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Evaluation of protein content and antioxidant activity of edible bird's nest by various methods

Siti Sarah Zamri [1], Mahani Mahadi [1], Faizuan Abdullah [2,*], Achmad Syafiuddin [3], Tony Hadibarata [4,*]

[1]Faculty of Pharmacy, Cyberjaya University College of Medical Sciences, 63000 Cyberjaya, Selangor, Malaysia
[2]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia
[3]Department of Water and Environmental Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia
[4]Department of [4]Environmental Engineering, Faculty of Engineering and Science, Curtin University, 98009 Miri, Sarawak, Malaysia

*corresponding author e-mail address: ***faizuan@utm.my; hadibarata@curtin.edu.my*** | Scopus ID 55053616200; 16233109100

ABSTRACT

The aim of this study was to evaluate the protein content and antioxidant activity of instant and raw Edible bird's nest (EBN). This study was conducted using two types of EBNs, which are instant and raw samples. All EBN samples were extracted via three types of extraction method, namely, salt, alkaline, and hot extractions. Lowry's method was used to analyze protein content and the antioxidant activities were analyzed via free radical scavenging assay 1,1-diphenyl-2-pycrylhydrazyl (DPPH), total phenolic content (TPC) assay and ferric reducing antioxidant power (FRAP). This study showed that the highest protein was 2165.90 µg/mL obtained from raw EBN extracted via alkaline solution. In addition, it was also found that the protein concentration of raw EBN was higher compared to instant EBN for all types of extraction procedures. Results from antioxidant assay showed that there was no significant difference between DPPH and FRAP of both EBN. Moreover, TPC results showed that the there was no phenolic compounds detected via all extraction procedures.

Keywords: *Antioxidant activity; protein; edible bird*

1. INTRODUCTION

Recently, natural products such as plants, animals, microorganisms, and marine organisms are essential for a wide range of technologies including medicine, engineering, cosmetics, and pesticides [1-3]. Among these, there is a growing body of literature that recognizes the importance of natural products in medicines to alleviate and treat diseases [4-6]. Since the establishment of modern technology, it becomes possible to determine the pharmacology properties of natural products as a modern medicine. By advancing, the theoretical background, therapeutic principles, and mechanism, a clearer understanding of the active compounds of natural products has become possible.

Edible bird's nest (EBN) is the nest produced by different swiftlet species [7, 8]. It is usually used as a traditional medicine in the Chinese communities [9, 10]. EBN has been widely used as a healthy food and beauty enhancer due to its high nutritional values and therapeutic benefits [11]. In addition, EBN can be used to treat malnutrition, boosting immune system, improve metabolism, enhancing skin complexion and alleviating asthma as it is rich with water soluble proteins, inorganic salts, amino acids, carbohydrates, and iron [9, 12, 13]. This was also proven by the previous study which found that EBN can enhance one's complexion, alleviate asthma, and strengthen the immune system [14]. EBN has been traditionally used for its health promoting benefits such as antioxidant, anti-inflammatory and bone strengthen properties [15]. EBN has health beneficial effects which can exhibit anti-oxidative, antimicrobial and antihypertensive activities. In addition, oral administration of EBN extract may help to improve bone strength and calcium concentration [16].

The nests are built during breeding season and are made almost entirely from saliva that contain glutinous material produced by the bird's sublingual salivary glands [17-21]. It is produced by several different swiftlet species in the genus of *Aerodramus* and *Collocolia* and the nest is mainly built by male swiftlets [22]. More than 24 species of swiftlets are distributed around the world, but only a few produced nests that are edible [23]. There are 4 species of swiftlets amongst *Collocalia* found in Southeast Asian, which are. are *Collocalia unicolor*, *Collocalia maxima*, *Collocalia germanis*, and *Collocalia fuciphaga*. Besides *Collocalia* species, several species of the *Aerodramus* genus also produces edible bird's nest such as *Aerodramus fuciphagus*. Most of EBN produced in Malaysia are belong to *Aerodramus fuciphagus*.

