Original Article

Egg Strings of *Dolabella auricularia*: Its *In Vitro* Antioxidants and Antibacterial Activities

Janeth Cuevas Tayone[1,*], Romeo Macaltao Del Rosario[2], Oliva Platero Canencia[2], Wilanfranco Caballero Tayone[1]

[1]Institute of Agriculture and Life Sciences, Davao Oriental State University, City of Mati, Davao Oriental, PHILIPPINES.
[2]College of Science and Technology Education, University of Science and Technology of Southern Philippines, Lapasan, Cagayan de Oro, PHILIPPINES.

Submission Date: 01-11-2020; Revision Date: 15-07-2021; Accepted Date: 25-07-2021

ABSTRACT

This study aimed to evaluate the antioxidants concentration and antimicrobial activities of the different crude extracts of egg strings from sea hare (*Dolabella auricularia*) taken from Guang-guang, Pujada Bay, Davao Oriental. The determination of phenolics using the Aluminum chloride colorimetric method yielded 0.01 – 0.17 mg gallic acid equivalent / gram. On the other hand, Folin-ciocalteau assay for the total flavonoids gave a range of 6.27 – 8.83 mg quercetin/ gram. Furthermore, the antibacterial activity assay using the agar disk diffusion method gave a promising zone of inhibition for ethyl acetate extract against *Staphylococcus aureus* and *Escherichia coli* (20 mm and 26.56 mm, respectively). The findings of this work may add to the overall value of the nutritional and medicinal potential of the egg strings from sea hare.

Key words: Antibacterial, Antioxidants, *Dolabella auricularia*, Flavonoids, Phenolics.

Correspondence:
Dr. Janeth C Tayone,
Institute of Agriculture and Life Sciences, Davao Oriental State University, City of Mati-8200, Davao Oriental, PHILIPPINES.
Phone no: +63- 093 09359177458

Email: njtayone2005@yahoo.com

INTRODUCTION

Egg strings of the sea hare, *Dolabella auricularia* (Figure 1) 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.[1,2] Researches show that it also contained secondary metabolites, which include phenols and flavonoids.[3] These compounds are reported to have multiple biological effects, including anti-bacteria and antioxidant activity that neutralizes the toxic effects of free radicals.[4,5]

Phenolics are one of the main secondary metabolites. Most of them come from the deamination of amino acid phenylalanine and tyrosine with the introduction of one or more hydroxyl groups into its phenyl ring. The structure of phenolic compounds varies from simple phenols (volatile phenols) to complex polymers, both with an aromatic ring. These include cinnamic acids, benzoic acid, flavonoids, proanthocyanidins, coumarins, stilbenes, lignans, and lignins.[6,7] On the other hand, flavonoids are known to be potent antioxidants that are dependent on their molecular structures. This is the most common group of polyphenolic compounds characterized by phenylbenzopyran chemical structure.[7]

The antioxidant and free radical scavenging activities of phenols and flavonoids depend on the hydroxyl groups attached to the large ring system.[8] Free radicals are defined as atoms or molecules that possess unpaired electrons. Examples of these free radicals are reactive oxygen species (ROS) and reactive nitrogen species (RNS).[4] An individual lifestyle is a great contributing factor to these radicals. Smoking, exposure to certain pollutants, preservatives, pesticides for food production are just a few to mention.

The ROS can cause lipid peroxidation not only in food but also in the lipid membrane. Its major and secondary products can react with other biomolecules. Excess

SCAN QR CODE TO VIEW ONLINE

www.ajbls.com

DOI: 10.5530/ajbls.2021.10.46

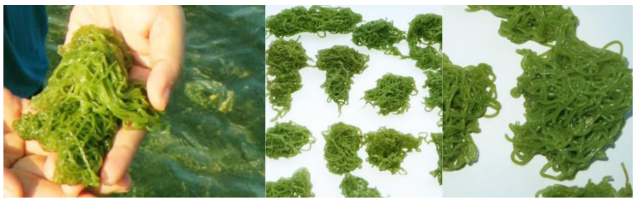

Figure 1: Egg strings of the sea hare, *D. auricularia* collected from Guang-guang, Pujada Bay, Davao Oriental, Philippines.

production of ROS beyond the antioxidant capacity of the organism can lead to various types of pathological diseases.[4] This includes acute and chronic disorders such as diabetes, atherosclerosis, immunosuppression, aging, and even cancer.[9,10] Antioxidants greatly reduce the damage caused by these radicals or oxidants through neutralizing the free radicals before it can attack the cells. These can prevent damage to lipids, proteins, enzymes, carbohydrates, and DNA. With these, the beneficial effects of phenols and flavonoids on human health in fighting diseases have led to the considerable interest of the compounds' antioxidant activity.

