95% ethanol for 24 to 48 hours. The mixture was filtered and washed with fresh portions of ethanol. The washings were combined with the first filtrate taking note of the total volume of ethanol used. After the filtration process, the residue was discarded and the filtrate was concentrated under *vacuo* at temperature below the boiling point of ethanol using rotary evaporator. The extract was stored in a tightly stoppered vial at 0 to 5^{0}C until its analysis. The same procedure was followed using ethyl acetate, and hexane as the extracting solvent.

Approximately 10 mL of the extract was transferred using a pipet into a previously weighed empty dish. It was placed in the oven at less than 50^{0}C for one hour, cooled and weighed. The process was repeated until constant weight was obtained. The crude extract concentration (CEC) was expressed as mg crude extract per mL of crude extract. Moreover, the crude extract yield (CEY) was determined by multiplying the CEC with the total volume of the concentrate divided by the weight of the dried egg strings.

2.3. Total antioxidant activity

Antioxidants scavenge DPPH radicals by donating a proton. Its proton or hydrogen donating ability is directly proportional to the free radical scavenging potentials of a sample. The radical scavenging activity increases with increasing percentage of free radical inhibition. The reduction reaction of DPPH is exhibited by the change of color from purple to yellow at 517 nm absorbance. As the electrons become paired off stoichiometrically, the solution loses its color which is dependent on the number of electrons being taken (Pallab et al., 2013).

A solution of DPPH was prepared by dissolving 6 mg DPPH in 50 mL methanol. One mL of DPPH solution (100 µL in methanol) was mixed with 1 mL of the extract with varying concentrations (100 - 1000 µg/mL) of the different fractions. The mixture was incubated at 37^{0}C for 30 minutes. The decrease in absorbance was measured at 517 nm using a spectrophotometer. The radical scavenging activity was determined by comparing the absorbance with the blank (100%) containing only DPPH and solvent. All analyses were done in three replicates (Pallab et al., 2013).

2.4. Zoochemical Analysis

The zoochemical analysis for the determinations of secondary metabolites alkaloids, anthraquinones, coumarins, flavonoids, glycosides, phenols, saponins, steroids, quinones, tannins, terpenoids were done using selected established procedure (Guevara, 2005).

2.5. Toxicity testing

Brine shrimp bioassay was carried out to investigate the cytotoxicity of the crude extracts of egg strings. Brine shrimps, *Artemia salina* Leach (1 g per liter) were hatched using a 22 cm x 32 cm rectangular dish filled with artificial sea water which was prepared by dissolving 3.8 grams of rock salt and with 100 mL distilled water. A plastic divider with several 2mm holes was placed in the dish to provide two unequal compartments. The brine shrimp eggs were sprinkled into the larger compartment and covered to keep away from light while the smaller compartment was leaved open and illuminated. After 48 hours, the hatched brown orange nauplii from the illuminated small compartment of the dish were pipetted. Using a 10

mL pipet, ten nauplii were transferred into each vial that is labeled 1, 2, 3 and control containing 4.5 mL of brine solution and 0.5 mL of the extract that was previously added and dried. A drop of yeast suspension that served as food was added that was prepared by dissolving 3 mg of yeast in 3 mL artificial seawater. The vials were illuminated. The survivors were counted after 24 hours. The experiments were conducted using different concentrations (10 – 1,000 µg/mL) of the crude extracts in a set of three tubes per dose (Krishnaraju et al., 2005). The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes.

2.6. Statistical analysis

All experimental measurements were carried out in triplicate and were expressed as the mean ± standard deviation. Data were analyzed using analysis of variance followed by Fisher Pairwise comparison test. P values < 0.05 were considered statistically significant.

3. Results and discussion

3.1. Crude extract yield of egg strings from sea hare

Table 1 shows the yield of the crude extract of egg strings from sea hare for ethanol, ethyl acetate, and hexane fractions using the dried samples. Using, anlaysis of variance at p<0.05, the crude extract yield of the different crude fractions showed significant difference.