Harvesting of the edible bird's nest for human consumption needs a hard work and dangerous for local collectors [23]. Most nests are built hundreds of feet on cave walls and it require use of temporary scaffolding made from bamboo and ironwood. While, for the preparation of EBN for market, the cleaning process takes 8 hours to finish 10 nests. The detail process of preparing EBN for market has been well explained [11]. There are a lot of impurities such as swiftlet dropping, twigs, and dirt in raw EBN. Thus, a series of cleaning progress need to be done to remove these impurities. Extract of EBN is usually obtained by extraction method using organic solvents such as methanol, chloroform, and DMSO which target the non-water soluble active compound. EBN is a thick and dried sample, thus mechanical method is required to breakdown the structure as protein extraction efficiency is strongly depends on the fineness of EBN powder. Aqueous extraction was

the easiest and commonly used method as EBN contains mostly water-soluble proteins [11]. The EBN extract was prepared by water extraction and stored at 4 ^{o}C in order to maintain the uniformity and quality of the extract.

Recently, researchers have shown an increased interest in EBN's protein content analysis. Half of the EBN composition consists of protein and the most abundant amino acids are serine, threonine, aspartic acid, glutamic acid, proline, and valine [24] . However, several researchers also found that the composition of EBN may be influenced by seasonal variation and even breeding sites. This result also proven in 2005, according two types of EBN which is the red "blood" nest and the white nest, the percentages of lipid, carbohydrate and protein were 0.14%–1.28%, 2.1%, 25.62%–27.26% and 62%–63%, respectively [23]. Moisture and fat content also found in EBN which is within the range of 7.0-9.34% and 0.05-0.09% respectively [23]. EBN is well known for source of protein and antioxidant. Several studies have been done for the identification of protein and antioxidant properties of EBN. However, most of the studies investigate the unprocessed EBN samples which can be collected directly from the cave and swiftlet's house. It is noted that study on the evaluation of protein and antioxidant activity using processed EBNs which are commercially available in the market is missing.

Thus, this study aims to investigate the protein content and the antioxidant activities of the processed EBN using several methods. Although various methods to estimate protein and antioxidant of materials have been established, no comprehensive work was dedicated to evaluating the suitability and practicality of existing methods for the analysis of raw and commercial EBNs. In the current contribution, the novelty of the study is that various established methods were tested and analyzed rather than a single method usually used for the analysis of protein content and the antioxidant activities. This study is useful for providing the most suitable method and condition for the protein and antioxidant evaluation particularly for EBN analysis.

2. MATERIALS AND METHODS

2.1. Materials

Materials used in this study were sodium carbonate (Na_2CO_3) (R&M Chemical, Malaysia), sodium hydroxide (NaOH) pellet (Bendosen, Malaysia), copper sulphate ($CuSO_4$) (R&M Chemical, Malaysia), potassium sodium tartarate ($KNaC_4H_4O_6{\cdot}4H_2O$) (R&M Chemical, Malaysia), Folin-ciocalteau reagent (R&M Chemicals, Malaysia), bovine serum albumin (BSA) (R&M Chemicals, Malaysia), sodium chloride (NaCl) (R&M Chemicals, Malaysia), 2,2-diphenyl-1-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany), methanol (R&M Chemicals, Malaysia), gallic acid ($C_7H_6O_5.H_2O$) (R&M Chemicals, Malaysia), ferrous(ii) sulphate ($FeSO_4$) (Bendosen, Malaysia), acetate buffer (R&M Chemicals, Malaysia), TPTZ (2,4,6-tri[2-pyridyl]-s-triazine) (Sigma-Aldrich, Germany), hydrochloric acid (HCl) (R&M Chemicals, Malaysia), and ferric chloride (FeCl3) (Bendosen, Malaysia).

2.2. Salt extraction

In this study, the salt extraction was carried out based on method proposed by the previous work [9]. In the preparation, the proteins were extracted by suspending 1 g of the grounded raw EBN in 100 ml of distilled water containing 0, 5, 15 and 25 % (w/v) NaCl. While for instant EBN sample, the proteins were extracted by suspending 2 g of the blended EBN sample in 100 ml of distilled water containing 0, 5, 15 and 25 % (w/v) NaCl. The suspensions were then shaken at 150 rpm for 3 hr. The suspension then was centrifuged at 4000 rpm for 10 minutes and the supernatant was collected and stored in 4 ^{o}C.