Accordingly, the amount of flavonoids, phenols, the extent of antioxidant activity, and antibacterial properties are dependent on the type of material extract and the solvent used for extracting such compounds. Solvents with different polarities can include water, methanol, ethanol, acetone, ethyl acetate, carbon tetrachloride, chloroform, or hexane.[11] The different pattern of solvents' effect on the extraction of sample materials is also due to the different geographical location of plants or marine organisms subjected to such analysis.[12] Further, the extraction process can also affect the results.[13]

This study validates the medicinal and nutritional potential of egg strings of *D. auricularia* using the extracting solvent of different polarities. It also provides benchmark information for the conduct of further researches on sea hare egg strings leading to the possible development of medicinal substances. This information can be a basis to strengthen the implementation of policies to protect, conserve, and sustain such local resources. This will lead to an increase in the productivity of sea hare, thereby also increase the income of the local fisher folks and, eventually, contribute to the "no hunger" sustainable development plan of the region.

MATERIALS AND METHODS

Sample collection and preparation

Sample collection and preparation was done in the same way with the previous study of egg strings collected at the same sampling site.[18] The randomly handpicked 4 kilograms of egg strings from the coast of Guang-guang, Pujada Bay, City of Mati, Davao Oriental, Philippines were washed, cleaned with distilled water, drained, and dried through nitrogen blanketing at 60°C. About twenty grams of dried samples were immersed with 95% ethanol, ethyl acetate, and hexane, separately for 24 to 48 hrs. The mixture was filtered and washed with fresh portions of each solvent. The filtrate was concentrated under *vacuo* at a temperature below the boiling point of the extracting solvents using a rotary evaporator. The concentration of the extract (mg/mL) was determined by approximately weighing 10 mL of the extract in an empty dish. It was dried in an oven with a temperature less than 50°C for one hour, cooled, and weighed until a constant weight was obtained.

Total flavonoids assay: Aluminum chloride colorimetric method

The aluminum chloride method is based on the formation of stable acid complexes (Figure 2) with the C-4 keto group and either C-3 or C-5 hydroxyl group of flavones and flavonols with aluminum chloride.[10]

About 1 mL aliquot of the extract solution was added to a 10 mL volumetric flask with 4 mL distilled water. Sequentially, 0.3 mL of 5% $NaNO_2$ was added and allowed to stand for 5 min, followed by the addition of 0.3 mL $AlCl_3$ (10%). After the 6th min, 2 mL of 1M NaOH was added and diluted with distilled water up to the mark (10 mL). The solution was vigorously shaken, and the absorbance was recorded at 510 nm wavelength. The same wavelength was used for the generation of a standard calibration curve using known concentrations of quercetin in ethanol (50, 100, 150, 200, and 300 mg/L). The concentrations of flavonoids in the sample was calculated from the calibration plot and expressed as mg quercetin per gram of sample.[14]

Total phenolics assay: Folin – ciocalteau method

The Folin – Ciocalteau assay is based on the formation of a blue color (molybdenum blue) developed due to the complex redox reaction of phenols with phosphomolibdic acid present in Folin - Ciocalteau reagent.[15]

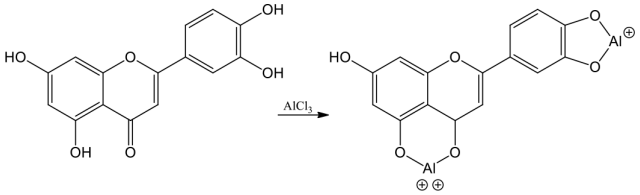

Figure 2: The chemical reaction of aluminum chloride method for flavonoids assay.

