International Food Research Journal 22(5): 1953-1960 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antioxidative and acetylcholinesterase inhibitor potential of selected honey of Sabah, Malaysian Borneo

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Article history

Received: 29 September 2014
Received in revised form: 19 January 2015
Accepted: 29 January 2015

Keywords

Wild honey
Commercial honey
Antioxidant
Anti-cholinesterase

Abstract

Honey is a sweet substance that can be obtain from flower nectar and secretes through transformations process by honey bees. The aim of this study was to compare the antioxidant and anti-cholinesterase activities of 4 wild honey (wild honey produced by different species of bees: *Apis cerana, Apis andreniformis, Apis koschevnikovi* and *Apis nuluensis*) collected from the secondary forest and 6 commercial (young and old Mangrove, young and old Upper mountain, Tropical, Potiukan) honey of Sabah, Malaysian Borneo. Antioxidant activities were determined using FRAP (Ferric Reducing Antioxidant Power), DPPH free radical scavenging and ABTS decolourization assays. Acetylcholinesterase inhibition effect was determined by enzyme inhibition method. The results of this study showed that wild honey produced by *A.cerana* collected from the *Acacia* tree extracted using 80% methanol displayed the highest DPPH free radical scavenging activity and ABTS decolorization assays. Whereas wild honey produced by *A.nuluensis* collected from the Chestnut tree extracted using 80% methanol displayed the highest FRAP activity. The wild honey produced by *A. nuluensis* also displayed the highest acetylcholinesterase properties as the antioxidant compounds contributed to the inhibitory properties of the enzyme. Manuka honey (produced from tea tree) found to be higher in antioxidant and acetylcholinesterase properties as compared to all other samples. The same trend of bioactivity was also observed in absolute methanol extracts. Strong positive correlation was found between antioxidant and acetylcholinesterase activity. Therefore, honey has the potential as natural antioxidant and acetylcholinesterase inhibition sources with promising potential benefits to human's health.

Introduction

Honey has been used as natural medicine since long time ago (Adewumi and Ogunjinmi, 2011). Honey is one of the natural products from flower nectar that contain a rich source of phenolic bioactive molecules and serves as natural antioxidants (Irina *et al*., 2010). Honey is effective in combating certain chronic diseases including heart disease, cancer, autism, immune system performance, asthma, infected wounds, neurological diseases and skin problems (Molan *et al*., 2009).

The presence of free radicals such as Reactive Oxygen Species (ROS) like oxygen and peroxides ion in the cells may be harmful and lead to biological complications such as carcinogenesis, mutagenesis, atherosclerosis and many other health problems related to advancing aging (Cadenas and Davies, 2000). The presence of small molecule of antioxidants either naturally or synthetically assist in preventing ROS damage by scavenging the free radicals (Madsen and Bertelsen, 1995). However the use for natural antioxidants are more desirable as free radicals generated naturally as byproduct of the body metabolism. Therefore, replacements of synthetic antioxidants which possess carcinogenic activity with the naturally occurring antioxidants in plants such as phenolic compounds, nitrogen compounds, carotenoids as well as ascorbic acids are necessary to keep stabilize, minimum damage and safer (Madsen and Bertelsen, 1995). Honey has relatively linked as a source of antioxidants with the use in food preservations (Irina *et al.*, 2010), such as enzymatic browning of fruits and vegetables (Chen *et al*., 2000) and lipid oxidation in meat (Nagai *et al.*, 2006).

In Sabah, there are five types of honey bees that have been identified under the family of *Apidae* including *Apis dorsata, Apis cerana* (black honey bee), *Apis koschevnikovi* (red honey bee), *Apis nuluensis* (mountain honey bee) and *Apis andreniformis* (dwarf honey bee). *Apis dorsata* or known as the giant honeybee has the largest size

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while *Apis andreniformis* has the smallest size (Nikolaus *et al*., 2010). These honey bee can be differentiated by the type of nesting sites, body size of worker bees and several other characteristics. Their colonies are preferably to be found in huge tall trees such as *Koompassia excelsa, Acacia* and rubber. Thus, the botanical resources, seasonal effect and environmental factors of the honey bee colonies contribute significantly to the antioxidant properties of honey which is important in combating damage caused by oxidizing agents such as heart disease, cancer, anti-inflammatory, antithrombotic and immune system performance (Gheldof *et al*., 2001).

