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Antioxidant Activity Synergy Between Stingless Bee Honey and Sea Cucumber Extract Combination for Food Supplement

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Abstract: Contemporary research has shown that the local tradition of stingless bee honey consumption has potential for additional value in modern medicine and has a higher therapeutic value than other species of bee. Meanwhile, modern food and pharmaceutical industries are inspired to develop certain functional foods and nutraceuticals from various parts of the marine sea cucumber such as to avoid inflammation and other therapeutic potential. Both the stingless bee and the sea cucumber are present in Malaysia, along with native species. The aim of this research is to investigate the synergy of antioxidants by combining sea cucumber extract with stingless bee honey for potential food supplement. The antioxidant effect of sea cucumber extract and stingless bee honey individually and in combination will be determined using total phenolic content (TPC) and antioxidant DPPH assay. From this study, the TPC value of stingless bee honey is similar to TPC value of gallic acid, while the TPC value of sea cucumber is lower than TPC value of gallic acid standard. Meanwhile, the TPC value for stingless bee honey: sea cucumber is very low compared to gallic acid standard. From the DPPH assay, the value of IC50 for stingless bee honey is 45.57, which indicates its satisfactory antioxidant content. The IC50 value for sea cucumber is 76.84, which indicates that the value is in the moderate category. The IC50 reading of stingless bee honey and sea cucumber combination is 44.48 and it is also in the category of very strong antioxidant. From this experiment, we have managed to characterize the TPC content and antioxidant profiles of sea cucumber extract and stingless bee honey individually and in combination. There is excellent potential for this combination to be marketed and diversified further through combination with other natural product to produce novel supplement combinations. It is hoped that this study could be a starting point to investigate the potential of combining kelulut honey with sea cucumber extract as a novel functional supplement.
Keywords: Antioxidant, Kelulut, Stingless Bee Honey, Sea Cucumber, Gamat, Synergy

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1. Introduction

Honey is a natural nutrition made from flower nectar collected by bees and is used frequently as a sweetener. Honey is famous for its nutritional value. There are many types of honeys and sea cucumbers. The combination of stingless bee honey and sea cucumber are unique for the use as food supplement. Besides, the quality and the durability of nutritious natural products have been increasingly identified. All of this encourages scientists and researchers to look for natural and traditionally used plants/herbs as supplements with a high level of antioxidant activity.

1.1 Natural source of antioxidant

Marine environments have high biodiversity and contain various chemical activity sources. Marine organisms contain a number of chemical compounds that are valuable for industrial sectors such as medicines, cosmetics and food [1]. Marine biotechnologists are aiming to make use of marine biodiversity to produce inexpensive medicines and functional food sources [2]. The idea of using foods to optimize health has provided a practical approach to apply natural products with physiological advantages to lowering the risk of different chronic diseases [3]. Several distinct biological and pharmacological properties have been assigned to several species of sea cucumbers, including anti-angiogenic, anti-cancer, anti-hypertension, anti-inflammatory, anti-inflammatory, anti-oxidant, antithrombotic, anticancer and injury cure.

1.2 Honey as traditional application

Honey is one of the sources of traditional medicine that can be used regardless of age from children up to the elderly [4]. With the exception of diabetic individuals, it also does not appear to give any adverse effects if taken in excess. Honey is considered as the most valuable gift in India, this is because it can treat a weak digestive system. It has also been emphasized that the use of honey is very beneficial in the treatment of cough and is considered to be an effective remedy for maintaining healthy teeth and gums [5].

Furthermore, it has been used for the treatment of insomnia for generations because of its hypnotic effect. In the case of skin problems such as cuts and bruises, heart discomfort and palpitation, lung imbalances and anemia, ancient Ayurvedan doctors also suggest honey. Ayurvedic usage of honey in certain eye conditions has a long history.

1.2 Sea Cucumber

Malaysian sea cucumber is also known as Gamat. In Asian nations, they are economically important especially in China, where various species are used or consumed in the traditional medicine [6]. Currently, their potential health features and other biological characteristics have made them widely recognised as naturally sourced marine antioxidant chemicals. There are currently thousands of plant species being investigated for potential antioxidants source.