One mL of the extract was added to a flask with 9 mL distilled water followed by one mL of Folin – Ciocalteu's phenol reagent. The mixture was thoroughly mixed. The addition of 10 mL of 7% sodium carbonate followed, and the mixture was finally diluted to 25 mL with distilled water and allowed to stand at room temperature for 90 min. A blue color (molybdenum blue) complex was developed.[15] The absorbance was measured at 750 nm. A standard calibration curve was prepared using 25 to 150μg/mL in 80% methanol of Gallic acid. The total phenolics were expressed as μg Gallic acid equivalents (GAE)/g samples.[16]

Antibacterial activities

The prepared crude extracts of samples were subjected to antimicrobial test against pathogenic gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* using agar well diffusion method. Ampicillin and penicillin were used as standard drugs, respectively, for studying the activities of the extract.

The melted agar was poured into dry sterile petri dishes and solidified. A sterile cotton swab moistened with the test suspension that was previously incubated in Muller-Hinton Broth for 24 hrs at 37°C was used to steak over the entire surface of the agar. It was allowed to stand for 5 min. A cork borer was sterilized by immersing it in an erlenmeyer flask containing ethanol and heated over an alcohol flame. The agar was stabbed using the cooled cork borer to the bottom of the dish to create a well. The well was filled with the previously prepared crude extract samples. The incubation period was 24 hrs at 37°C. The activity of the extract was determined by measuring the diameter of the zone of inhibition and compared with the values produced from the standard drugs.[17]

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.

RESULTS

Total Flavonoids from the Egg Strings of Sea Hare

The total flavonoids were expressed in terms of quercetin equivalent per grams of egg strings (d.w.), as shown in Table 1 with the linear equation based on the standard curve: $y = 0.0005x + 0.0027$, $r^2 = 0.9947$. The concentration of flavonoids in the egg strings sample had a significant difference at $p<0.05$. It ranged from 6.27 mg/g to 8.83 mg/g in this order: ethyl acetate > ethanol > hexane. Using Fisher Pairwise Comparisons, each extract differs significantly from each other.

Table 1: Total Flavonoids in the Egg Strings of Sea Hare Expressed in Terms of mg Quercetin per Gram of Dried Egg Strings.

Crude Extract	mg Quercetin/ g of egg strings (d.w.)
Ethanol Extract	7.28 ±0.32
Ethyl Acetate Extract	8.83 ±0.56
Hexane Extract	6.27 ±0.35

Each value was the average of three analyses ± standard deviation in d.w.

Table 2: Total Phenolics from the Egg Strings of Sea Hare Expressed in Terms of mg Gallic Acid Equivalents/ g of Dried Egg Strings.

Crude Extract	mg GAE/ g of dried egg strings
Ethanol Extract	0.17 ±0.05
Ethyl acetate Extract	0.13 ±0.00
Hexane Extract	0.01 ±0.00

Each value was the average of three analyses ± standard deviation.

Total Phenolics Extracted from the Egg Strings of Sea Hare

The total phenolics (Table 2) was expressed in terms of Gallic acid equivalent and calculated with a linear equation based on a standard curve: $y = 0.0025x + 0.0291$, $r^2= 0.9814$. The highest concentration of the total phenolics was measured in ethanol extract (0.17 mg GA/ g). This was followed by ethyl acetate extract with total phenolics content (TPC) of 0.13 mg GAE/ g and lastly hexane extract with 0.01 mg GAE/ g. A significant difference was observed at $p<0.05$ with hexane showing significantly different from the other two extracts.

Antimicrobial Activity of egg Strings

The egg strings extract (1 mg/mL) using different solvents showed different values of the zone of inhibition against *E. coli* (gram-negative) and *S. aureus* (gram-positive), as shown in Table 3. It was the ethyl acetate crude extract that gave promising results that were almost comparable with the positive control.

DISCUSSION

This study quantifies the previous result which showed the presence of different secondary metabolites that include phenols and flavonoids. It was the phenols and flavonoids in the egg strings' extract

Table 3: Antimicrobial activity of Egg Strings Using Different Extracting Solvents.

