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Full Length Research Paper

Comparison of total phenolic content and antioxidant activities of freeze-dried commercial and fresh fruit juices

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Fruits contain various natural antioxidants and secondary metabolites which play an important role in human health. Fruit juices have been proposed as a convenient substitute for fresh fruit. In this study, the total phenolic content and antioxidant activity of various freeze-dried fresh fruit blends or fruit juices (Apple, lime, guava, mango, orange) were compared. Total phenolic content of the extracts was determined using Folin-Ciocalteu assay. Antioxidant capacity of the extracts was determined by 1,1-diphenyl-2picryl hydrazyl (DPPH) scavenging assay and tyrosinase inhibition assay. Guava contained the highest amount of total phenolic content among all the tested fresh fruit blend, while mango contained the highest amount of total phenolic content for the commercial fruit juice. Apple contained the lowest content of total phenolic in both fresh and commercial fruit juice. In the DPPH scavenging test, guava and mango still possessed the strongest antioxidant activity for fresh and commercial fruit juices. Commercial lime juice possessed the weakest antioxidant activity where no IC_{50} was able to obtain even up to 10 mg/ml. All of the commercial and fresh fruit did not show tyrosinase inhibition activity. The present study demonstrates the potential value of commercial fruit juice as the replacement of fresh fruit. However, only commercial mango and orange juices were able to maintain similar antioxidant activity and total phenolic content as fresh fruit.

Key words: Fruit juices, 1,1-diphenyl-2picryl hydrazyl (DPPH), tyrosinase inhibition activity.

INTRODUCTION

Plant food, especially vegetables and fruits, have been given great attention due to their health benefits nowadays. In the past decade, numbers of studies have found that they are the great source of natural antioxidant (Tezcan et al., 2009). Fruit is normally consumed fresh and also in salads and desserts. They are sometimes blended to consume as fruit juice. Antioxidants have long been known to protect biological systems via inhibition or prevention of oxidation stress induced by reactive oxygen substances generated from normal metabolic activity or environmental factors (Hwang et al., 2010). Thus, insufficient level of antioxidants in human may lead to the damage of DNA, lipids and proteins which may induce various diseases such as cancer (Hodzic et al., 2009).

Associated with the rise of the economy, commercial fruit juices have been proposed as the substitute of fresh

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fruit juice due to their convenience. Consumers nowadays are more aware on the health-promoting quality of their food and drink. As a result, food industry has intensified their effects to provide better quality and more convenient food and drink to fulfill the requirement of the consumer (Michalczyk et al., 2009). However, the level of antioxidant preserved in the commercial fruit juices when compared to fresh fruit juice is still unclear. Commercial fruit juices manufacturer normally advertise and educate their consumer that their fruit juices are rich in multivitamin (such as vitamins A, C and E). All of these vitamins are normally synthetic and added during the process of the fruit juices. In addition, some of the synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroxyquinone (TBHQ) are used as permitted preservative in the commercial beverages. These synthetic antioxidants may cause genotoxicity and carcinogenicity at high concentration (Ndhlala et al., 2010). Most of the vitamins which are heat sensitive are normally deactivated by the heat during the evaporation step. Study has found that phenolic compounds but not the vitamins from the fruits are more important to provide antioxidant activity toward the host (Bermudez-Soto and Tomas-Barberan, 2004). To date, the value and believe of purchasing the expensive commercial fruit juice as healthy drink or source of antioxidant is still unclear.

The purpose of this study was to analyze and compare the antioxidant activities of five different types of fresh and commercial fruit juices in Malaysia. By studying the total phenolic content and compare with DPPH scavenging activity, the concentration and the effect of this health promoting phenolic content can be identified and used by the consumer as a factor to consider before they make up their mind to go for fresh fruit or ready to drink fruit juice.