2.3. Alkaline extraction

The alkaline extraction was done using a method proposed by the previous study with a little adjustment [9] as a basis. The proteins were extracted by suspending 1 g of the grounded raw EBN in 100 ml of distilled water containing 0, 0.05, 0.5, 1.5 M NaOH. While for instant EBN sample, the proteins were extracted by suspending 2 g of the blended EBN sample in 100 ml of distilled water containing 0, 0.05, 0.5, 1.5 M NaOH. The suspensions were then shaken at 150 rpm for 3 hours then centrifuged at 4000 rpm for 10 minutes. Finally, obtained supernatant were stored in refrigerator at 4 ^{o}C.

2.4. Heat extraction

The heat extraction used in this work was based on method proposed by the previous work [9]. The extraction was initiated by suspending 1 g of grounded raw EBN in 100 ml of distilled water. For the extraction of instant EBN, 2 g of the sample was also suspended in 100 ml of distilled water. Next, the solution was boiled at 40°C, 60°C, 80°C, and 100 °C for 45 min. The suspension was then centrifuged at 4000 rpm for 10 minutes. Finally, the obtained supernatants were then stored in refrigerator at a temperature of 4 ^{o}C.

2.5. Protein analysis

The protein content analysis of the instant and raw EBN was estimated based on Lowry's method. Reagents A, B, C, and D were prepared as follows. Reagent A is a 2 g of Sodium Carbonate dissolved in 100 ml 0.1 N Sodium Hydroxide. Reagent B contains 0.5% of Copper Sulphate with 1% of Potassium Sodium Tartarate. Reagent C was prepared by mixing 100 ml of reagent A in 2 ml of reagent B. Next, reagent D was prepared by diluting 200 ml Folin-Ciocalteau reagent in 200 mL of 0.1 N NaOH. Reagent D must be prepared 5 minutes before use as these solutions are light sensitive. Then, 100 mg of BSA was dissolved in 100 ml of distilled water to prepare the stock standard. Next, 20 ml of the stock standard was diluted to 100 ml for working standard solution. One ml of this working standard solution contains 200 µg protein.

For the assay procedure, the working standard was diluted to series of concentration and work as the standard. Then, 5 ml of reagent C was added in all test tubes including test tubes containing 1 ml of EBN extracts, and the standard. Next, 0.5 ml of reagent D was added, and the test tube was incubated at room temperature for 30 min before measuring the absorbance at 660 nm against blank (distilled water). Standard curve was plotted and the protein concentration of EBN extract was determined.

2.6. DPPH free radical scavenging

In this procedure, 2,2-diphenyl-1-1-picrylhydrazyl (DPPH) assay was performed based on procedure proposed by the previous work [25]. The stock solution of 1 M DPPH was prepared in

methanol and kept at -20°C until analysis. Fresh 0.1 mM DPPH working solution was prepared by diluting 10 ml with 90 ml methanol. Gallic acid were used as a positive control. Fresh DPPH reagent was added into each test tube that containing the extracts and the control. The test tube was then incubated at ambient temperature in the dark for 40 minutes before measuring the absorbance using UV-VIS spectrophtometer at 540 nm.

2.7. Total phenolic content

In this evaluation, Folin-Ciocalteu colorimetric method was used for the analysis of total phenolic content [26]. Aliquot of 0.5 ml of EBN extract was mixed with 0.5 mL of Folin-Ciocalteu phenol reagent. After 5 min, 2 ml of 6 % of sodium carbonate was added and the mixture was allowed to stand at room temperature for 60 min. The absorbance of the mixture was measured at 650 nm. The total phenolic content was calculated from the calibration curve.

2.8. Ferric reducing antioxidant power

For anti-oxidant analysis, ferric reducing anti-oxidant power (FRAP) was employed based on the previous report [9]. This analysis was initiated by preparing aqueous ferrous sulphate solution (1 mM). It was then followed by transferring the solutions into cuvettes, which is positioned in a spectrophotometer (593 nm). It is noted that acetate buffer (300 mM, pH 3.6), HCl (40 mM), TPTZ (2,4,6-tri[2-pyridyl]-s-triazine) (10 mM), and ferric chloride (20 mM) were prepared and used for this analysis. Next, Mixing 200 mL of acetate buffer, 20 ml of TPTZ solution, 20 ml of $FeCl_3$ solution and 24 ml of distilled water was carried out to prepare FRAP reagent before incubating at 37°C for 4 min. Moreover, the mixture was then transferred to cuvette and placed in the spectrophotometer following by recording the absorbance of the mixture.