Microorganism	Zone of Inhibition, mm		
	Ethanol Extract	Ethyl acetate Extract	Hexane Extract
Staphylococcus aureus	6 26.67*[a]	20 27.33* [a]	6 26.67* [a]
Escherichia coli	8.33 24.67*[b]	26.56 26.67* [b]	8.0 26.00* [b]

*Average value for the positive control (a- ampicillin, b-penicillin)

that is responsible for the antioxidant activity that was measured using DPPH assay.[18] Accordingly, the secondary metabolites flavonoids, which include flavones, flavanols, and tannins, have an antioxidant property that greatly depends on the hydroxyl group, specifically the 3-OH.[19] It can scavenge reactive oxygen species (ROS) or reactive nitrogen species (RNS) because of the presence of phenolic hydroxyl groups in its structure.[11]

The present study showed that the ethyl acetate extract had high total flavonoids at $p < 0.05$ in the egg strings of sea hare. This is an indication that ethyl acetate maximized the extraction of the total flavonoids in egg strings. These results only prove that natural products like total flavonoid compounds can be obtained from marine mollusks. Similarly, a marine mollusk (sea cucumber) has total flavonoids content (TFC) with values ranging from 0.029 to 0.598 mg of rutin equivalents per 100 gram. This flavonoids content in sea cucumber is significantly correlated with antioxidant activity.[20]

In comparison, the TFC in egg strings of sea hare in this present study was low compared to the TFC content in the ink of sea hare collected from the same locality.[21] It is believed that secondary metabolites of sea hare organisms are obtained from their algal diet. With these, the flavonoids, phenolics, and other bioactive compounds in the ink and egg strings of sea hare may also be obtained from the same algal diet. Accordingly, algae are one of the richest sources of bioactive compounds with a wide range of therapeutic applications. Compounds with strong potential in treating cancer are isolated from algae, which include dolastatin 10, which is also isolated from sea hare.[22]

Usually, the total phenolics and total flavonoids in plants using different extracting solvents will range from 5 to 10 mg quercetin / g of dry plants. The total flavonoids in the egg strings, especially for ethyl acetate extract, were generally much higher compared to the three known Nigerian medicinal plants that only contain flavonoids ranging from 3.67 g QE/ 100g dw) to 5.32 g QE/ 100 g dw for its ethyl acetate extract.[11]

On the other hand, ethanol solvent was more efficient in extracting the total phenolics from egg strings of sea hare. The efficiency of the extraction of the total phenolic was greatly dependent on the type of sample and the kind of extracting solvent used.[23,24] Moreover, the differences can also be explained by the different dielectric constants (ε) (EtOH, ε = 24.55, EtOAc = 6.02, Hex = 1.89) and different polarities (EtOH = 0.654 EAOAc = 0.228, hex = 0.009).[25] Hence, the extractability of the targeted phenolics compound from the sample materials may vary with the different polarities of the solvent used.

Relatively, the total phenolics from the ethanol extract of egg strings were higher compared to the ethanolic extract from the ink of sea hare.[21] However, this result was lower compared with that of sea cucumber, *Cucumaria frondosa* (0.225 to 2.36 mg of gallic acid per gram), which is still concluded to be a useful source of antioxidants for human consumption.[20]

Phenolics are generally known to have antioxidant activity. It has redox properties that absorb and neutralize free radicals, scavenging singlet or triplet oxygen, as well as decomposing peroxides facilitated by the presence of a hydroxyl group. Hence total phenolic concentration rapidly estimates the antioxidant activity.[4,21] In the study of Cherif *et al.*[26] showed that *Aplysia depilans* ink, a depilatory sea hare, contains a large number of compounds with antioxidant properties. Moreover, the aqueous ink extract was effective in scavenging DPPH radicals and exhibited reducing power activity with an IC_{50} at 0.94 mg/mL and 39.4 μg/mL, respectively. Although these are lower than the IC_{50} of the standards used, it is still said to be the strongest antioxidant activity of sea hare.

The previous study of egg strings collected from the same sampling site[18] and these present results showed that egg strings extracts had a proton-donating ability, which served as a free radical inhibitor or scavenger and could act as a primary antioxidant.[4] With these, the 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.