MATERIALS AND METHODS

Selection and preparation of commercial and fresh fruit juices samples

Fresh fruits and commercial fruit juices of apple, guava, lime, mango and orange were purchased from hypermarket in state of Selangor, Malaysia. Twenty different samples of each type of fruits which were completely ripe were selected. Skin of lime, mango and orange were removed. All fruits were washed and 5 g of each sample was blended in 100 ml of deionized water. The extracts were then centrifuged at 10,000 rpm for 30 min and the supernatants were filter with Whatman filter paper to remove the non-soluble fiber. The commercial fruit juices were selected based on the label of nutritional fact. Only one brand that stated the percentage of fruit concentrated in the juice (5%) was used for this study. Twenty different samples of each type of fruits juices were selected, pelleted at 10,000 rpm for 30 min and filter through Whatman filter paper. All the filtrated was stored overnight in -80°C freezer. Then, the samples were placed in a single layer on solid anodized aluminum trays and freeze-dried for 22 h. The freeze-dried extracts were stored in -80°C freezer prior to further use in the bioassay.

Determination of total phenolic content in commercial and fresh fruit juices samples

Folin-Ciocalteau test was used to determine total phenolic content in the tested fruit juices because this reagent was sensitive to reduce compounds including polyphhenols to produce blue colour which can be quantified by using spectrophotometer (Singleton et al., 1999). Gallic acid (1 µM) was used as the positive control. Standard curve was plotted from the absorbance of different concentration of gallic acid and results of each extracts were expressed as mg of gallic acid equivalents (GAE)/L.

In vitro DPPH radical-scavenging activity

1,1-diphenyl-2picryl hydrazyl radical (DPPH) was used to determine the free radical-scavenging activity of freeze-dried commercial and fresh fruit juice (Ebrahimzadeh et al., 2010). Freeze-dried commercial and fresh fruit juice powders were dissolved in methanol at different concentration (5000, 2500, 1250, 625, 312.5, 156.3 and 78.13 µg/ml) were tested in triplicate and vitamin C (Sigma, USA) and vitamin E (SimeDarby, Malaysia) were used as standard controls. IC_{50} values which represent concentration required to scavenge 50% of DPPH free radicals was compared among freeze-dried commercial and fresh fruit juices, vitamins C and E.

Tyrosinase inhibition activity

Inhibition of tyrosinase activity was measured by using L-tyrosine as substrate (Lee et al., 1997). The results were expressed as percentage of inhibition of tyrosinase activity. Kojic acid and L-cysteine was used as the standard tyrosinase inhibitor in this assay.

Statistical analysis

Results were expressed as mean ± standard error (S.E.M).

RESULTS

Total phenolic content in commercial and fresh fruit juices samples

Figure 1 represents the correlation of the garlic acid concentration toward the absorbance in the Folin-Ciocalteau assay. Based on this standard curve, total phenolic content of various freeze-dried fruit juices was determined. Total phenolic content was different among the fresh and commercial fruit juices. Table 1 summarized the total phenolic content of various freeze-dried commercial and fresh fruit juices. Fresh guava contained the highest amount of phenolic content among all the tested samples. Both fresh and commercial apple juices contain the lowest amount of phenolic content in their representative group. Unlike the fresh lime, commercial lime juices contain five times lower phenolic content when compared to fresh lime juices. The phenolic content of mango and orange remain high in both

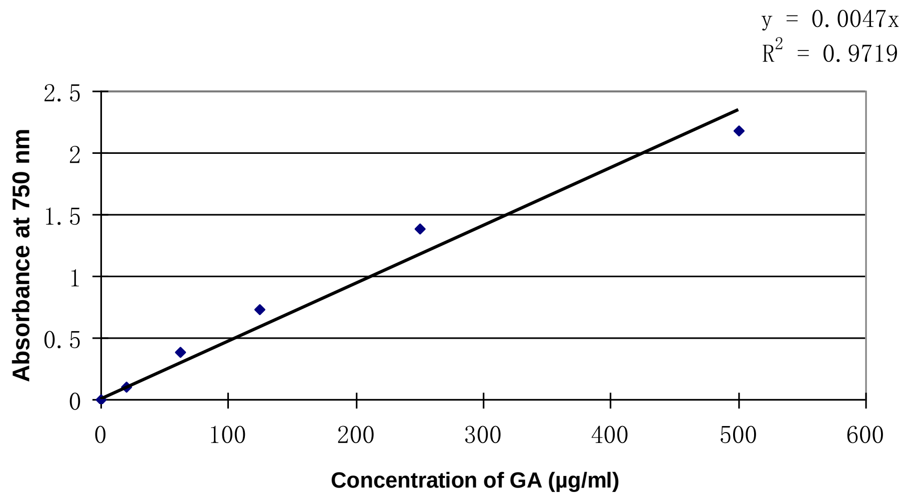

Figure 1. Folin-Ciocalteau garlic acid standard curve. Each value represents the means ± S.E.M. triplicate in three independent experiments.