Table 1: The crude extract yield of egg strings in different solvents

Crude Extract	Crude Extract Concentration (mg/mL)	Crude Extract Yield (mg/g)
Ethanol	2.73 ±0.02	28.96 ± 0.25
Ethyl acetate	2.76±0.08	29.21 ± 0.87
Hexane	1.74±0.03	18.38 ± 0.35

Each value is the average of three measurements; ±: standard deviation

The extraction yield is only one of the criteria used for evaluating the effectiveness of a particular extraction method and suitability of the solvent (Sreejamole and Radhakrishnan, 2010). A good extracting solvent must give high yield with minimal changes of the extract's property (Dhanani et al., 2017). The yields of extraction by various solvents in this study decreased in the following order: ethyl acetate > ethanol > hexane. This order did not follow the result of Do et al. (2014) in which the extraction yield increases with increasing polarity of the solvent used in extraction. However, it had the same trend with the extraction yield of Anokwuru et al. (2011). This difference may be due to the fact that the efficiency of extraction and its yield are affected not only by the extraction method, particle size of the sample, solvent used and presence of interference but also the chemical composition of the sample (Do et al., 2014).

3.2. Antioxidant activity

The antioxidant activity of the three crude extracts was measured using the change in absorbance produced by reducing DPPH. This is based on the principle of the test samples ability to scavenge the DPPH radicals. Fig. 2 shows the dose-response curves of the different crude fractions. The solvent had significant difference at p<0.05. Furthermore, using Fisher Pairwise comparison, ethanol extract had a significant effect towards the percentage inhibition. The variable degrees of free radical scavenging property increased in a dose-dependent manner. The percentage inhibition of DPPH radical formation ranged from 21.97% to 24.86% at the highest tested dose of 1000 µg/mL and from 20.81% to 23.51% at the lowest dose (100 µg/mL) with IC_{50} >1000 µg/mL for all extracts. The present study also showed that the scavenging percentage of the different crude extracts at the same concentrations were as follows: ethanol > ethyl acetate > hexane.

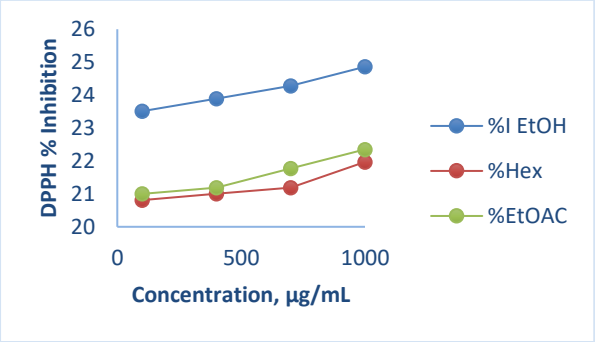

Fig. 2: The percentage inhibition of the three different crude extracts of egg strings from sea hare

The results showed that the antioxidant activity of the extracts was dependent on the extracting solvent used. Alam et al. (2013) showed that ethanol is the most frequent solvent being used in the extraction of antioxidants because of its high polarity and hence can favorably extract polar compounds such as phenolic and flavonoids that are effective antioxidants.

Moreover, this study showed that the extracts had proton-donating ability which served as free radical inhibitor or scavenger and could act as primary antioxidant (Adedapo et al., 2011). With these, egg string of sea hare can be a potential source of natural antioxidants and can be recommended to be part of our diet to protect human health and promote general wellness.

3.3. Zoochemical analysis

The zoochemical analysis of egg strings using different extracting solvents showed the presence of secondary metabolites in different test as summarized in Table 2. Test for alkaloids reported

positive for the ethanol extract only. The presence of phenols was confirmed in all extracts. Saponins, steroids, and terpenoids were found to be in increasing concentration as revealed in the intensity of color change per solvent. Hexane fractions gave higher concentration compared to ethyl acetate and ethanol. Tannins, on the other hand were present in moderate amount in ethyl acetate, trace amount in hexane but absent in the ethanol fraction. Anthraquinones, coumarins, flavonoids, glycosides, quinones on the other hand were not present in the three extracts. The antioxidant activity exhibited by the different crude extracts may be attributed to the secondary metabolites present in the egg strings.