In spite of numerous research on the antioxidant property of honey globally, the evaluation of the in vitro antioxidant and anti-cholinesterase activity of honey and its relationship to active biological activities have not been fully explained. Thus the aim of this study is to evaluate the antioxidant activities of selected honey of Sabah, Malaysia that are potentially could be linked as antioxidants and anti-cholinesterase inhibitor agents.

Materials and Methods

Honey samples

There are two types of honey (wild and commercial) used in this experiment (Table 1). The wild honey produced by selected honey bee species was collected from Agriculture Research Station (ARS), Tenom, Sabah (wild honey produced by *A.cerana, A.koschevnikovi, A.andreniformis* which collected from secondary forest); Mesilau (wild honey produced by *A.nuluensis* collected from secondary forest) and Kg.Bundu Tuhan, Ranau, Sabah (wild honey produced by *A.cerana* collected from secondary forest) (Table 1). Meanwhile for the commercial honeys, they were supplied by the Sabah Raw Honey (SRH) company (i.e, younger and older Mangrove honey, younger and older Upper Mountain honey) and the Rural Development Corporation (RDC) agency (Potiukan and Tropical honey). Manuka honey (from Australia) and sugar analogue (consist of 40% fructose, 30% glucose, 10% maltose and 20% water) were used as positive and negative controls for this study.

Extraction

Samples (0.7 g) were extracted for 1 h with solvents (80% methanol and absolute methanol) with a ratio of 1:10 at room temperature on a rotary evaporator (Mervat and El-Gendy, 2010). The extract was decanted into a 15 ml vial and store at -4^{0}C for analysis of total antioxidant activity and anti-cholinesterase inhibitors assay.

DPPH free-radical scavenging assay

The scavenging activity of the extract was measured by using 1,1-diphenyl-2-pycrylhydrazyl (DPPH) as a free radical model and a method adapted from Magalhaes *et al*., (2006). An aliquot (300 µl) of samples or control (80% methanol or distilled water) was mixed with 3.0 ml of 500 µM (DPPH) in absolute ethanol. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min in the dark. Absorbance of the mixture was measured spectrophotometrically at 517 nm, and the free-radical scavenging activity was calculated as follows:

Scavenging effect (%) = [1- {absorbance of sample/ absorbance of control}] x 100

The scavenging percentage of all samples was plotted. The final result was expressed as the concentration of sample producing percentage scavenging of the DPPH radical (µg/m).

FRAP (Ferric reducing/antioxidant power) assay

This procedure was conducted according to Benzie and Strain (1996) with slight modification. The working FRAP reagent was produced by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s- triazine (TPTZ) solution and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 ratio prior to use and heated to 37°C in a water bath. A total of 3.0 ml FRAP reagent was added to a test tube and a blank reading was taken at 593 nm using spectrophotometer. A total of 100 µl of the extracts and 300 µl of distilled water were added to the cuvette. A second reading at 593 nm was performed after 90 min of incubation at 37°C in water bath right after the addition of the sample to the FRAP reagent. The changes in absorbance after 90 min from initial blank reading were compared with standard curve. Standard of known Fe (II) concentrations were run using several concentrations between 0 and 1000 µg/ml. A standard curve was then plotted. The final result was expressed as the concentration of antioxidant having a ferric reducing ability in 1 gram of sample (µM/g).