The usage of sea cucumber directly reduces wound healing time and helps to build and regenerate new tissues in humans, as does the capacity of the sea cucumber to swiftly rebuild its own tissue if destroyed [7]. A multitude of bio-actives have been found in a variety of biological activities including antimicrobials. In summary, sea cucumber can be examined for use consumed as food and nutritional source as well as potential provider of other valuable compounds for the biotechnology sector.

1.4 Problem statements

In Malaysia, there are still reported cases of nutrient deficiency among poor rural households, among them are cases of underweight and stunting among young children, anemia and vitamin A deficiency in children under 5 years old [8]. One of the reasons is due to poor macro- and micro

nutritional intake among Malaysians, notably for calcium, zinc, iron, magnesium, copper and protein [9]. Food synergy consisting of mixtures of nutrients have potential health benefits for human well-being, preventing chronic diseases and infections as well as increasing the immune system. The aim of this research is to investigate the potential synergistic of antioxidants activity by combining sea cucumber extract with stingless bee honey and to determine the appropriate ratio between stingless bee honey and sea cucumber in producing an effective antioxidant.

2. Methodology

2.1 Antioxidant testing of reactivity

The antioxidant information on sea cucumber extract and stingless honeybee may be found on a separate basis in the preceding chapter. A few methods are thus utilised to assess the antioxidant activity of both agents in this chapter. Individual, combined, and total phenolic content, DPPH assay will evaluate the antioxidant activity of the sea cucumber mineral and stingless bee honey.

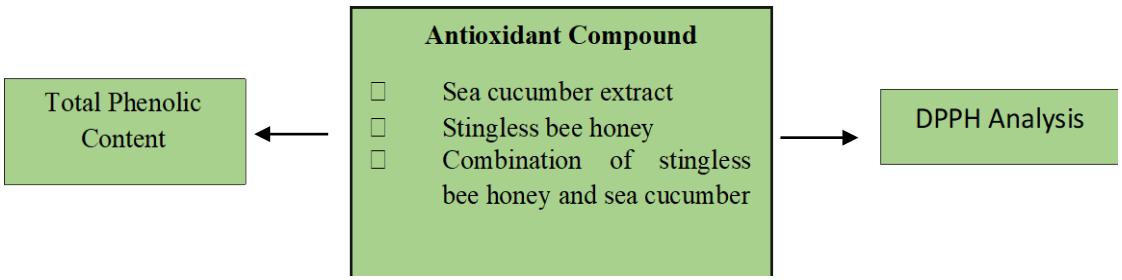

Figure 1: Method used for antioxidant activities

2.2 Step of total phenolic content

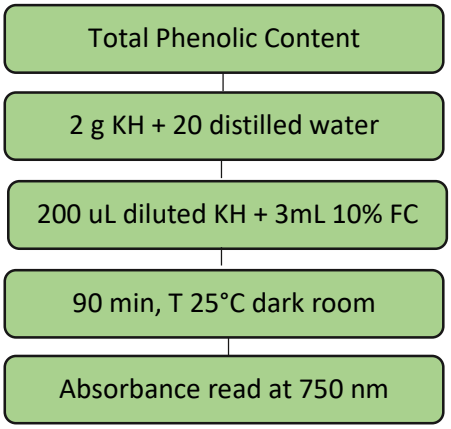

Figure 2: Flow chart representing step in total phenolic content

2.3 DPPH assay

The test produces a strong violet solution that is constant without direct light exposure at room temperature. In this work, a Multiskan GO spectrophotometer was identified to radically scavenge Stingless bee honey with the radical DPPH [10]. One ml ethanolic Stingless bee honey solution was added into 2 mL of DPPH solution after DPPH solution was prepared by dissolving 2 mg of DPPH in 100 mL of ethanol. The reaction mixture was forcefully shaken by using hands to give the excellent mix and then kept for 30 minutes at room temperature without exposure to light.