Table 2: Zoochemical analysis of egg strings in different crude extracts

Zoochemicals	Ethanol	Ethyl Acetate	Hexane
Alkaloids	+	-	-
Anthraquinones	-	-	-
Coumarins	-	-	-
Flavonoids	-	-	-
Glycosides	-	-	-
Phenols	+	+	+
Quinones	-	-	-
Saponins	+	++	+++
Steroids	+	++	+++
Tannins	-	++	+
Terpenoids	+	++	+++

(-): absence; (+): trace amount; (++): moderate; (+++): abundant
Each trial was done in three replicates

3.4. Cytotoxic activity

The brine shrimp lethality assay is a simple, rapid, reliable test for assessing the bioactivities of extract sample which usually correlates well with cytotoxic and anti-tumor properties. It allows determining the LC_{50} values in µg/ mL of active constituents in the brine medium (Krishnaraju et al., 2005). The lethality of crude extracts in this present study was determined using the procedure of Meyer et al. (1982). Table 3 below shows the results of the brine shrimp lethality of each crude extracts.

Table 3: Effects of crude extracts from egg strings on brine shrimp lethality assay

Crude Extracts	Concentration (µg/mL)	Total Number of Survivors	%Mortality
Control	0	30	0
	10	30	0
Ethanol	100	30	0
	1000	29	3.33
	10	30	0
Ethyl acetate	100	30	0
	1000	29	3.33
	10	30	0
Hexane	100	30	0
	1000	29	3.33

Values are the mean of three replicates

All extract showed the same activity for all concentrations tested. The highest concentration gave only 3.33% mortality. The low mortality result of the different crude extracts indicated the absence of potent cytotoxic and antitumor components in the egg strings from sea hare, *Dolabella auricularia* in this study. The results gave LC_{50} greater than 1000 ppm. According to Meyer's toxicity index, crude extract is toxic (active) if it has an LC_{50} value of less than 1000 µg/mL while non-toxic if it is greater than 1000 µg/mL (Meyer et al., 1982). Contrary to the results of Kawsar et al., (2010), the egg strings of sea hare *Aplysia kurodai* showed mortality of 63.33% at 32 µg/mL. This high mortality is due to the isolated and purified lectin. Lectins are a group of sugar-binding proteins which are known to be cytotoxic and mitogenic agents. Hence, isolation and purification of compounds from egg strings of this study can be recommended for future researches on its bioactivities.

4. Conclusion

Based on the antioxidant activity assay conducted on the different crude extracts, compounds with nutritional and medicinal potential may be isolated from egg strings. These potentials may be due to the secondary metabolites present like alkaloids, phenols, steroids, tannin, saponins, and terpenoids which are known for their biological activities. On the other hand, the low mortality of brine shrimp at high concentration indicated the absence of cytotoxic compounds and confirms the edible nature of egg strings.

The result of this study increases the nutritional and medicinal potential values and provide baseline information for another possible source of future novel antioxidants that can be used in food and pharmaceutical industries. However, further investigations must be conducted to explore other biological activities of the extracts.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

References

Adedapo A, Jimoh F, and Afolayan A (2011). Comparison of the nutritive value and biological activities of the acetone, methanol and water extracts of the leaves of Bidens pilosa and Chenopodium album. Acta Poloniae Pharmaceutica - Drug Research, 68(1): 83-92.

Adedapo AA, Jimoh FO, Koduru S, Afolayan AJ, and Masika PJ (2008). Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of Calpurnia aurea. BMC Complementary and Alternative Medicine, 8(1): 53-61. https://doi.org/10.1186/1472-6882-8-53
PMid:18803865 PMCid:PMC2556645

Alam MN, Bristi NJ, and Rafiquzzaman M (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity.