ABTS decolorization assay

The ABTS decolorization assay was carried out according to the method described by Re *et al.* (1999), with slight modification. Working ABTS solution (7 mM) and potassium persulfate (2.45 mM) were added into a beaker, and the mixture allowed standing for 15 hours in the dark to generate an ABTS free radical cation solution. The mixture was diluted

Table 1. Type of honey, location and type of tree data collection for the wild honey collected from Sabah, Malaysian Borneo

Type of honey	Location	Type of tree
Wild *A.cerana*	Agriculture Research Station (ARS), Tenom,Sabah.	*Acacia*
	Kg.Bundu Tuhan, Ranau, Sabah.	*Acacia*, Rubber
Wild *A.andreniformis*	Agriculture Research Station (ARS), Tenom,Sabah.	*Acacia*
Wild *A.nuluensis*	Sub-station Mesilau, Ranau, Sabah.	Chestnut
Wild *A.koschevnikovi*	Agriculture Research Station (ARS), Tenom,Sabah.	*Acacia*, Rubber

with 80% methanol or distilled water in order to obtain absorbance of 0.7±0.2 units at 734 nm. Then, 2 ml of this working ABTS free radical cation solution was added 200 µl of solvent exracts (80% or absolute methanol) the mixture vortexed for 45 seconds, and the resulting absorbance value read at 734nm using microtiter plate reader. Standards of ascorbic acid in the concentration range 0 to 100 µg/ml were run with the test samples, from which a standard curve was plotted. The final result was expressed as mg ascorbic acid equivalent antioxidant capacity in 1 g of sample (mg AEAC/g).

Anti-cholinesterase inhibition assay

The anticholinesterase inhibition assay was done according to the previous method described by Atta ur-Rahman *et al.*, (2005). 250µl phosphate buffer 200 mM pH 7.7 that contained sample extracts were preincubated. 80 µl of DTNB (3.96 mg of DTNB and 1.5 mg sodium bicarbonate dissolved in 10 ml phosphate buffer pH 7.7) and 10 µl enzyme (2 U/ml) were added to the mixture. The mixture was incubated for 5 minutes at 25ºC. 15 µl of the substrate that contained 10.85 mg acetylcthiocholine iodide in 5 ml phosphate buffer were added and incubated for 5 minutes. The colour develop have been measured by using microwell plate reader at 412 nm. The percent of inhibition was calculated by using the formula below.

$$\% \text{ inhibition} = \frac{\text{control absorbance} - \text{sample/ tested absorbance x100\%}}{\text{control absorbance}}$$

Statistical analyses

All experiments were carried out in 3 replicates in 3 independent experiments. The results were presented as mean ± standard deviation (SD). Pearson's coefficient correlation analysis was done to correlate between the antioxidants and anti-cholinesterase inhibitors potential in the samples. The level of statistical significance was set at $p \leq 0.05$.

Results and Discussion

Scavenging activity on 2,2-diphenyl-2-picrylhydrazyl radical

The utilization of selected wild and commercial honey of Sabah, Malaysia in this research was due to the strong evidence of honey and its medicinal potential. Therefore, the quality of honey is mainly depended on its sensorial, chemical, physical and substrate characteristics (Susana *et al*., 2010). The electron or hydrogen donating process by antioxidant resulting in changes of 2,2-diphenyl-1-picrylhydrazyl (DPPH) purple colour into a pale yellow hydrazine (Alvarez-Suarez *et al*., 2010). The stable DPPH free radical has the ability to be undisturbed by certain side reactions such as enzyme inhibition and metal ion chelation (Leticia *et al*., 2008) compared to unstable free radical like hydroxyl group. The scavenging activity of the sample towards the free radicals can be measured spectrophotometrically at 518 nm. Therefore, the faster decreases of absorbance, the stronger the antioxidant activity of the extract.

For the 80% methanolic extracts, honey produced by wild *A.cerana* bee displayed the highest radical scavenging activity as compared to other samples (Table 2). The scavenging activity of the absolute methanol extract displayed the same trend with higher scavenging activity observed in wild honey produced by *A.cerana* bee as compared to other samples (Table 2). The results for 80% methanol extracts displayed relatively higher free radical scavenging activity as compared to absolute methanol extracts. Methanol-

Table 2. Antioxidant properties of extracts of selected honey of Sabah, Malaysia evaluates by different type parameter of antioxidant assays

