Study on Some Physicochemical Analysis and Antioxidant Activity of Nuts of *Dracontomelon dao* (Blanco) Merr. & Rolfe (Nga bauk) and *Prunus dulcis* (Mill.) D. A. Webb (Badam)

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Abstract

Natural antioxidants are widely used in the food industry to enhance the sensory, promote health and maintain the quality of food. Consumption of food rich in natural antioxidants protects from certain types of cancer and reduces the risk of cardiovascular events. Several nuts such as *Dracontomelon dao* (Blanco) Merr. & Rolfe (Nga bauk) and *Prunus dulcis* (Mill.) D. A. Webb (Badam) are known to have significant antioxidant contents. The purpose of this research is to study some physicochemical analysis and antioxidant activity of nuts of Nga bauk and Badam. The preliminary phytochemical tests were performed by appropriate reported methods. The physicochemical analysis of both samples was carried out by standard analytical techniques. In addition, the qualitative elemental analysis was detected by using EDXRF spectrometry. The soluble matter contents of Nga bauk and Badam were also determined by the solvent extraction method. Moreover, antioxidant activity of pet-ether, ethyl acetate, ethanol and watery extracts of both samples were investigated by using DPPH free radical scavenging assay.

Key words : *Dracontomelon dao* (Blanco) Merr. & Rolfe (Nga bauk), *Prunus dulcis* (Mill.) D. A. Webb (Badam), Physicochemical analysis, EDXRF elemental analysis, antioxidant activity

Introduction

Antioxidants are first line of defense against free radical damage and are critical for maintaining optimum health and wellbeing. All living organisms contain antioxidant enzymes and chemicals. The reactive oxygen species play an important role related to degenerative or pathological processes such as aging, cancer, coronary heart disease, atherosclerosis, cataracts, and inflammation (Krishnaiah *et al*., 2011). The use of traditional medicine is widespread and plants still present a large source of natural antioxidants that might serve as leads for the development of novel drugs. The natural antioxidants are phenolic compounds, nitrogen compounds, carotenoids or ascorbic acid. They are constituents of many fruits, nuts, vegetables and they have attracted a great deal of scientific attention. Almonds, walnuts and peanuts are known to have potent antioxidant properties (Sies *et al*., 2005).

Dracontomelon dao (Blanco) Merr. & Rolfe (Nga bauk) is a large tree in the flowering plant and belongs to family of Anacardiaceae. It grows in the lowlands and swamps across southern Asia where annual rainfall is high and the dry season is quite short. It occurs from sea level to about 1600 ft. Young leaves and mature fruits are eaten as vegetable or used as food flavouring. The seed surface typically displays an intricate pattern with an approximate five-fold symmetry and its five rhombic protrusions are reminiscent of primitive Buddha images. Nga bauk nut is an excellent source of hydrocarbon (80 % of total oil). It is also a good source of phytochemicals (α - tocopherols). In additon, it is a source of minerals such as potassium, calcium, sulphur, phosphous, iron, zinc, copper and strontium.

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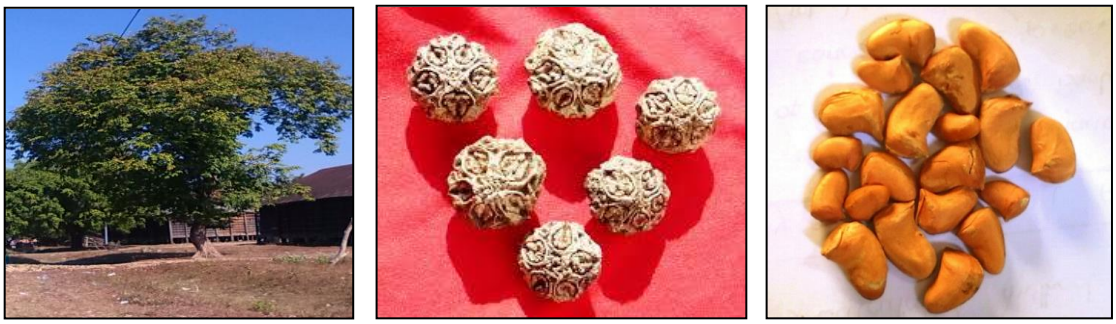
The bark is used against dysentery. Nga bauk nut is good source of antioxidant that protects from heart disease and cancer. In addition, it has been used in beauty culture to remove the skin of the face (Orwa *et al*., 2009).