2.9. Statistical Analysis

Readings of the protein concentration, DPPH, FRAP, and TPC were expressed as means ± standard deviation. The analysis was done by using SPSS IBM Statistics 23. Results were analyzed using Independent Sample T-test. The test is considered significant if the P value were less than 0.05 ($P<0.05$).

3. RESULTS

3.1. Protein concentration

Figure 1 shows the BSA standard curve. It is noted that Figure 1 demonstrated that as the concentration of protein in BSA increased, and the absorption also increased. Since the graph produced a good determination coefficient ($R^2 = 0.8568$) between concentration and its absorption spectrophotometrically at 660 nm, and the equation for this graph: $y = 0.0013x + 0.015$ was used to calculate the protein concentration of each EBN extract.

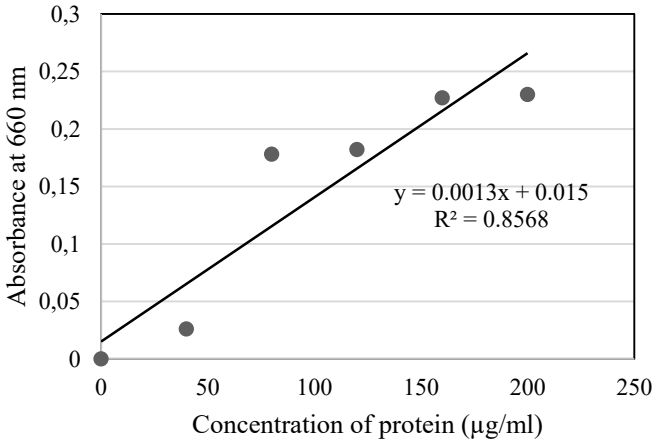

Figure 1. Concentration of protein (µg/ml) at different absorbance at 660 nm

The protein concentration of EBN extracts was determined for each type of EBN extraction, salt, alkaline, and hot extract. The protein concentration was calculated as mean ± SEM and tabulated in Table 1. Instant EBN in 5% concentration of NaCl shows the highest protein concentration as compared to others in instant EBN. However, raw EBN in 15% of NaCl shows the highest level of protein compared to others concentration. For the alkaline, concentration of the instant EBN in distilled water compared to other NaOH concentration has the highest concentration. While for the raw EBN, the highest protein concentration was obtained in mixing of raw EBN with 0.05 M NaOH. As the concentration of NaOH increases, the protein concentration decreases. For the hot extract, for both instant and raw EBN, the highest protein concentration was observed in the highest temperature. As the temperature increase, the protein concentration also increases. All variables were tested using kurtosis and skewness test.

Table 1. Protein concentration of instant and raw EBN extract

Extraction procedure		Type of EBN	
		Instant (µg/ml)	Raw (µg/ml)
S	EBN + DW	38.46 ± 2.22	127.44 ± 7.96
	EBN + 5 % NaCl	68.72 ± 6.22	224.87 ± 2.85
	EBN + 15 % NaCl	23.85 ± 1.33	416.41 ± 0.68
	EBN + 25 % NaCl	37.69 ± 2.03	326.92 ± 25.40
A	EBN + DW	59.49 ± 1.13	641.54 ± 30.00
	EBN + 0.05 M NaOH	41.28 ± 0.68	2165.90 ± 2.23
	EBN + 0.5 M NaOH	27.43 ± 0.68	1498.46 ± 15.08
	EBN + 1.5 M NaOH	23.33 ± 1.85	711.03 ± 41.30
H	40°C	59.49 ± 1.12	73.85 ± 0.77
	60°C	44.10 ± 1.12	116.67 ± 2.71
	80°C	99.74 ± 2.24	237.69 ± 2.77
	100°C	117.18 ± 1.12	382.05 ± 6.47

Note: S, A, and H refer to salt, alkaline, and hot extractions.

Table 2 lists statistical analysis of EBN extract. An independent sample t-test was conducted to compare the protein concentration of instant EBN and raw EBN at all concentration of the extract. There were significant differences between raw and instant EBN in all type of extract. It was found that the protein concentration may varies between raw and instant EBN. The protein concentration in raw EBN was higher than instant EBN in all types of extraction.

The results may suggest that, protein can be denatured during the processing or addition of other ingredients such as rock sugar and preservative into the instant EBN.