Type of honey	DPPH Assay (%)[1]	FRAP Assay[2]	ABTS Assay[3]
80% methanol			
Wild *A.cerana*	83.68 ± 0.18	36.87 ± 0.14	8.04 ± 0.23
Wild *A.nuluensis*	80.03 ± 0.15	43.87 ± 1.30	7.44 ± 0.29
Wild *A.andreniformis*	78.24 ± 0.28	29.01 ± 0.24	7.04 ± 0.36
Wild A.koschevnikovi	77.02 ± 0.30	23.16 ± 0.42	7.76 ± 0.04
Old Upper Mountain	73.32 ± 0.28	25.68 ± 0.14	6.52 ± 0.23
Old Mangrove	69.88 ± 0.45	22.66 ± 0.03	6.29 ± 0.32
Young Mangrove	65.22 ± 0.28	20.03 ± 0.04	5.87 ± 0.0
Young Upper Mountain	62.49 ± 0.21	18.52 ± 0.08	5.55 ± 0.01
Potiukan	56.64 ± 0.30	13.12 ± 0.16	4.78 ± 0.04
Tropical	53.16 ± 0.21	7.72 ± 0.04	4.04 ± 0.08
Manuka	84.14± 0.25	46.73 ± 0.06	8.18 ± 0.28
Absolute Methanol			
Wild *A.cerana*	80.02 ± 0.28	32.28 ± 0.04	7.27 ± 0.23
Wild *A.nuluensis*	77.16 ± 0.15	34.88 ± 0.30	5.98 ± 0.04
Wild *A.andreniformis*	76.27 ± 0.28	27.87 ± 0.03	5.83 ± 0.36
Wild *A.koschevnikovi*	74.46 ± 0.30	19.88 ± 0.26	6.16 ± 0.04
Old Upper Mountain	69.29 ± 0.28	22.72 ± 0.04	5.37 ± 0.23
Old Mangrove	67.88 ± 0.35	17.81 ± 0.02	5.31 ± 0.44
Young Mangrove	63.11 ± 0.18	16.31 ± 0.04	4.98 ± 0.01
Young Upper Mountain	60.52 ± 0.48	15.11 ± 0.02	4.79 ± 0.09
Potiukan	51.04 ± 0.30	10.52 ± 0.06	4.37 ± 0.05
Tropical	48.21 ± 0.21	6.79 ± 0.02	3.64 ± 0.23
Manuka	81.53 ± 0.25	42.59 ± 0.29	7.47 ± 0.09

Values are presented as mean ± standard deviation (SD).
[1] DPPH free radical scavenging activity was expressed as % of scavenging (µg/ml).
[2] FRAP was expressed as mM ferric reduction to ferrous in 1 g of dry sample.
[3]ABTS free radical scavenging activity was expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC) in 1 g of dry sample.

water mixture solvent is preferable due to high polarity, solubility and greater efficacy towards the extraction of polar phytochemicals such as phenolic and flavonoid (Farooq *et al*.,2010) while polar solvent like methanol is often recommended for the extraction of phenolic antioxidants components from a plant material (Peschel *et al.*, 2006).

Most of honey produced by wild honey bees from this study, displayed higher antioxidant activity as compared to commercial honey due to availability and access of honey bee to floral source (Gheldof *et al.*, 2003). The location of wild honey bee in secondary forests helps to assure unlimited botanical resources compared to agriculture botanical garden (Chye and Ng, 2008).

Ferric reduction based on FRAP assay

FRAP is one of the method used to examine the antioxidant activity in the samples. FRAP assay is suitable for determination antioxidant in food and beverages (Irina *et al*., 2010). The presence of reducers (antioxidants) causes the reduction of ferricyanide complex to ferrous (II) form in low pH at 593 nm (Gheldof *et al*., 2003). The reducing ability of polyphenols by FRAP assay depends on the degree of hydroxylation and the extent of conjugation of the phenolic compounds (Jasna *et al*.,2007). This type of reaction is non-specific reaction, simple, rapid and directs (Aljadi and Kamaruddin, 2004).