The absorption of the mixes has been reported thereafter. The scavenging of Stingless bee honey with radical DPPH was measured on the basis of the following equation:

$$DPPH\ radical\ scavening\ activity\ (\%) = \frac{(Abs\ control - Abs\ sample)}{Abs\ control} \times 100\ \ Eq.\ 1$$

Preparation of antioxidant activity:

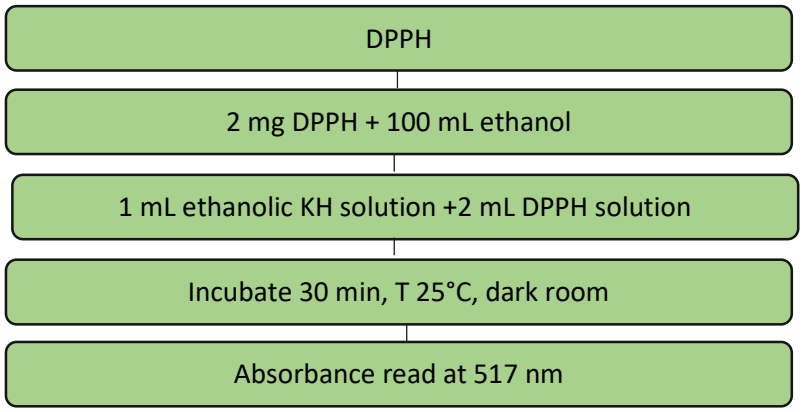

Figure 3: Shows flowchart representing step in DPPH assay analysis

3. Results and Discussion

3.1 Determination of total phenolic content

In order to find the values for Gallic acid equivalent, concentration of Gallic acid in both samples were calculated by using equation below:

$$y = mx + c\ \ Eq.\ 2$$

Where y = Absorbance value, m= gradient of the Total Phenolic Content (TPC) graph and c = intercept at Absorbance value.

From the standard calibration curve, the equation established was y = 0.0043x + 0.0196 with correlation coefficient of 0.9653. This indicates that the model is 96.53 % fit to the data obtained from this experiment has been presented in Figure 4.

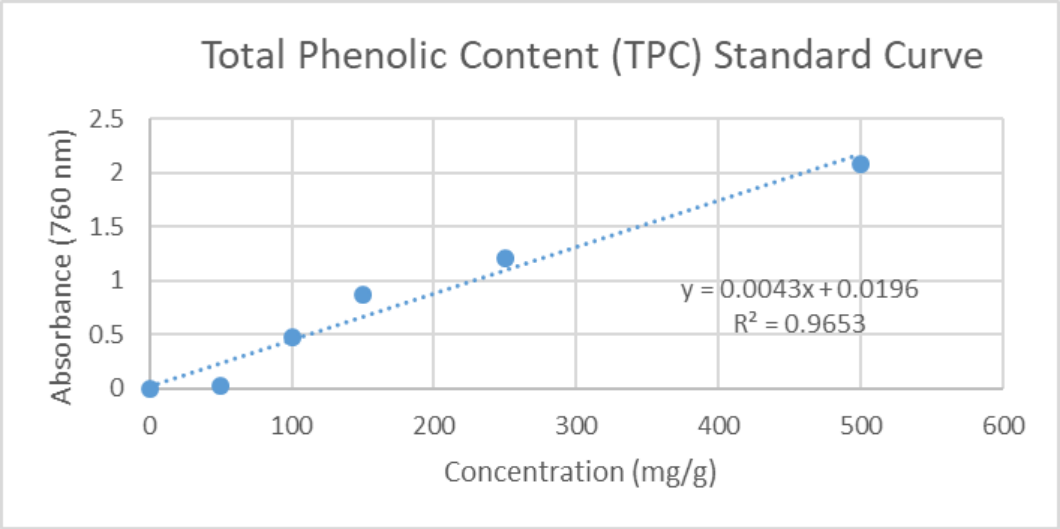

Figure 4: Gallic acid standard curve

In the experiments that have been done, three graphs were plotted: (1) TPC of stingless bee honey, (2) TPC of sea cucumber, and (3) TPC of stingless bee honey and sea cucumber combination. The total phenolic content was determined by Folin Ciocalteu reagent.