Table 1. Total phenoolic content distribution of freeze-dried commercial and fresh fruit juice. Each value represents the means ± S.E.M. triplicate in three independent experiments.

Type of fruit juices	Total phenolic concentration (GAE mg/L or mg/g)
Fresh apple	5.85±0.83
Fresh guava	15.74±1.79
Fresh lime	12.13±1.44
Fresh mango	9.26±0.77
Fresh orange	13.19±0.92
Commercial apple	1.90±0.04
Commercial guava	12.31±0.63
Commercial lime	3.10±0.07
Commercial mango	12.56±1.33
Commercial orange	12.02±1.56

commercial and fresh fruit juices.

In vitro DPPH radical-scavenging activity of commercial and fresh fruit juices samples

The DPPH scavenging activity of freeze-dried commercial and fresh fruit juice has been shown to be strongly correlated with their phenolic content. All of the fresh fruit juices gave IC_{50} in this assay. Among them, fresh apple juice gave the lowest antioxidant activity with the highest IC_{50} value (2.5 mg/ml) (Table 2). Commercial apple and lime juice did not show any IC_{50} while commercial mango and orange juices possessed compatible inhibitory effect as the fresh fruit juices (Figure 2). However, all of the freeze-dried fruit juices gave the much higher IC_{50} value when compared to vitamin C (0.005 mg/ml) and vitamin E (0.03 mg/ml).

Tyrosinase inhibition activity of commercial and fresh fruit juices samples

In tyrosinase inhibition test, positive control L-cysteine was able to inhibit 50% of tyrosinase activity at 0.3 mg/ml while none of the freeze-dried commercial and fresh fruit juice was able to inhibit autooxidation of tyrosinase.

DISCUSSION

In recent years, consumers have shown increasing interest in food materials that are rich in natural ingredients which are good for human health (Alitheen et al., 2010a, b). Antioxidants are substances that inhibit the oxidation of easily oxidisable biomolecules such as lipids, proteins and DNA. It can be grouped into enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants

Table 2. IC_{50} of various freeze-dried commercial and fresh fruit juices in DPPH scavenging activity. Each value represents the means ± S.E.M. triplicate in three independent experiments.

Type of fruit juices	IC_{50} (mg/ml)
Fresh apple	2.500±0.268
Fresh guava	0.156±0.031
Fresh lime	0.313±0.143
Fresh mango	0.220±0.067
Fresh orange	0.290±0.057
Commercial apple	>10 mg/ml
Commercial guava	0.250±0.097
Commercial lime	>10 mg/ml
Commercial mango	0.270±0.081
Commercial orange	0.480±0.152
Vitamin C	0.005±0.002
Vitamin E	0.032±0.006