The reducing ability of wild honey was higher than commercial honey in 80% methanol extract, with honey produced by wild *A.nuluensis* (which the nectar collected from Chestnut tree in secondary forest) displayed strong reducing capacity followed by honey produced by wild *A.cerana*, wild *A.andreniformis* honey, old Upper Mountain honey, wild *A.koschevnikovi* honey, old Mangrove honey, young Mangrove honey, young Upper Mountain honey, Potiukan honey and Tropical honey orderly. The same trend also observed in the absolute methanol extract (Table 2).

Chye and Ng (2008) reported that wild Malaysian honey has high antioxidant activity. The wider botanical origin proved to provide abundant

Table 3. The anti-cholinesterase activity (%) of selected honey of Sabah, Malaysia in both 80% and absolute methanol extracts

Type of honey	Anti-Cholinesterase activity (%)[1]	Type of honey	Anti-Cholinesterase activity (%)[1]
80% methanol		Absolute Methanol	
Wild *A.cerana*	73.22 ± 0.10%	Wild *A.cerana*	61.28 ± 0.08%
Wild *A.nuluensis*	75.21 ± 0.14%	Wild *A.nuluensis*	64.51 ± 0.17%
Wild *A.andreniformis*	70.29 ± 0.24%	Wild *A.andreniformis*	60.22 ± 0.27%
Wild *A.koschevnikovi*	69.02 ± 0.06%	Wild *A.koschevnikovi*	58.98 ± 0.09%
Old Upper Mountain	66.29 ± 0.18%	Old Upper Mountain	52.22 ± 0.05%
Old Mangrove	64.29 ± 0.12%	Old Mangrove	46.88 ± 0.05%
Young Mangrove	61.29 ± 0.22%	Young Mangrove	46.89 ± 0.12%
Young Upper Mountain	61.00 ± 0.08%	Young Upper Mountain	45.09 ± 0.08%
Potiukan	49.21 ± 0.32%	Potiukan	39.12 ± 0.10%
Tropical	47.02 ± 0.09%	Tropical	38.91 ± 0.11%
Manuka	76.91 ± 0.21%	Manuka	71.22 ± 0.22%

Values are presented as mean ± standard deviation (SD).
[1] Anti-cholinesterase activity was expressed as percentage (%).

amount of pollen nectar (*Acacia* and rubber trees) which is suspected to contribute to the higher reducing capabilities in the honey produced by wild *A.cerana* in this study. Higher antioxidant properties in honey produced by wild *A.cerana* are in line with the study by Gheldof *et al.* (2003), which showed higher reducing activity in wild Buckwheat honey as compared to commercial honey type. Therefore, the ability of honey bee to access and collects the nectar and also geographical habitat plays apart in differences between the antioxidants activity of the samples (Huang *et al*., 2005; Yap *et al*., 2015). Meanwhile maturity of the honey also affected their quality as the younger and older of honey contained different level of physicochemical and phytochemical contents such as younger and older Mangrove and Upper Mountain honey (Taormina *et al*., 2001).

ABTS scavenging assay

Proton radical (ABTS) has the ability to stable in aqueous or in organic solvents due to the hydrophilic and lipophilic nature of the compounds in the samples and measured at 734 nm (Arnao, 2000). This was supported by the stereo selectivity of the radicals and solubility of the extracts plays apart in quench against different radicals (Yu *et al*., 2002). The chemistry involves the direct generation of the ABTS radical monocation without intermediary radical. Meanwhile, the radical cation is preformed rather than generation of radical in the presence of antioxidant (Adedapo *et al.*, 2009). Hence, the results obtained may not directly comparable with those obtained using the original TEAC assay.

For both extracts, the higher scavenging activity was found in the honey produced by wild *A.cerana* (which the nectar collected from *Acacia* tree in secondary forest), followed by wild *A.Koschevnikovi* honey, wild *A.nuluensis* honey, wild *A.andreniformis* honey, old Upper Mountain honey, old Mangrove honey, young Mangrove honey, young Upper Mountain honey, Potiukan honey and Tropical honey orderly (Table 2). The wild honey was found to exhibit higher ABTS scavenging activity compared to commercial honey which in agreement with previous study (Chye and Ng, 2008). Besides, the present result also supported by previous study where wild honey contained numerous amounts of byproducts resources and minerals as compared to commercial honey type (Wang *et al.*, 2004; Yap and Abu Bakar, 2014).