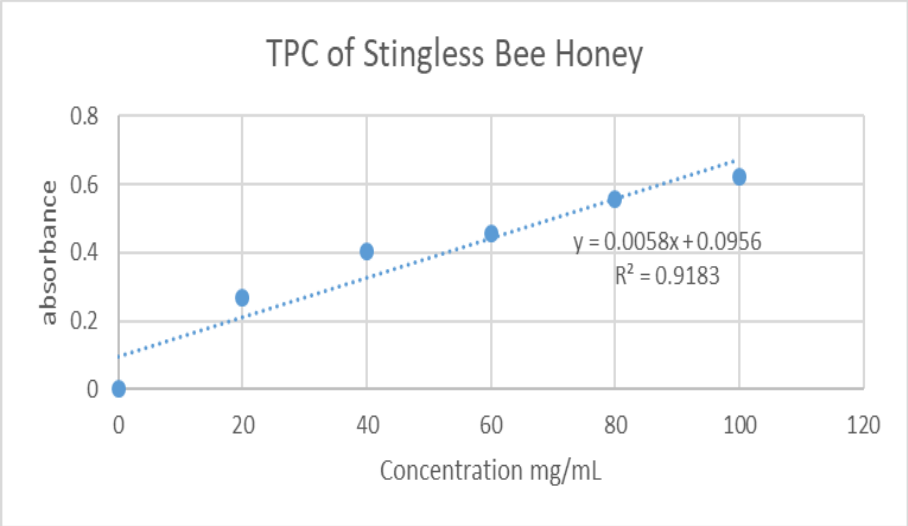

Figure 5: Total phenolic content of stingless bee honey

From Figure 5, the absorbance value increased linearly with increasing concentration of stingless bee honey, indicating increasing total phenolic content in increasing concentration of stingless bee honey. The linear regression reading from this stingless bee honey is close to the standard graph. This proves that it contains high phenol value.

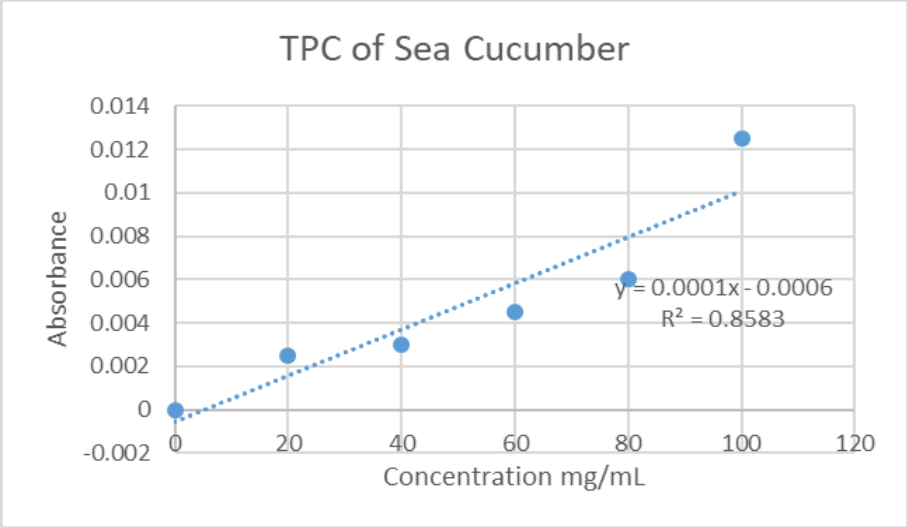

Figure 6: Total phenolic content of sea cucumber

Figure 6 above shows the absorbance value plotted against increasing concentration of sea cucumber extract. A linear increase was observed, with a difference in linear regression of 0.107. This is because the linear regression reading rate for standard curve Gallic acid in Figure 4 is 0.9653 and the reading rate for sea cucumber is 0.8583. Therefore, the total phenolic content of sea cucumber is slightly lower than standard curve gallic acid.