Table 2. Statistical analysis of protein concentration between raw and instant EBN

Extraction procedure		P-value
S	EBN + DW	0.001*
	EBN + 5 % NaCl	0.002*
	EBN + 15 % NaCl	0.001*
	EBN + 25 % NaCl	0.001*
A	EBN + DW	0.001*
	EBN + 0.05 M NaOH	0.001*
	EBN + 0.5 M NaOH	0.004*
	EBN + 1.5 M NaOH	0.001*
H	40°C	0.005*
	60°C	0.001*
	80°C	0.002*
	100°C	0.001*

In addition, the type of solvent extraction used may affect the protein extracted from the EBN. This research found that the alkaline solution which is sodium hydroxide may increase the solubility of the raw EBN. Thus, the highest protein concentration was detected in the alkaline solution. Alkaline extraction is one of the significant parameters that affects the protein extraction from EBN. Protein contains amino acid side chains chargers that can be soluble in solution at certain pH [9].

Another important finding was that the protein concentration increased in raw EBN with salt extraction method as increased of the concentration of NaCl. Moreover, the protein concentration extracted also increased in this condition. It was observed that, both raw and instant EBN was not soluble in the salt solution which suggest that salt condition is not the suitable condition to extract protein from the EBN as the protein extraction depends on the solubility behavior on the EBN. In hot condition, it was found that as the temperature increases, the protein concentration also increases for both raw and instant EBN. The protein solubility shows the highest value which is 3302.56 µg/ml when heated at 100 ^{o}C for 180 min. However, in this study, the highest protein concentration, 382.05 µg/ml was observed at temperature 100 ^{o}C. In addition, the EBN used in this study is commercialized processed EBN sample while the previous researcher used unprocessed EBN.

The purity of the sample also may influence the protein content of both EBN samples. For example, in commercially available EBN, there are three major adulterants which is karaya gum, read seaweed and tremella fungus and the composition ranges from 2-10%. Some adulterants which usually incorporated during the processing stages may reduce the overall protein content of the genuine EBN by as much as 1.1-6.2%. The nutrient contents of EBN also may be affected by seasonal variation and breeding sites due to EBN's are produces by swiftlets whose diet was composed of food from the local environment [24, 27]. In this study, the seasonal and breeding site of the raw and instant EBN sample was unknown.

3.2. DPPH Scavenging Activity

Free radical scavenging activity of EBN extracts was measured as percentage of inhibition following the trapping of unpaired electron of DPPH. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. The percentage of radical scavenging activity was calculated and tabulated in the Table 3. In addition, Table 4 summarizes the statistical analysis of DPPH scavenging activity between raw and instant EBN. In Table 3, the highest percentage of DPPH scavenging activity was obtained in raw EBN with 5% concentration of NaCl which is 58.29 ± 3.30 %. While the lowest percentage of DPPH in instant EBN with the highest concentration of NaCl (25%) which is 28.63 ± 9.60 %. It found that when concentration of NaCl increase, the DPPH scavenging activity in both raw and instant were decrease. For the alkaline extract, the highest DPPH scavenging activity obtained from raw EBN in 0.05 M NaOH. However, for the instant EBN, the highest scavenging activity was observed in 1.5 M NaOH. In hot extract, the highest DPPH scavenging activity for both instant and raw EBN were observed at 100 ^{o}C. As the temperature of extract was increase, the DPPH Scavenging activity also increase.

Table 3. Percentage of DPPH radical scavenging activity of instant and raw EBN

Extraction procedure		Type of EBN	
		Instant (%)	Raw (%)
S	EBN + DW	53.77 ± 3.27	41.01 ± 2.43
	EBN + 5 % NaCl	52.41 ± 2.43	58.29 ± 3.30
	EBN + 15 % NaCl	45.04 ± 2.69	47.47 ± 3.77
	EBN + 25 % NaCl	28.63 ± 9.60	38.25 ± 13.04
A	EBN + DW	49.10 ± 0.82	47.82 ± 4.21
	EBN + 0.05 M NaOH	50.46 ± 3.16	48.36 ± 3.14
	EBN + 0.5 M NaOH	49.91 ± 6.06	58.10 ± 11.61
	EBN + 1.5 M NaOH	51.72 ± 8.79	43.72 ± 4.03
H	40°C	40.75 ± 14.34	44.06 ± 9.67
	60°C	45.51 ± 6.84	47.41 ± 8.46
	80°C	46.09 ± 9.80	50.24 ± 6.17
	100°C	55.61 ± 2.96	51.76 ± 1.08

The result may vary due to different concentration of protein extracted from the EBN sample. Protein have good potential as antioxidant in foods because they can inhibit lipid oxidation through several pathways [28]. There was no significant differences of the DPPH scavenging activity between raw and instant EBN for all type of extract although the protein concentration of the raw EBN was higher than the instant EBN. This is might be due to added ingredient such as rock sugar and stabilizer in the instant EBN which may increase the antioxidant content of the instant EBN.