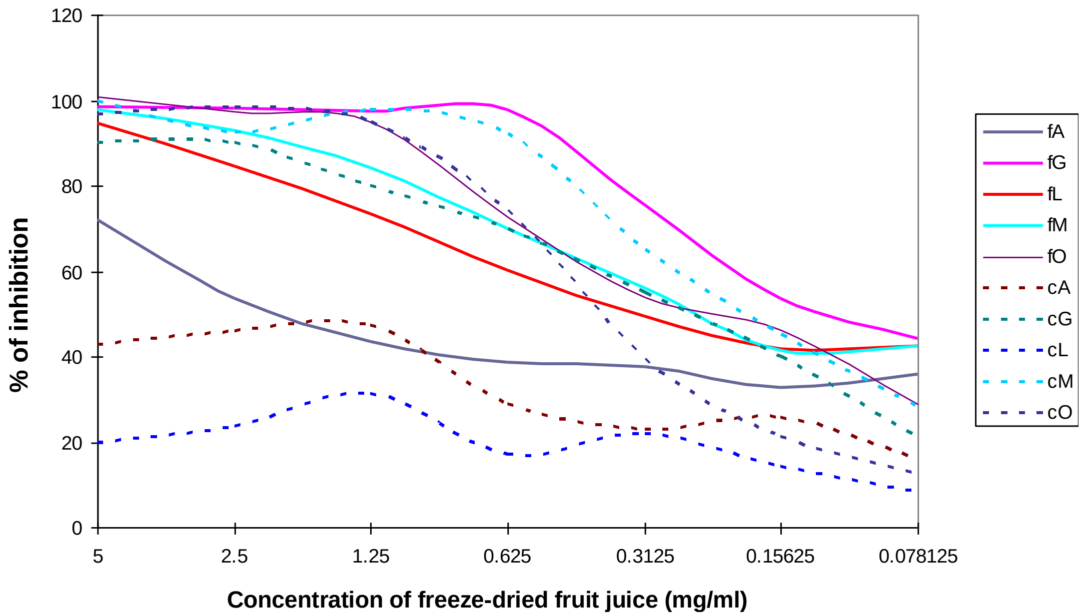

Figure 2. DPPH scavenging activity of various freeze-dried commercial and fresh fruit juices. Each value represents the means ± S.E.M. triplicate in three independent experiments. fA: fresh apple, fG: fresh Guava, fL: fresh lime, fM: fresh mango, fO: fresh orange, cA: commercial apple, cG: commercial guava, cL: commercial lime, cM: commercial mango, cO: commercial orange.

include superoxide dismutase and glutathione reductase while non-enzymatic antioxidants are water soluble (vitamin C and polyphenolic compounds) and lipid-soluble (vitamin E and pre-vitamin A) biomolecules (Ndhlala et al., 2010). Plant food materials contain high level of numerous redox-active antioxidants such as polyphenols, tocopherols, carotenoids and ascorbic acid (Pisoschi et al., 2009; Ho et al., 2009; Yeap et al., 2010). These antioxidant substances are believed to carry health-promoting properties including inhibition on the neoplasms, coronary disease and other degenerative diseases (Michalczyk et al., 2009). Fruit is the most widely consumed plant food due to their taste and the present of antioxidant ingredients. Apple is commonly consumed in Europe and North America as the source of phenolic compounds in their daily diet. In United State, the major fruit phenolic among the consumers was apple (Wolfe et al., 2003). Besides apple, guava (*Psidium guajava*) is an important tropical fruit which contained high level of phenolic compound that contributed to high antioxidant capacity of guava fruit (Chen and Yen, 2007; Jimenez-Escrig et al., 2001). Musk lime (or scientifically call as *Citrus microcarpa*) or more commonly known as limau kasturi is a type of lime that is not generally eaten fresh but is commonly used as a flavour for food and beverages. Vitamin C was previously identified as the principle antioxidant ingredient in musk lime (Hoyle and Santos, 2010). Mango (*Mangifera indica* L.) is an important commercial crop worldwide that contains dietary antioxidants such as ascorbic acid, carotenoids and phenolic compounds that help to protect the study animal from cytotoxic effect induced by dimethyldrazine (Ribeiro et al., 2007). Orange is a type of citrus fruit that rich in bitter limonoids and vitamins including ascorbic acid (vitamin C), carotenoids, folates and phenolic compounds. Although orange is well known as the source of vitamin C, scientific study has found that the antioxidant activity of orange juice greatly depends on their phenolic compounds rather than their vitamin C content (Riso et al., 2005). On the other hand, fruit juice concentrate is the result of an industrial process where fruit juice is subjected to evaporation to remove majority of the water to provide better condition for storage, transport and preservation of liquid food materials with lowest handling cost. Besides, this process which normally involves heating was believed to be able to increase the phenolic content of the fruit through the extra extraction step. Therefore, fruit juice concentrate or commercial fruit juice that reconstitute from the concentrate was believed can be served as good source of functional food to replace carbonated soft-drink (Bermudez-Soto and Tomas-Barberan, 2004; Mahdavi et al., 2010).