Prunus dulcis (Mill.) D. A. Webb (Badam) is a medium-sized to large evergreen tree, perennial with a straight trunk and belongs to family Rosaceae. The Badam tree is one of the earliest domesticated trees. It is a major commercial nut crop of the world. Badam is thought to have originated in Western Asia and North Africa. It origins to the deserts and lower mountain slopes of central and southwest Asia and spreads by human populations along trade routes. Badam nut contains about 54 % of total oil but the amount can be as high as 62 % depending on the variety. It is a rich source of unsaturated fatty acids, mainly oleic acid and linoleic acid. It contains a high level of α - tocopherols and a significant amount of various amino acids. The high concentration of unsaturated fatty acids, minerals, total phenols, flavonoids and phenolic acids gives powerful antibacterial, antioxidant, anticancer and antifungal properties. Badam nut is edible and popular snack. It is also used as an ingredient in many processed foods such as nut butters, bakery and salads. It also helps in maintenance of healthy hair, skin care and dental care (Takeoka and Dao, 2002).

The present work focused on some physicochemical analysis and antioxidant activity of nuts of *Dracontomelon dao* (Blanco) Merr. & Rolfe (Nga bauk) and *Prunus dulcis* (Mill.) D. A. Webb (Badam). The photographs of trees, fruits and nuts of Nga bauk and Badam are shown in Figure 1 and 2.



(a) (b) (c)

Figure 1. Photographs of Nga bauk (a) tree (b) fruits and (c) nuts

(a) (b) (c)

Figure 2. Photographs of Badam (a) tree (b) fruits and (c) nuts

Materials and Methods

All chemicals used in this research work were obtained from British Drug House (BDH). All other reagents and solvents used were analytical grade. The apparatus used in the work consist of glass ware and other supporting facilities. Instrument employed in the experiment was JENWAY, 6300 UV-visible spectrophotometer, England.

The two samples : Nga bauk and Badam nuts were selected for physicochemical analysis and antioxidant activity. Nga bauk nuts were collected from Mohnyin Township and Badam nuts from Myitkyina Township, Kachin State. They were cleaned and stored in the refrigerator. Then, they were freshly grated by blender prior to extraction.

Preliminary Phytochemical Tests of Nga bauk and Badam Nuts

A few grams of each of the powder sample of Nga bauk and Badam nuts were subjected to the test of alkaloids, α-amino acids, carbohydrate, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponin glycosides (Shriner *et al*., 1980), starch, steroids, tannins and terpenoids (Marini *et al.*, 1981) as the preliminary phytochemical test according to appropriate reported methods.

Determination of Some Nutritional Values of Nga bauk and Badam Nuts

The physicochemical analyses such as nutritional values; moisture, ash, crude fibre and energy value were carried out in triplicate using the methods described in AOAC. The nitrogen was determined by the micro Kjeldahl method and the nitrogen content was converted to protein by multiplying by a factor of 6.25. The total carbohydrate content was calculated from the difference between 100 and the sum of percentages of moisture, protein, fat, ash and crude fibre.

Qualitative Elemental Analysis by Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometry

For this measurement, pellets of each sample were first made. X-ray spectrometer permits simultaneous analysis of light element to heavy element. Energy dispersive X-ray fluorescence spectrometer (Shimadzu EDX-720) can analyze the elements from Na to U under vacuum condition. The individual elements in each sample are detected by using semiconductor [Si-Li] that permits multi-elements, simultaneous analysis. In this way, EDX-720 spectrometer determines elements that are present in the sample. Qualitative analysis of some elements in Nga bauk and Badam nuts were measured by EDXRF method using EDX-720 instrument at the Universities' Research Centre (URC), Yangon.