3.3. TPC Assay

Figure 2 shows the Gallic acid standard curve. Increasing the concentration of gallic can rise the absorption property. It is noted that the graph performed a good coefficient (R^2 = 0.913)

between the concentration and its absorption. Next, the equation was used to estimate the gallic acid of raw and instant EBN.

Table 4. Statistical analysis of DPPH scavenging activity between raw and instant EBN

Extraction procedure		P-value
S	EBN + DW	0.854
	EBN + 5 % NaCl	0.770
	EBN + 15 % NaCl	0.839
	EBN + 25 % NaCl	0.618
A	EBN + DW	0.100
	EBN + 0.05 M NaOH	0.514
	EBN + 0.5 M NaOH	0.807
	EBN + 1.5 M NaOH	0.964
H	40°C	0.390
	60°C	0.366
	80°C	0.498
	100°C	0.095

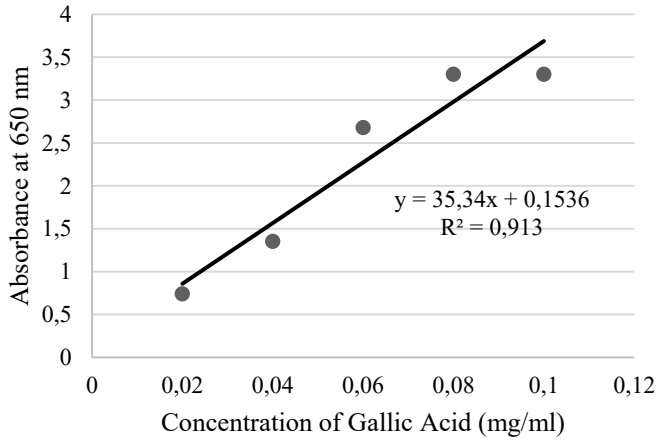

Figure 2. Absorbance at 650 nm against concentration of gallic acid (mg/ml)

Table 5 shows the total phenolic content (TPC) of instant and raw EBN in different type and concentration of extract. It was observed that smallest amount of phenolic compound was traced in the alkaline raw EBN extract. This result indicated that less amount of phenolic compounds were available in both instant and raw EBN. This is may be due to EBN was derived from saliva of the swiftlets and phenolic are the most abundant secondary metabolites of plant and broadly distributed in the plant kingdom [29].

Table 5. Total phenolic content (GAE/g) of instant and Raw EBN

Extraction procedure		Type of EBN	
		Raw (GAE/g)	Raw (GAE/g)
S	EBN + DW	Undetected	Undetected
	EBN + 5 % NaCl	<0.00	Undetected
	EBN + 15 % NaCl	Undetected	Undetected
	EBN + 25 % NaCl	Undetected	Undetected
A	EBN + DW	Undetected	<0.00
	EBN + 0.05 M NaOH	Undetected	0.04 ± 0.01
	EBN + 0.5 M NaOH	<0.00	0.08 ± 0.01
	EBN + 1.5 M NaOH	<0.00	0.06 ± 0.02
H	40°C	Undetected	Undetected
	60°C	Undetected	Undetected
	80°C	Undetected	<0.00
	100°C	Undetected	<0.00

3.4. FRAP between Raw and Instant EBN

Figure 3 shows the standard curve for the FRAP assay. The graph provided a good relation by providing R^2 = 0.8471. The equation was then used for the estimation of the ascorbic acid of raw and instant EBN.