Anti-cholinesterase activity

Acetylcholinesterase (AChE) is a specific substrate enzyme that degraded neurotransmitter acetylcholine in nerve synapses (Penpan *et al*., 2007). The enzyme activity is determined through the steadily increases of yellow colour formed through thiocholine reactions with dithiobisnitrobenzoate ion (Ellman *et al*., 1961). The changes in the yellow colour formed, indicates the ability of the samples to become acetylcholinesterase inhibitors potential (Vinutha *et al*., 2007).

The present study showed all of selected honey of Sabah, Malaysian Borneo has anti-cholinesterase effects when tested at concentration of 10-100 µg/ml. Most of the wild honey (wild honey produced by A.nuluensis which collected from secondary forest) as compared to the commercial honey (the lowest by

Tropical honey) displayed the highest percentage of inhibition against the enzyme reaction. The highest anti-cholinesterase activity was observed in honey produced by wild *A.nuluensis* collected at secondary forest of Mesilau, Ranau in both extracts (80% and absolute methanol) with the values of 75.21% and 64.51%; respectively. All samples extracted with 80% methanol solvent showed higher anti-cholinesterase activity (47-75%) as compared to absolute methanol extract (38-64%). These results were comparable with previous study on Turkish medicinal plants whereby it exhibited wide range of anti-cholinesterase activity (50-93%) (Orhan *et al.*, 2004).

The ability of the sample to inhibit the acetylcholinesterase enzyme are in dose-dependent trend which the higher the concentration of the samples, the anti-cholinesterase of the sample also increase in both 80% methanolic (Figure 1) and absolute methanol extracts (Figure 2). This was supported with methanolic extracts contained some active compounds that exhibited the anti-cholinesterase activity (Houghton *et al*., 2006). However, it is also can be occurred due to synergistic effects of the compounds with their chemical structures such as alkaloids and flavonoids (Lee *et al*., 2011).

Relation between antioxidants and anti-cholinesterase

Previous studies showed the correlation between antioxidants and anti-cholinesterase activity in herbs and vegetables (Haque *et al.*, 2012). The correlation analysis was performed and showed that there is strongly positively correlation between the antioxidants parameter with anti-cholinesterase activity. FRAP, DPPH and ABTS resulted positively correlated with anti-cholinesterase activity with the values of ($r = 0.724$, $p<0.001$), ($r = 0.802$, $p<0.001$) , ($r = 0.811$, $p<0.001$); respectively.

The results of this study were in agreement with earlier previous study (Shapira *et al.*, 2001) which reported that 95% of acetylcholinesterase accounted in inhibitory activity of nurse and foragers honey bees brain, therefore it simultaneously correlated with the antioxidant activity presence among the samples tested. The presence of acetylcholinesterase in plant-insect particularly, serves as protection by paralyzing and killing insects of the non-adapted species (Jo *et al*., 2012).

Conclusion

From the results of this study, it can be concluded that wild honey displayed higher antioxidant and anti-cholinesterase activity as compared to commercial honey. Honey produced by wild *A.cerana* and *A.nuluensis* possess significant antioxidant and anti-cholinesterase activity that can be beneficial for human health.

Acknowledgements

We are indebted to Mr Herbert Lim from Agriculture Research Station (ARS), Tenom, Sabah, for the for the technical assistance; Institute for Tropical Biology and Conservation (ITBC); and School of Science and Technology (SST) University Malaysia Sabah, Malaysia for the use of the laboratory facilities and technical assistance.

References

Adedapo, A. A., Florence, O.J., Anthony, J.A. and Patrick, J.M. 2009. Antioxidantproperties of methanol extracts of the leaves and stems of *Celtis Africana*. Journal of Natural Products 3(1): 23-31.