Phenolic compounds are derived from the shikimate and phenylpropanoid pathway, which contains a diverse and ubiquitous class of plant secondary metabolites, characterized by an aromatic ring and one or more hydroxyl groups (Ndhlala et al., 2010; Bermudez-Soto and Tomas-Barberan, 2004). A study has found that content of polyphenols has great correlation with the antioxidant capacity of natural food especially food (Walkowiak-Tomczak, 2007). Although it is wide accepted that vitamin C, E and pro-vitamin A were responsible for the health benefits of fruits and vegetable, long term clinical studies have recorded that vitamins C and E did not reduce the risk of various diseases such cardiovascular disease and stroke which related to stress and oxidation. Phenolic antioxidants were found to possess even more biological effects than vitamins in terms of health-promoting effects as antioxidants (Vinson et al., 2005). In this study, total phenolic content of both fresh and commercial fruit juice was considered as low, since the daily polyphenolic compounds of the diet was estimated to range between 150 mg to 1 g/day (Ribeiro et al., 2007). Nutritionally, the daily intake of 100 ml of commercial fruit juice (with 5% or 5 g of fruit concentrate) only contributes to maximum 65 mg of phenolic compounds (13 mg of phenolic compound in 1 g of mango juice). This result reflects that relying on either fresh fruit or commercial fruit juice does not contribute to enough daily phenolic compounds intake.

In this study, fresh apple and guava were blended with peel. This is because peel was previously found to contain additional flavonoids which are not found in the flesh of the fruits (Wolfe et al., 2003). However, this step did not help to preserve the antioxidant and phenolic content of fresh apple juices. The phenolic content of fresh apple was comparatively lowest among all the fresh fruit juices. On the other hand, keeping peel of the guava may contribute to the additional antioxidant capacity since fresh guava contain the highest amount of polyphenol content and was able to inhibit DPPH scavenging activity with the lowest inhibition concentration.

The antioxidant activity of the tested fruit juice in DPPH scavenging assay was correlated with the total phenolic content that was quantified using Folin-ciocalteu test. This result suggested that phenolic content but not the major vitamins (such as vitamin C, vitamin E and pro-vitamin A) may be the major contributor of antioxidant activity of various fruits.

Lime and orange are the examples of citrus fruits which are high in vitamin C and phenolic compounds. In this study, the antioxidant activity of both fresh lime and orange juices are similar in both total phenolic quantification and DPPH scavenging test. This result postulated that both lime and orange may contain similar bioactive compounds which contribute to their antioxidant effect.

Decrease of antioxidant activity in commercial fruit juice may be due to storage of the raw fruit or the fruit concentrate before the production and packaging of the ready-to-drink fruit juice. Walkowiak-Tomczak (2007) has found that increase of oxygen, pH and temperature during storage reduced the antioxidant activity of the fruit concentrate.

Previous study has found that short term *in vivo* consumption of orange juice was not able to affect the oxidation stress that related to cardiovascular risk (Riso et al., 2005). This may be due to the insufficient daily phenolic compounds intake since the concentration of phenolic compounds was too minute as reported in this study.

Although antioxidant activity was always associated to the whitening effect, all of the freeze-dried fruit juices, unlike the positive control L-cysteine, were not able to inhibit auto-oxidation of tyrosinase. This means that taking either fresh or commercial fruit juice did not help in protection against skin darkening.

This investigation clearly shows the potential value of commercial fruit juice as the substitute for fresh fruit. Various fruits contain different level of antioxidant capacity and total phenolic content. Commercial mango and orange juice still preserved high level of phenolic content and antioxidant capacity as compared to fresh mango and orange blend. Therefore, commercial mango and orange juice can be considered a good source of natural antioxidants besides fresh fruit. However, fresh fruit still contain much higher phenolic content and antioxidant capacity. Thus, commercial fruit juice which is normally more expensive as compared to fresh fruit should be considered as the secondary choice of dietary fruit, when fresh fruit is not available.

REFERENCES

Alitheen NB, Manaf AA, Yeap SK, Shuhaimi M, Nordin L, Mashitoh AR (2010a). Immunomodulatory effects of damnacanthal isolated from roots of *Morinda elliptica.* Pharm. Biol., 48:446-452.

Alitheen NB, Mashitoh AR, Yeap SK, Shuhaimi M, Abdul Manaf A, Nordin L (2010b). Cytotoxic effect of damnacanthal, nordamnacanthal, zerumbone and betulinic acid isolated from Malaysian plant sources. IFRJ 17:711-719.