The antibacterial activity results of the ethyl acetate extract were very promising. The secondary metabolites present in the egg strings could be responsible or had contributed to its antimicrobial activity since these compounds generally have an antibacterial factor.[14,15] In general, the antibacterial activity of egg strings was

higher as compared to other bivalves and gastropods from the same sampling area.[27]

Recently, researches are redirected to marine organisms that are rich in phenolics. These organisms are now being considered in the food industry as a natural source of antioxidants, which are good for human health. The presence of phenolics and flavonoids shows a broad spectrum of antimicrobial agents with high antifungal activity against gram-positive and gram-negative bacteria. It is the partial hydrophobic property of phenols that make it effective against microbes. It either inactivates microbial adhesions or inhibits hydrolytic enzymes such as proteases.[19]

CONCLUSION

Based on the measured antioxidants and antibacterial activity assay conducted on the different crude extracts, compounds with nutritional and medicinal potential may be isolated from egg strings of *D. auricularia*. These potentials may be due to the presence of phenols, flavonoids, and other secondary metabolites. The findings of this work may add to the overall value of the nutritional and medicinal potential of the egg strings from sea hare.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Research Development and Extension Division of Davao Oriental State University for providing the funds for this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ROS: Reactive Oxygen Species; **RNS:** Reactive Nitrogen Species; **GAE:** Gallic Acid Equivalents; **TPC:** Total Phenolics Content; **TFC:** Total Flavonoids Content; **DDPH:** 2,2-diphenyl-1-picrylhydrazyl.