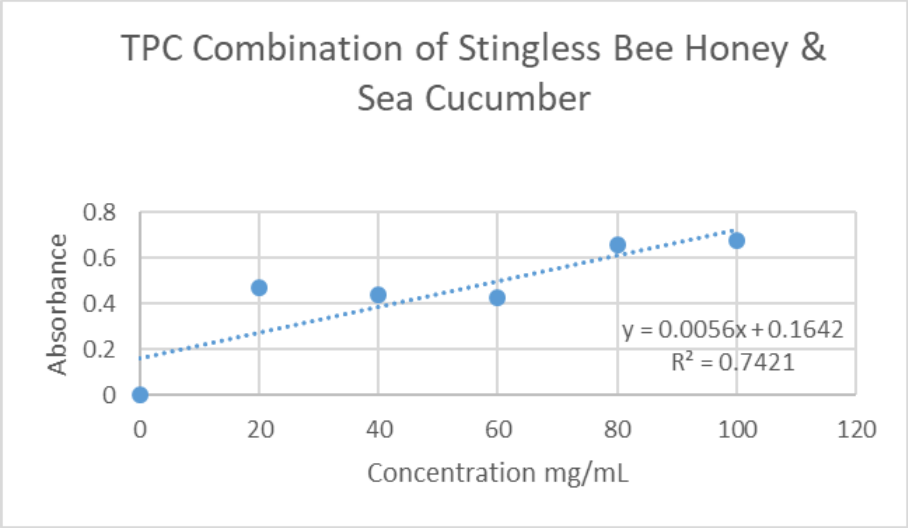

Figure 7: Total phenolic content of stingless bee honey & sea cucumber

Considering phenolic compounds are obtained from plants, the phenolic content of honey is heavily influenced by the nectar source picked by the bees. Figure 7 showed the total phenolic content for a mixture of stingless bee honey and sea cucumber. The linear regression reading rate is observed to be too low compared to the standard reading rate of Gallic acid and stingless bee honey by itself (Figure 5 and Figure 6 respectively).

Therefore, from the standard curve, the concentration of total phenolic in each sample was obtained. Total phenolic content from the mean and standard deviation of Stingless bee honey and Sea cucumber from this method were expressed as mg GAE / g as recorded in the Table 1.

Table 1: Total of phenolic content (mg GAE/g) and means with SD different sample

Stingless Bee Honey	**Mean ± SD**	**Total Phenolic Content (mg GAE/g)**
Sample 1	0.266 ± 0.0085	100 ± 0.0085
Sample 2	0.4015 ± 0.0148	400 ± 0.0148
Sample 3	0.4565 ± 0.0077	900 ± 0.0077
Sample 4	0.555 ± 0.0071	1600 ± 0.0071
Sample 5	0.6215 ± 0.0148	2500 ± 0.0148
Sea Cucumber	**Mean ± SD**	**Total Phenolic Content (mg GAE/g)**
Sample 1	0.0025 ± 0.0007	100 ± 0.0007
Sample 2	0.003 ± 0.0014	400 ± 0.0014
Sample 3	0.0045 ± 0.0021	900 ± 0.0021
Sample 4	0.006 ± 0.0028	1600 ± 0.0028
Sample 5	0.0125 ± 0.0035	2500 ± 0.0035
Stingless Bee Honey and Sea Cucumber	**Mean ± SD**	**Total Phenolic Content (mg GAE/g)**
Sample 1	0.4705 ± 0.0091	133.3 ± 0.0091
Sample 2	0.4395 ± 0.0035	266.7 ± 0.0035
Sample 3	0.427 ± 0.0056	400.0 ± 0.0056
Sample 4	0.6575 ± 0.0063	533.3 ± 0.0063
Sample 5	0.6775 ± 0.0077	666.7 ± 0.0077

3.2 Antioxidant activity of stingless bee honey and sea cucumber

At room temperature, the free radical assay (DPPH) is stable. When an antioxidant molecule is present, a violet coloured solution is converted into a colourless methanol solution [11]. The DPPH test provides a straightforward and quick technique to analyse antioxidants using spectrophotometry [11], making it ideal for evaluating many materials at the same time.

Figure 8 showed the increasing trendline for ascorbic acid standard. The calculated IC50 value is specifically used to determine the antioxidant category as either strong, very strong, moderate or weak. If the IC50 value obtained is less than 50 ppm, that means the antioxidant rate is very strong. Next, the IC50 value of 50 ppm is categorized as strong, moderate if the IC50 value is calculated to be in the range between 50-100 ppm, and categorized as very weak if the IC50 value is within the range of 100-200 ppm.