Adewumi, A.A. and Ogunjinmi, A.A. 2011. The healing potential of honey and propolislotion on septic wounds. Asian Pacific Journal of Tropical Biomedicine 55(3): 40-46.

Aljadi, A.M. and Kamaruddin, M.Y. 2004. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honey. Food Chemistry 4(85): 513- 518.

Alvarez-Suarez, J.M., Tulipani, S., Diaz, D., Estevez, Y., Romandini, S., Giamperi, F.,Damiani, E., Astolfi, P., Bompadre, S. and Battino, M. 2010. Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. Food and Chemical Toxicology 48(21): 2490-2499.

Arnao, M.B. 2000. Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practice case. Trends in Food Science Technology 11: 419-421.

Atta-ur-Rahman., Choudary, M.I. and Thomsen, W.J. 2005. Bioassay techniques for drug development. Harwood Academic Publishers 2: 142-143.

Benzie, I.F. and Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP Assay. Analytical Biochemistry 239(1): 70-6.

Cadenas, E. and Davies, K.J. 2000. Mitochondrial free radical generation, oxidative stress, and aging. Free Radical Biology and Medicine 29(3): 222-30.

Chen, L., Mehta, A., Berenbaum, M., Zangerl, A.R. and Engeseth, N.J. 2000. Honey from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. Journal of Agricultural and Food Chemistry 2(48): 4997-5000.

Chye, F. Y. and Ng, X.W. 2008. Antioxidant and antibacterial properties of honey. UMS Publication, Malaysia.

Ellman, G. L., Courtney, K.D., Valentino, A.J. and Featherstone, R.M. 1961.A new and rapid colourimetric determination of acetylcholinestrase activity. Biochemical Pharmacology 7(2): 88-95.

Farooq, A., Hafiz, M.Q.A., Abdullah, I.H. and Shahid, I.2010. Antioxidant activity of 100% and 80% methanol extracts from barley seeds (*Hordeum vulgare* L.): stabilization of sunflower oil. Food Chemistry 61(3):1-7.

Gheldof, N., Wang, X. and Engeseth, N. 2001. Characterization of the antioxidants in honey from different floral sources. Presented at Annual Meeting, Institute of Food Technologist, Dallas, USA, 11th-14th June.

Gheldof, N., Wang, X.H. and Engeseth, N.J. 2003. Buckwheat honey increases serum antioxidant capacity in humans. Journal of Agricultural and Food Chemistry 5(51): 1500-1505.

Haque, M. A., Rahman, M.M., Sayeed, M.S., Hassan, M.M. and Ashraful S.M. 2012. Phytochemical screening, antioxidant, anti-alzheimer and anti-diabetic activities of Centella asiatica. Journal of Natural Product and Plant Resources 2(4): 504-511.

Houghton, P.J., Ren, Y. and Howes, M.J. 2006. Acetylcholinesterase inhibitors from plants and fungi. Natural Product Reports 23: 181-199.

Huang, D., Ou, B. and Prior, R.L. 2005. The chemistry behind antioxidant capacity assay. Journal of Agriculture and Food Chemistry 53: 1841-1848.

Irina, D., Georgiana, G., Livia, P., Alina, M.E. and Rodica, S. 2010. The antioxidant activity of selected Romanian honeys. Journal of Food Technology 34(2): 38-43.

Jasna, B., Urska, D., Mojca, J. and Terezija, G. 2007. Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chemistry 105(2): 822-828.

Jo, Y.H., Yuk, H.G., Kim, J.H., Kim, R. and Lee, S.C. 2012. Antioxidant, tyrosinase inhibitory and acetylcholinesterase inhibitory activities of green tea (*Camellia sinensis* L.) seed and its pericarp. Food Science and Biotechnology 21(3): 761-768.

Lee, S-H., Sancheti, S.A., Bafna, M.R., Sancheti, S.S. and Seo, S-Y. 2011. Acetylcholinesterase inhibitory and antioxidant properties of Rhododenron yedoense var. Poukhanense bark. Journal of Medicine Plants Research 5(2): 248-254.