Bermudez-Soto MJ, Tomas-Barberan FA (2004). Evaluation of commercial red fruit juice concentrates as ingredients for antioxidant functional juices. Eur. Food Res. Technol. 219:133-141.

Chen HY, Yen GC (2007). Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves. Food Chem. 101:686-694.

Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bahramian F, Bekhradnia AR (2010). Antioxidant and free radical scavenging activity of *H. officinalis L. var. angustifolius, V. odorata, B. hyrcana* and *C. speciosum.* Pak. J. Pharm. Sci. 23:29-34.

Ho WY, Ky H, Yeap SK, Rahim RA, Omar AR, Ho CL, Alitheen NB (2009). Traditional practice, bioactivities and commercialization potential of *Elephantopus scaber* Linn. J. Med. Plant Res. 3:1212-1221.

Hodzic Z, Pasalic H, Memisevic A, Srabovic M, Saletovic M, Poljakovic M (2009). The influence of total phenols content on antioxidant capacity in the whole grain extracts. EJSR 28:471-477.

Hoyle CHV, Santos JH (2010). Cyclic voltammetric analysis of antioxidant activity in citrus fruits from Southeast Asia. IFRJ 17:937-946.

Hwang PA, Wu CH, Gau SY, Chien SY, Hwang DF (2010). Antioxidant and immune-stimulating activities of hot-water extract from seaweed *Sargassum hemiphyllum*. J. Mar. Sci. Tech-Japan 18:41-46.

Jimenez-Escrig A, Rincon M, Pulido R, Saura-Calixto F (2001). Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. J. Agric. Food Chem. 49:5489-5493.

Lee KT, Kim BJ, Kim JH (1997). Biological screening of 100 plant extracts for cosmetic use (I): Inhibitory activities of tyrosinase and DOPA auto-oxidation. Int. J. Cosmet. 19:291-298.

Mahdavi R, Nikniaz Z, Rafraf M, Jouyban A (2010). Determination and comparison of total polyphenol and vitamin C contents of natural fresh and commercial fruit juices. Pak. J. Nutr. 9:968-972.

Michalczyk M, Macura R, Matuszak I (2009). The effect of air-drying, freeze-drying and storage on the quality and antioxidant activity of some selected berries. J. Food Process Pres. 33:11-21.

Ndhlala AR, Moyo M, Staden JV (2010). Natural antioxidants: Fascinating or mythical biomolecules? Molecules 15:6905-6930.

Pisoschi AM, Cheregi MC, Danet AF (2009). Total antioxidant capacity of some commercial fruit juices: Electrochemical and spectrophotometrical approaches. Molecules 14:480-493.

Ribeiro SMR, Queiroz JH, Lopes ME, Queiroz R, Campos FM, Sant'ana HMP (2007). Antioxidant in Mango (*Mangifera indica* L.) Pulp. Plant Foods Hum. Nutr. 62:13-17.

Riso P, Visioli F, Gardana C, Grande S, Brusamolino A, Galvano F, Galvano G, Porrini M (2005). Effects of blood orange juice intake on antioxidant bioacailability and on different markers related to oxidative stress. J. Agric. Food Chem. 53:941-947.

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Method Enzymol., 299:152-178.

Tezcan F, Gultekin-Ozguven M, Diken T, Ozcelik B, Erim FB (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. Food Chem. 115:873-877.

Vinson JA, Zubik L, Bose P, Samman N, Proch J (2005). Dried fruits: Excellent *in vitro* and *in vivo* antioxidants. JACN., 24:44-50.

Walkowiak-Tomczak D (2007). Changes in antioxidant activity of black chokeberry juice concentrate solutions during storage. Acta Sci. Pol. Technol. Aliment. 6:49-55.

Wolfe K, Wu X, Liu RH (2003). Antioxidant activity of apple peels. J. Agric. Food Chem., 51:609-614.

Yeap SK, Ho WY, Beh BK, Liang WS, Ky H, Yousr AHN, Alitheen NB (2010). *Vernonia amygdalina*, an ethnoveterinary and ethnomedical used green vegetable with multiple bioactivities. J. Med. Plant Res. 4:2787-2812.