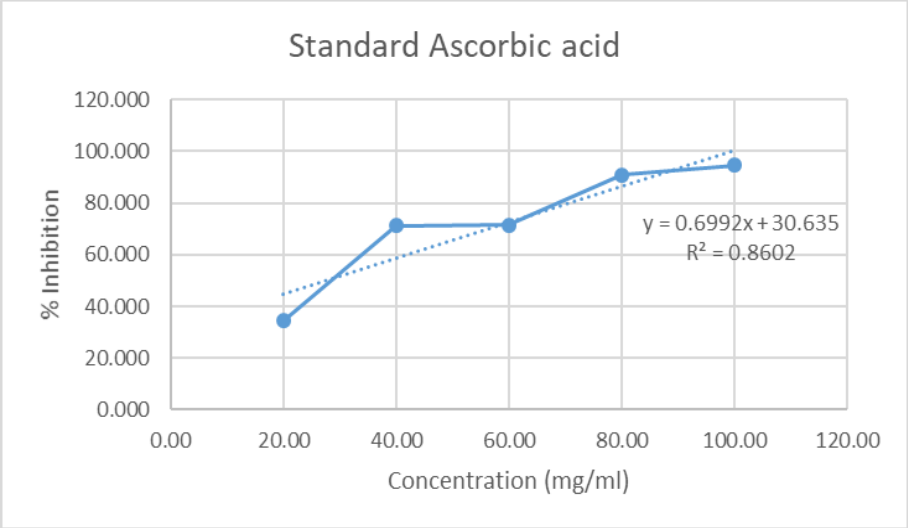

Figure 8: Standard calibration curve of ascorbic acid

Based on Figure 8, the IC50 value that has been calculated is 28.4137. This indicated that the antioxidant rate in standard ascorbic acid is very strong because its value is less than 50 ppm. The reading for linear regression is 0.8602 which indicates that it is not too far from the set value.

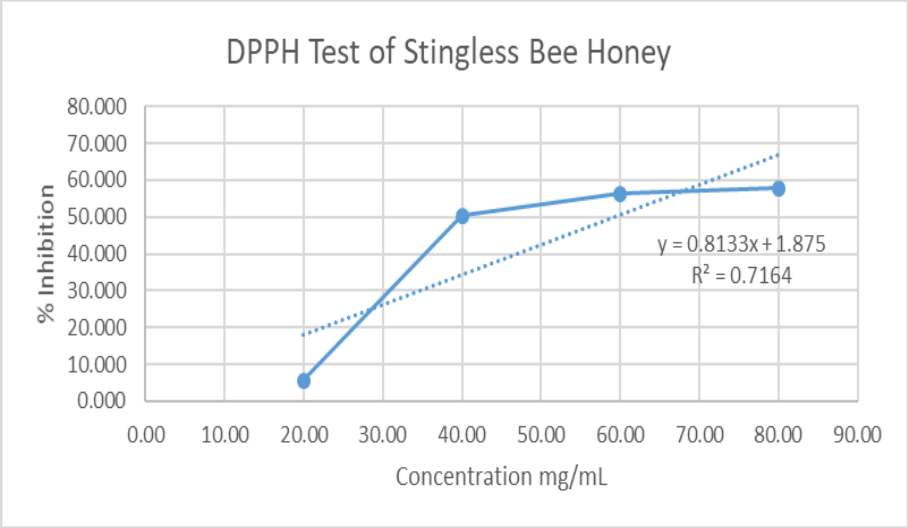

Figure 9: DPPH test of stingless bee honey

In the case of stingless bee honey sample, Figure 9 shows reading of linear regression of 0.7164 that is less than the standard calibration ascorbic acid. The calculated IC50 value is 45.57, which is still in the very strong category and also proves that stingless bee honey has a good antioxidant content.