Saudi Pharmaceutical Journal, 21(2): 143-152. https://doi.org/10.1016/j.jsps.2012.05.002 **PMid:24936134 PMCid:PMC4052538**

Anokwuru CP, Anyasor GN, Ajibaye O, Fakoya O, and Okebugwu P (2011). Effect of extraction solvents on phenolic, flavonoid and antioxidant activities of three Nigerian medicinal plants. Nature and Science, 9(7): 53-61.

Chojnacka K, Saeid A, Witkowska Z, and Tuhy L (2012). Biologically active compounds in seaweed extracts—the prospects for the application. The Open Conference Proceedings Journal, 3(1): 20-28. https://doi.org/10.2174/1876326X01203020020

Dhanani T, Shah S, Gajbhiye NA, and Kumar S (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. Arabian Journal of Chemistry, 10(1): S1193-S1199. https://doi.org/10.1016/j.arabjc.2013.02.015

Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, and Ju YH (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. Journal of Food and Drug Analysis, 22(3): 296-302. https://doi.org/10.1016/j.jfda.2013.11.001 **PMid:28911418**

Guevara BQ (2005). A guidebook to plant screening: phytochemical and biological. University of Santo Tomas Publishing House, Manila, Philippines.

Kawsar S, Aftabuddin S, Yasumitsu H, and Ozeki Y (2010). The cytotoxic activity of two D-galactose-binding lectins purified from marine invertebrates. Archives of Biological Sciences, 62(4): 1027-1034. https://doi.org/10.2298/ABS1004027K

Krishnaraju AV, Rao TV, Sundararaju D, Vanisree M, Tsay HS, and Subbaraju GV (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (Artemia salina) lethality assay. International Journal of Applied Science and Engineering, 3(2): 125-34.

Lozada PWM, Flores LAJ, Tan RM, and Dy DT (2005). Abundance and ingestion rate of the sea hare, Dolabella auricularia (Lightfoot 1786) in a shallow embayment (Eastern Mactan Is., Cebu, Central Philippines). Philippine Scientist, 42: 67-78.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, and McLaughlin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica, 45(5): 31-34. https://doi.org/10.1055/s-2007-971236

Pallab K, Tapan BK, Pal TK, and Kalita R (2013). Estimation of total flavonoids content (TFC) and anti oxidant activities of methanolic whole plant extract of Biophytum sensitivum Linn. Journal of Drug Delivery and Therapeutics, 3(4): 33-37.

Pennings SC, Nadeau MT, and Paul VJ (1993). Selectivity and growth of the generalist herbivore Dolabella auricularia feeding upon complementary resources. Ecology, 74(3): 879-890. https://doi.org/10.2307/1940813

Pepito AR, Delan GG, Asakawa M, Ami LJ, Yap EES, Olympia MS, and Lamayo MHA (2015). Nutritional quality of the egg mass locally known as "lukot'of the wedge seahare dolabella auricularia (Lightfoot, 1786). Tropical Technology Journal, 1(19): 1-6.

Pereira DM, Valentão P, Pereira JA, and Andrade PB (2009). Phenolics: From chemistry to biology. Molecules, 14(6): 2202-2211. https://doi.org/10.3390/molecules14062202

Saeed N, Khan MR, and Shabbir M (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complementary and Alternative Medicine, 12(1): 221-233. https://doi.org/10.1186/1472-6882-12-221 **PMid:23153304 PMCid:PMC3524761**

Simmons TL, Andrianasolo E, McPhail K, Flatt P, and Gerwick WH (2005). Marine natural products as anticancer drugs. Molecular Cancer Therapeutics, 4(2): 333-342. **PMid:15713904**

Sreejamole KL and Radhakrishnan CK (2010). Preliminary qualitative chemical evaluation of the extracts from mussel Perna viridis. International Journal of Pharmaceutical Sciences Review and Research, 5(2): 38-42.