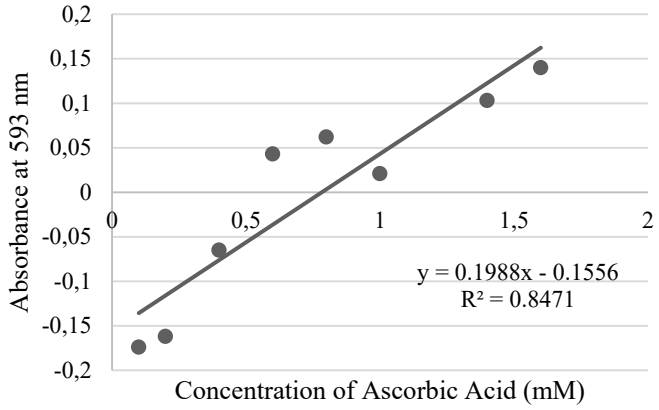

Figure 3. Concentration of ascorbic acid (mM) against absorbance 593 nm

Table 6 shows the FRAP (mM/g EBN) of instant and raw EBN in different type and concentration of extract. In the salt extract, highest FRAP value was observed in 15% NaCl and 5% NaCl for instant and raw EBN respectively. For the alkaline extract, both of EBN has the highest antioxidant activity in 0.05 M NaOH. In hot extract, for both instant and raw EBN, it was observed that the highest antioxidant activity is when heated at 100 ^{o}C. In this study, ascorbic acid is used as the standard, to compare with the absorbance of the EBN extract.

It was found that type of extract may affect the antioxidant power. It was observed that the FRAP value was the highest in alkaline solution for both raw and instant EBN. This is possibly due to the hydrolyzed proteins that exposed more amino acids in the solution [9]. For the hot extraction, it was observed that for both instant and raw EBN, as the temperature was increases, the FRAP value also increases and the highest antioxidant power which are 0.81 mg AAE/g and 1.06 mg AAE/g EBN was observed when instant and raw EBN sample was boiled at 100 ^{o}C respectively.

Table 6. FRAP (AAE mM/g sample) of instant and raw EBN

Extraction procedure		Type of EBN	
		Raw (mM/g)	Raw (mM/g)
S	EBN + DW	1.09 ± 0.14	1.07 ± 0.27
	EBN + 5 % NaCl	0.63 ± 0.05	1.81 ± 0.24
	EBN + 15 % NaCl	2.61 ± 0.01	0.75 ± 0.00
	EBN + 25 % NaCl	1.07 ± 0.02	0.38 ± 0.01
A	EBN + DW	1.93 ± 0.16	1.11 ± 0.24
	EBN + 0.05 M NaOH	3.41 ± 0.34	2.23 ± 0.05
	EBN + 0.5 M NaOH	3.32 ± 0.30	5.13 ± 0.02
	EBN + 1.5 M NaOH	1.96 ± 0.18	1.95 ± 0.02
H	40°C	0.43 ± 0.01	0.82 ± 0.02
	60°C	0.68 ± 0.09	0.80 ± 0.00
	80°C	0.73 ± 0.03	0.94 ± 0.02
	100°C	0.81 ± 0.00	1.06 ± 0.00

Based on Table 7, this study also found that there was no significance difference of FRAP value between the raw and instant EBN which suggest that the antioxidant power is similar for both type of EBN although there was a significance difference of the protein concentration between them. This is might be due to added ingredient such as rock sugar and stabilizer in the instant EBN which may increase the antioxidant content of the instant EBN.

Table 7. Statistical analysis of FRAP between raw and instant EBN

Extraction procedure		P-value
S	EBN + DW	0.592
	EBN + 5 % NaCl	0.006*
	EBN + 15 % NaCl	0.743
	EBN + 25 % NaCl	0.218
A	EBN + DW	0.238
	EBN + 0.05 M NaOH	0.054
	EBN + 0.5 M NaOH	0.041*
	EBN + 1.5 M NaOH	0.310
H	40°C	0.069
	60°C	0.178
	80°C	0.186
	100°C	0.062

4. CONCLUSIONS

The aim of this study was to evaluate the protein content and antioxidant activity of EBNs. This study revealed that both raw and instant EBNs that are available in the local market contained significant amount of protein. The raw EBN has higher protein content compared to the instant EBN. It was also approved that there were no significant differences of the antioxidant activity based on results obtained from assays of DPPH scavenging activity and FRAP. Salt, alkaline, and hot extraction methods affected the protein extraction. The highest protein concentration was obtained when the alkaline extraction was used. Similar finding was also found for the antioxidant content analysis. The highest antioxidant content can be obtained by using alkaline extraction compared to other employed methods. Moreover, finding from this work exhibited that the there was no phenolic compounds detected using the employed extraction.

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6. ACKNOWLEDGEMENTS

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