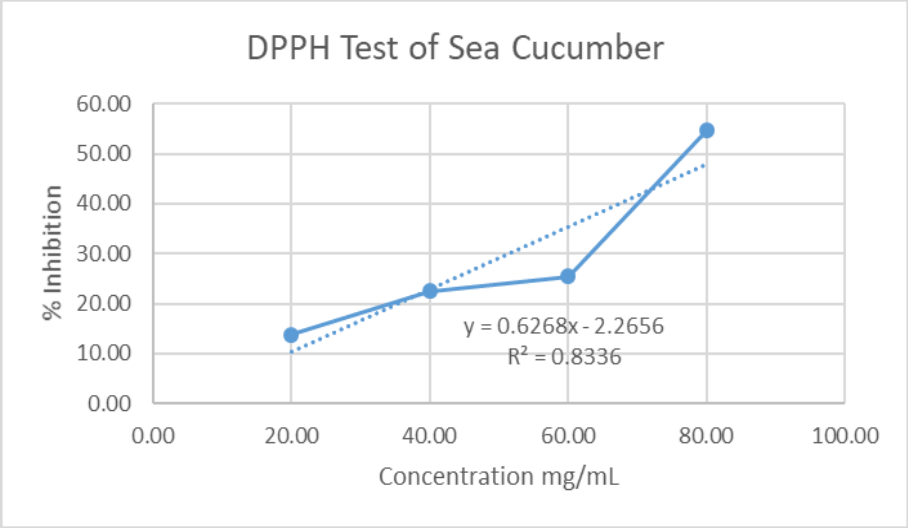

Figure 10: DPPH test of sea cucumber

DPPH test in Figure 10 shows a linear regression in sea cucumber very close to the standard ascorbic acid graph (Figure 8). The calculated IC50 value for sea cucumber is 76.84, which indicates that the antioxidant potential in the moderate category.

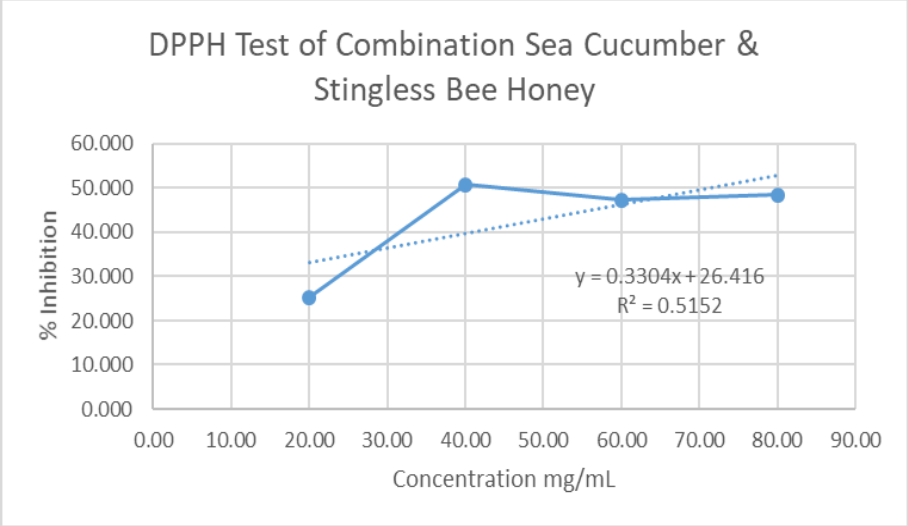

Figure 11: DPPH test of combination stingless bee honey & sea cucumber

Next, the DPPH test result for stingless bee honey and sea cucumber combination (Figure 11 above) showed the linear regression value of 0.5152. This is too far if compared to the standard reading of ascorbic acid. Abnormalities were found for antioxidant levels of stingless bee honey and sea cucumber combination. However, the calculated IC50 reading of the mixture is 44.48, which is also in the category of very strong antioxidant. The result indicated stingless bee honey has a better antioxidant property than sea cucumber extract, and in combination with sea cucumber, a good antioxidant mixture can be obtained.