Leticia, E., Ana, P.P., Leandro, M., Luis, G. D. and Ermelinda, P. 2008. Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food and Chemical Toxicology 46: 3774-3779.

Madsen, H.L. and Bertelsen, G. 1995. Spices as antioxidants. Food Science and Technology 6(2): 271-277.

Magalhaes, L. M., Segundo, M.A., Reis, S. and Lima, J.L.F.C. 2006. Automatic method for determination of total antioxidant capacity using 2,2-diphenyl-1-picrylhydrazyl assay. Analytica Chimica Acta 558(2):310-318.

Mervat, M.A. and El-Gendy. 2010. In vitro evaluation of medicinal activity of Egyptian honey from different floral sources as anticancer and antimycotic infective agents. Journal of Microbial and Biochemical Technology 2(5): 1-6.

Mckibben, J. and Engeseth, N.J. 2002. Honey as a protective agent against lipid oxidation in ground Turkey. Journal of Agricultural and Food Chemistry 50 (11): 592-595.

Molan, P.C., Adams, C.J. and Manley-Harris, M. 2009. The origin of methylglyoxal in New Zealand Manuka *(Leptospermum scoparium)* honey. Carbohydrate Research 344(11):1050-1053.

Nagai, T., Inoue, R., Kanamori, N., Suzuki, N. and Nagashima, T. 2006. Characterization of honey from different floral sources, its functional properties and effects of honey species on storage of meat. Food Chemistry 97: 256-262.

Nikolaus, K., Gudrun, K. and Salim, T. 2010. Honey Bees of Borneo: Exploring the Centre of Apis Diversity Sabah. Natural History Publications, Borneo, Sabah.

Orhan, I., Sener, B., Choudhary, M.I. and Khalid, A. 2004. Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some Turkish medicinal plants. Journal of Ethnopharmacology 91: 57-60.

Peschel, W., Sanchez-Rabaneda, F.D.N., Plescher, A., Gartzia, I. and Jimenez, D. 2006. An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chemistry 97: 137-150.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Radical Biology and Medicine 26(9): 1231-1237.

Shapira, M., Thompson, C.K., Soreq, H. and Robinson, H.E. 2001. Changes in neuronal acetylcholinesterase gene expression and division of labor in honey bee colonies. Journal of Molecular Neuroscience 17: 1-12.

Sharon, K. and Ryan, M.F. 1999. Purification, characterization, and inhibition by monoterpenes of acetylcholinesterase from the waxmoth, G*alleria mellonella* (L.). Insect Biochemistry and Molecular Biology 29: 1097-1104.

Susana, G., Luis, D.G., Leandro, M.L., Paula, R. and Leticia, E. 2010. Physiochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. Food and Chemical Toxicology 48: 544-548.

Taormina, P.J., Niemira, B.A. and Beuchat, L.R. 2001. Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. International Journal of Food Microbiology 69(31): 217-225.

Vinutha, B., Prashnth, D., Salma, K., Sreeja, S.L., Pratiti, D., Padmaja, R., Radhika, S., Amit, A., Venkateshwarlu, K. and Deepak, M. 2007. Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. Journal of Ethnopharmacology 109: 359-363.

Wang, X. H., Gheldof, N. and Engeseth, N. 2004. Effect of processing and storage on antioxidant capacity of honey. Journal of Food Science 69(11): 96-101.

Yap, P., Lim, H., Carrier, D. and Abu Bakar, M. F. 2015. Antibacterial activity of polyphenol rich extract of selected wild honey collected in Sabah, Malaysia. Journal of Apicultural Research (in press): 1-29.

Yap, P. and Abu Bakar, M. F. 2014. Physicochemical, phytochemical and antimicrobial properties of wild honey collected at mangrove and mountain areas in Sabah, Malaysian Borneo. International Journal of Pharmacy and Pharmaceutical Sciences 6(7): 287-292.

Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J. and Qian, M. 2002. Free radical scavenging properties of wheat extracts. Journal of Agricultural and Food Chemistry 50(27):1619-1624.