Determination of Soluble Matter Contents by Direct Extraction Method

Each dried powdered sample (50 g) was extracted with 150 ml of pet-ether (60-80 ^{o}C) by using Soxhlet extractor for 6 hr and filtered. The filtrates were placed in a weighed porcelain basin and then evaporated to dryness on a water-bath until it was completely dried. The residue with the basin was weighed. If there is difference in weights of basin before and after,\ then the experiment was taken to be the pet-ether soluble matter content. Similarly, ethyl acetate and ethanol soluble matter contents were also determined by the method described above procedure by using ethyl acetate and ethanol as solvents. Watery extract of each dried powdered sample was prepared by boiling (50 g) of each sample with 150 ml of distilled water for 6 hr and filtered. The filtrates were placed in a weighed porcelain basin and then evaporated to dryness on a water bath until it was completely dried. The residue with the basin was weighed. If there is difference in weights of basin before and after, then the

experiment was taken to be the watery soluble matter content. Each extract was stored under refrigerator for screening of antioxidant activity.

Screening of Antioxidant Activity of Nga bauk and Badam Nuts

Antioxidant activity of pet-ether (PE), ethyl acetate (EtOAc), ethanol (EtOH) and watery (H_2O) extracts of both samples were carried out by DPPH (1,1- Diphenyl-2-picryl-hydrazyl) radical scavenging assay using UV-visible spectrophotometer.

(a) Preparation of Solutions

For test sample solutions, 2 mg of each crude extract was separately dissolved in 10 ml of ethanol and thoroughly mixed. The mixture solution was filtered and filtrate was used as a stock solution. Desired concentrations (40, 20, 10, 5, 2.5, 1.25 μg/ml) of sample solution were prepared from the stock solution by serial dilution with appropriate amount of ethanol. In addition, 2.364 mg of DPPH was thoroughly dissolved in 100 ml of ethanol to obtain 60 μM DPPH solution. It was freshly prepared in brown coloured flask and kept in refrigerator for no longer than 24 hr. Moreover, 2 mg of ascorbic acid was dissolved in 10 ml of ethanol and used as a standard solution. Blank solution was also prepared by mixing 1.5 ml of the test sample solution with1.5 ml of ethanol.

(b) Procedure

The control solution was prepared by mixing 1.5 ml of 60 μM DPPH solution and 1.5 ml of ethanol. The sample solution was also prepared by mixing 1.5 ml of 60 μM DPPH solution and 1.5 ml of test sample solution thoroughly. The solution was allowed to stand at room temperature for 30 minutes. After 30 minutes, the absorbance of these solutions was measured at 517 nm by using JENWAY, 6300 UV-visible spectrophotometer. Absorbance measurements were done in triplicate for each solution and then mean values so obtained were used to calculate the percent inhibition of oxidation by the following equation;

$$\% \text{ Oxidative Inhibition} = \frac{\text{Control} - (\text{Sample} - \text{Blank})}{\text{Control}} \times 100$$

% Oxidative Inhibition = percent inhibition of test sample
Control = absorbance of DPPH in EtOH solution
Sample = absorbance of sample and DPPH solution
Blank = absorbance of sample and EtOH solution

Then, IC_{50} (50 %oxidative inhibitory concentration) values were also calculated by linear regressive excel program.

Results and Discussion

Preliminary Phytochemical Tests

In order to find out the types of phytochemical constituents present in both samples, preliminary phytochemicals tests were carried out according to the procedure as mentioned in appropriate reported methods. According to these results, alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponin glycosides, starch, steroids, tannins and terpenoids were present in both Nga bauk and Badam nuts. These may contribute to possess bioactivities such as antibacterial, antioxidant, antidiabetic and anticancer.