Table 2: Comparison of Antioxidant, DPPH (%) of stingless bee honey and sea cucumber

Stingless Bee Honey	Mean ± SD	Percent (%) Inhibition	IC_{50}
Sample 1	0.556	57.879	45.57
Sample 2	0.577	56.288	45.57
Sample 3	0.6555	50.341	45.57

Sample 4	1.2455	5.644	45.57
Sea Cucumber	**Mean ± SD**	**Percent (%) Inhibition**	**IC_{50}**
Sample 1	0.581	54.61	76.84
Sample 2	0.955	25.39	76.84
Sample 3	0.992	22.50	76.84
Sample 4	1.1035	13.79	76.84
Stingless Bee Honey and Sea Cucumber	**Mean ± SD**	**Percent (%) Inhibition**	**IC_{50}**
Sample 1	0.428	48.434	44.48
Sample 2	0.4375	47.289	44.48
Sample 3	0.4085	50.783	44.48
Sample 4	0.6205	25.241	44.48

4. Conclusion

The study indicated that the combination of kelulut honey with sea cucumber extract showed a positive synergy, and a greater antioxidant activity can be obtained in combination than its individual antioxidant activity. From this experiment, we have managed to describe the antioxidant properties from combination of sea cucumber and stingless bee honey is in the very strong category compared to sea cucumber alone and stingless bee honey alone. Kelulut honey in Malaysia has been identified as an industry that can generate income and help boost the local economy. Malaysian kelulut honey has excellent potential to be marketed further if the products can be diversified further through combination with other natural product to produce a novel supplement combination. It is hoped that this study could be a starting point to investigate the potential of combining kelulut honey with sea cucumber extract as a novel functional supplement. This study could be the starting point for a balanced development of natural products between sea and land.

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References

[1] J. Tramper, C. Battershill, W. Brandenburg, G. Burgess, R. Hill, E. Luiten, … R. Wijffels, "What to do in marine biotechnology?" Biomolecular Engineering, 20(4-6), 467-471. doi:10.1016/s1389-0344(03)00077-7, (2003)

[2] G. Selvan, P., Ravikumar, S., A. Ramu, & P. Neelakandan, undefined. Asian Pacific Journal of Tropical Disease, 2, S724-S728. doi:10.1016/s2222-1808(12)60252-7, (2012)

[3] G. P. Webb, An overview of dietary supplements and functional foods. doi:10.1002/9781118788660.ch1, (2013)

[4] J. Quezada-Euán, J., Echazarreta, C. M., & R. J. Paxton, The distribution and range expansion of africanized honeybees (Apis mellifera) in the state of Yucatan, Mexico. Journal of Apicultural Research, 35(3-4), 85-95. doi:10.1080/00218839.1996.11100917, (1996)

[5] D. K. Ved, Demand and supply of medicinal plants in India (2008).

[6] B.H. Ridzwan, Sea Cucumbers, A Malaysian Heritage, 1st ed.; Research Centre of InternationalIslamic University Malaysia (IIUM): Kuala Lumpur Wilayah Persekutuan, Malaysia, 2007;pp. 1–15, 89–128.

[7] H.B. Yaacob, K.H. Kim, M.M. Shahimi, S.M.S. Jamalulail, Water extract of Stichopus sp. 1improves wound healing. J. Perubatan Univ. Kebangsaan Malays. 1994, 16, 19–29.

[8] W. M. W. Muda, J. K. Sundaram, & T. Z. Gen, Addressing malnutrition in Malaysia. Khazanah Research Institute. License: Creative Commons Attribution CC BY 3.0. (2019)

[9] A. Zieritz, S. Azam-Ali, A. Marriott, L., Ng, Q. N., Razak, N. A. A. B. A., & Watts, M. (2018). Biochemical composition of freshwater mussels in Malaysia: A neglected nutrient source for rural communities. Journal of Food Composition and Analysis, 72, 104-114.

[10] Y. Zhong, M. Ahmad Khan, F. Shahidi, "Compositional characteristics and antioxidant properties of fresh and processed sea cucumber (Cucumaria frondosa)". J. Agric. Food Chem.2007, 55, 1188–1192.

[11] Y. Ranneh, F. Ali, M. Zarei , A. M. Akim, H. Hamid, A., & H. Khazaai, "Malaysian stingless bee and Tualang honeys: A comparative characterization of total antioxidant capacity and phenolic profile using liquid chromatography-mass spectrometry". Journal of Food Science and Technology, 89, 1-9, (2017)