REFERENCES

1. Pepito A, Delan G, Asakawa M, Ami L, Yap E, Olympia M, Yasui K, Maningo A, Rica R, Lamay M. Nutritional quality of the egg mass locally known as 'Lukot' of the wedge sea hare *Dolabella auricularia* (Lightfoot, 1786). Trop Technol J. 2015;19(1):1-6.
2. Tayone J, Morales J, Jimenez L. Nutrient and chemical composition of sea hare, *Dolabella auricularia* in Pujada Bay, Davao Oriental. Davao Research [journal:2015];11:6-12.
3. Pereira RB, Andrade PB, Valentão P. Chemical diversity and biological properties of secondary metabolites from sea hares of Aplysia Genus. Mar Drugs. 2016;14(2):1-33. doi: 10.3390/md14020039, PMID 26907303.
4. Adedapo AA, Jimoh FO, Koduru S, Afolayan AJ, Masika PJ. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea*. BMC Complement Altern Med. 2008;8:53. doi: 10.1186/1472-6882-8-53, PMID 18803865.
5. Alam MN, Bristi NJ, Rafiquzzaman M. Review on *in vivo* and *in vitro* methods evaluation of the antioxidant activity. Saudi Pharm J. 2013;21(2):143-52. doi: 10.1016/j.jsps.2012.05.002, PMID 24936134.
6. Vábková J, Neugebauerová J. Determination of total phenolic content, total flavonoid content and FRAP in culinary herbs in relation to harvest time. Acta Univ Agric Silvic Mendelianae Brun. 2013;60(1):167-72. doi: 10.11118/actaun201260010167.
7. Pereira D, Valentão P, Pereira J, Andrade P. Phenolics: from chemistry to biology. Molecules. 2009;14(6):2202-11. doi: 10.3390/molecules14062202.
8. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem. 1999;47(10):3954-62. doi: 10.1021/jf990146l, PMID 10552749.
9. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complement Altern Med. 2012;12:221. doi: 10.1186/1472-6882-12-221, PMID 23153304.
10. Pallab K, Tapan B. Tapas P, Ramen K. Estimation of total flavonoids content (TFC) and antioxidant activities of methanolic whole plant extract of Biophytum sensitivum linn. J Drug Deliv Ther. 2013;3:33-7.
11. Anokwuru C, Anyasor G, Ajibaye O, Fakoya O, Okebugwu P. Effects of Extraction Solvents on phenolic, Flavonoid and Antioxidant activities of three Nigerian Medicinal Plants. Nat Sci. 2011;9(7):53-61.
12. Chojnacka K. Biologically Active Compounds in Seaweed Extracts - the Prospects for the Application. TOPROCJ. Proceedings of the journal. 2012;3(1):20-8. doi: 10.2174/1876326X01203020020.
13. Dhanani T, Shah S, Gajbhiye N, Kumar S. Effects of extraction methods on yield, phytochemical constituents, and antioxidant activity of Whithania somnifera. Arab J Chem. 2013;10:1193-9.
14. Ardekani M, Hajimahmoodi M, Oveisi M, Sadeghi N, Behrooz J, Ranjbar A, Moridi T. Comparative antioxidant activity and total flavonoid content of Persian pomegranate (*Punica granatum* L.) cultivars. Iran J Pharm. 2011;10(3):519-24. Available from https:.
15. Khatiwora E, Adsul V, Kulkarni M, Deshpande N, Kashalkar R. Spectroscopic determination of total phenol and flavonoid contents of *Ipomoea carnea*. Int J Chem Tech Res. 2010;2(3):1698-701.
16. Omar NF, Hassan SA, Yusoff UK, Abdullah NA, Wahab PE, Sinniah U. Phenolics, flavonoids, antioxidant activity and cyanogenic glycosides of organic and mineral-base fertilized cassava tubers. Molecules. 2012;17(3):2378-87. doi: 10.3390/molecules17032378, PMID 22370524.
17. Guevarra B. A guidebook to plant screening: phytochemical and Biological. Philippines: University of Santo Tomas; 2004.
18. Tayone JC, Del Rosario RM, Canencia OP. Extracts from egg strings of sea hare (*Dolabella auricularia*): yield, antioxidant activity, zoochemical profile, and toxicity. Int J Adv Appl sci. 2019;6(1):24-8. doi: 10.21833/ijaas.2019.01.003.
19. Baba SA, Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. J Taibah Univ Sci. 2015;9(4):449-54. doi: 10.1016/j.jtusci.2014.11.001.
20. Mamelona J, Pelletier É, Lalancette K, Legault J, Karboune S, Kermasha S. Quantification of phenolic contents and antioxidant capacity of Atlantic sea cucumber, *Cucumaria frondosa*. Food Chem. 2007;104(3):1040-7. Availavle from. doi: 10.1016/j.foodchem.2007.01.016.
21. Tayone JC, Del Rosario RMD. Crude extract yield, total phenolics, and total flavonoids from the ink of sea hare (*Dolabella auricularia*). Int J Adv Appl sci. 2017;4(11):17-21. doi: 10.21833/ijaas.2017.011.003.
22. Varshney A, Singh V. Effects of algal compounds on cancer cell line. J Exp Biol Agric Sci. 2013;1(5):337-46.
23. Jang H, Chang K, Huang Y, Hsu C, Lee S, Su M. Principal phenolic phytochemicals and antioxidant activities of three Chinese medicinal plants. Food Chem. 2007;103(3):749-56. doi: 10.1016/j.foodchem.2006.09.026.

24. Jakopič J, Veberič R, štampar F. Extraction of phenolic compounds from green walnut fruits in different solvents. Acta Agric Slov. 2009;93(1):11-5. doi: 10.2478/v10014-009-0002-4.
25. Dailey A, Vuong QV. Effect of extraction solvents on recovery of bioactive compounds and antioxidant properties from macadamia (*Macadamia tetraphylla*) skin waste. Cogent Food Agric. 2015;1(1):1-21. doi: 10.1080/23311932.2015.1115646.
26. Cherif E, Lahbib K, Charaf M, Yahaia M. Antioxidant activity of *Aplysia depilans* ink collected from Bizerte Channel (NE Tunisia). Alg J Nat Prod. 2015;3(1):138-45.
27. Tayone JC, Ortiz CC, Tayone WC. Selected Mollusks from Pujada Bay, Philippines: Heavy Metal Health Risk Assessment and Antibacterial Activities. AJBLS. 2020;9(2):177-84. doi: 10.5530/ajbls.2020.9.27.

Cite this article: Tayone JC, Rosario RMD, Canencia OP, Tayone WC. Egg strings of *Dolabella auricularia*: Its *in vitro* Antioxidants and Antibacterial Activities. Asian J Biol Life Sci. 2021;10(2):340-5.