Determination of Some Nutritional Values of Nga bauk and Badam Nuts

The determination of the nutrient values such as moisture, ash, fat, protein, fiber, carbohydrate and energy value of Nga bauk and Badam nuts were obtained by using the methods described in AOAC. The results obtained are shown in Table 1.

From these results, the fat contents were found to be highest in both samples. Fat is important in diets because it promotes fat soluble vitamin absorption. The protein, moisture and ash contents of Badam were respectively observed to be higher than that of protein, moisture and ash contents of Nga-bauk. But carbohydrate and fiber contents of Nga bauk were found to be respectively higher than those of Badam. In addition, The calorie of Nga bauk was higher than that of Badam due to the higher amount of carbohydrate in Nga bauk. The calculated metabolizable energy values showed that both nuts flour were concentrated source of energy.

Some Elements Present in Nga bauk and Badam Nuts by EDXRF Method

The relative abundance of elements present in Nga bauk and Badam nuts were determined by EDXRF spectrometer. The resultant data are shown in Table 2. It was observed that K, Ca, S, P, Fe, Zn and Cu were present as trace elements in both the samples. Among them, the relative abundances of K, Ca and Zn in Badam nuts were higher than that of these in Nga bauk nuts. But the relative abundances of S, P, Fe and Cu in Nga bauk nuts were found to be higher than these in Badam nuts. However, Sr was not detected in Badam nuts. A handful of Nga bauk and Badam nuts in the diet a day would provide enough of these minerals and prevent deficiency diseases and which function as co-factor for antioxidant enzymes.

Tale 1. Some Nutrient Values of Nga bauk and Badam Nuts

No.	Nutrients	Content	
		Nga bauk	Badam
1.	Moisture(%)	5.21	6.85
2.	Ash (%)	2.73	4.07
3.	Fat(%)	46.50	49.28
4.	Protein(%)	17.07	19.98
5.	Fiber(%)	5.56	5.38
6.	Carbohydrate(%)	22.93	14.44
7.	Energy(kcal/100g)	583	557

Table 2. Relative Abundance of Elements in Nga bauk and Badam Nuts By EDXRF Method

No.	Elements	Relative Abundance (%)	
		Nga bauk	Badam
1.	Potassium(K)	0.231	0.267
2.	Calcium(Ca)	0.023	0.100
3.	Sulphur(S)	0.078	0.053
4.	Phosphous(P)	0.157	0.150
5.	Iron(Fe)	0.004	0.002
6.	Zinc(Zn)	0.001	0.002
7.	Copper(Cu)	0.002	0.001
8.	Strontium(Sr)	0.001	-

Soluble Matter Contents of Nga bauk and Badam Nuts

In this research work, the soluble matters contents of Nga bauk and Badam nuts were performed by solvent extraction method. The resultant contents of these samples are shown in Table 3. From the results, it was observed that the amounts of nonpolar constituents present in Nga bauk were higher than that present in Badam. But Ethanol soluble matter content of Badam was found to be the highest (57.16 %). Consequently, the amounts of moderate polar and polar constituents in Badam nuts were higher than that present in Nga bauk nuts.

Table 3. Soluble Matter Content of Nga bauk and Badam Nuts

Extract	Yield(%)	
	Nga bauk	Badam
Pet-ether	48.99	46.08
Ethylacetate	35.75	53.92
Ethanol	40.12	**57.16**
Watery	3.92	9.12

Antioxidant Activity of Nga bauk and Badam Nuts

The antioxidant activity of Pet-ether, ethyl acetate, ethanol and watery extracts of the both samples were studied by DPPH assay. This method is based on the reduction of colored of free radical DPPH in ethanolic solution by different concentrations of the sample. The antioxidant activity was expressed as 50 % oxidative inhibitory concentration (IC_{50}). In this experiment, ascorbic acid (AA) was used as standard.

Each sample was dissolved in ethanol to get 0.2 mg/ml concentration and then it was diluted with ethanol to obtained 40, 20, 10, 5, 2.5, 1.25 μg/ml concentration. After mixing with DPPH solution, the absorbance of each solution was measured at 517 nm. On the basic of absorbance values, % inhibition of each sample in different concentrations was calculated and the results obtained are tabulated in Table 4 and Table 5. It was observed that as the concentrations were increased, the respective % inhibition was also increased in each sample. The antioxidative potential of each sample can be determined by IC_{50} (50 % inhibition concentration). The IC_{50} values for each sample were determined by linear regressive excel program. These values are also described in Table 4 and Table 5.

From this experiment, the decreasing order of antioxidant activity of Nga bauk nuts was EtOAc extract (IC_{50} = 2.74) > PE extract (IC_{50} = 3.35) > EtOH extract (IC_{50} = 5.87) > watery extract (IC_{50} = 9.23). In addition, the order of antioxidant activity of Badam nuts was found to be EtOH extract (IC_{50} = 1.92) > EtOAc extract (IC_{50} = 2.68) > PE extract (IC_{50} = 4.27) > watery extract (IC_{50} = 6.42).

According to these results, it was observed that the higher the concentration of crude extract, the lower the absorbance and the greater the % inhibition. Moreover, the lower the IC_{50} values, the higher the antioxidant activity of the sample. Thus, Badam nuts were found to possess more potent antioxidant potency than Nga bauk nuts.

Table 4. Oxidative Percent Inhibitions and IC_{50} Values of Nga bauk Nuts

Sample	% inhibitions in various concentration (μg/ ml)						IC_{50} (μg/ml)
	1.25	2.5	5	10	20	40	
PE	34.56	47.24	55.32	68.54	73.45	84.57	3.35
EtOAC	**36.19**	**49.56**	**54.08**	**80.15**	**89.48**	**96.02**	**2.74**
EtOH	20.31	34.83	49.09	54.32	64.54	72.18	5.87
H_2O	8.12	24.17	45.32	50.85	60.12	68.27	9.23
Ascorbic acid	44.96	79.61	82.98	90.74	95.57	98.15	1.43

Table 5. Oxidative Percent Inhibitions and IC_{50} Values of Badam Nuts

Sample	% inhibitions in various concentration (μg/ ml)						IC_{50} (μg/ml)
	1.25	2.5	5	10	20	40	

PE	19.62	39.89	54.15	61.37	64.24	71.15	4.27
EtOAC	22.25	49.45	56.99	65.74	72.24	76.12	2.68
EtOH	**38.93**	**59.71**	**73.56**	**80.35**	**86.43**	**90.78**	**1.92**
H_2O	12.45	29.19	47.32	56.79	60.12	68.27	6.42
Ascorbic acid	44.96	79.61	82.98	90.74	95.57	98.15	1.43

Conclusion

The preliminary phytochemical tests revealed the presence of alkaloids, α-amino acids, carbohydrate, glycosides, organic acids, reducing sugar, starch, steroids and terpenoids in both Nga bauk and Badam nuts. In Physicochemical analysis, nutritional values such as fat, protein, carbohydrate, fiber, moisture and ash contents in both samples were determined by AOAC methods. The fat contents were the highest in both samples. According to the EDXRF elemental analysis, K, Ca, S, P, Fe, Zn and Cu were present as trace elements. But, the contents of K in both samples were observed to be highest. By using the solvent extraction method, ethanol soluble matter content of Badam nuts was largest amount. The antioxidant activity of pet-ether, ethyl acetate, ethanol and watery extracts of both samples were investigated by using DPPH assay. All crude extracts in both samples were found to possess the antioxidant activity. But, ethanol extract of Badam exhibited the significant antioxidant activity. Consequently, it can be deduced that Nga bauk and Badam nuts may be used as antioxidants in reducing of oxidative stress related diseases as well as some age-related disorders and also contribute to positive health benefits.

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