PHYTOCHEMICAL AND PHYSICO-CHEMICAL STUDIES OF RIPE FRUIT PULP OF *BORASSUS FLABELLIFER* LINN. AND IT'S NATURAL COLOURANTS

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Abstract

To day, the demand for natural colorants is increasing worldwide due to the increased awareness on therapeutic and medicinal properties and their benefits and also because of profound effects of synthetic dyes. This study was conducted to evaluate the food colourants from ripe fruit pulp of *Borassus flabellifer* L. (Htan), family Arecaceae. These plants are abundantly found in Myanmar and have many medicinal and economic values. The plant specimens used in this research were collected from Dalla Township, Yangon Region. Botanical identification of the plant was recorded with photo images. In this study, the colorants produced from fresh fruit pulp and powdered sample of *Borassus flabellifer* L. were used with powdered rice and agar to get rice cakes and agar desserts. The present study deals with the phytochemical and physicochemical screenings and nutritional values of ripe fruit pulp of *Borassus flabellifer* L. The phytochemical screenings revealed the presence of several phytochemicals. The phytochemical tests show the presence of alkaloids, glycosides, flavonoids, carbohydrates, reducing sugar and absence of cyanogenic glycosides. The physico-chemical properties of powdered ripe fruit pulp were investigated by using different solvents according to WHO guidelines. Nutritional value of ripe fruit pulp was investigated and shown that the presence of carbohydrate, sugar, protein, fats and vitamin C.

Key words: *Borassus flabellifer,* phytochemicals, natural colorants, nutritional values

Introduction

In recent years there has been an increasing demand for materials of natural origin, particularly regarding natural colourants (Trease and Evans, 2009). The present research work includes the extraction of natural colourants from a resource plant which is used in making of rice desserts in Myanmar.

Natural colourants are derived from naturally occurring sources such as plants, insects, animals and minerals. This study was carried out on

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carotenoids from fruit pulp in ripe fruits of *Borassus flabellifer*L. (Htan),used as food colourants in Myanmar. The sample used in this research were collected from Dalla Township,Yangon Region.

Borassus flabellifer L. is known as 'Htan' in Myanmar and belongs to the family Arecaceae. *Borassus* is one of the most widespread palm genera. Most species grow in low sandy Costal plains exposed to sun and wind. However some species such as *Borassus flabellifer* L. can occur in mountain districts of India and on river banks. In Ceylon, the soft yellow, pulpy tissue, under the outer skin of ripe fruit, is squeezed out and the juice is dried in thick layers into an edible preparation called punatoo (Dassanayake, 2000). The yellow coloured fruit pulp of *Borassus flabellifer* L. ripe fruit is applied externally in skin diseases (Kirtikar, 1975).

Natural dyes not only release medicinal properties but also improve the aesthetic value of the product and they are unique and ecofriendly (Grover and Panti, 2011). Carotenoids are natural pigments, comprising a class of hydrocarbons (carotenes) and their oxygenated derivatives (xanthophyll). Carotenoids are excellent singlet oxygen scavenger and are used as food colourants, food additives, cosmetics and nutraceuticals.

Carotenoids are a family of compounds of over 600 fat soluble plant pigments that provide much of the colour in nature. They are important nutritions for the human body owing to their provitamin A and antioxidant activity (Krinsky & Johson, 2005).

Dietary carotenoids are thought to provide health benefits in decreasing the risk of disease, particularly certain cancer and eye disease. In part, the beneficial effects of carotenoids may be due to their role as antioxidants.

The purpose of this research is to produce natural colourants from plant for use in pharmaceutical and food preparations. These colourants produced from natural plant sources, have less toxicity and more stability for human. Therefore the natural colourants of *Borassus flabellifer* L. were investigated in this research.

Aim and objectives of present research are (i) to verify the resource plant, *Borassus flabellifer* L. and investigation of its natural colourants, (ii) to examine the qualitative and quantitative analysis of ripe fruit pulp of *Borassus*

flabellifer L. and (iii) to explore the preparation of rice and agar dessert by using natural colourants from ripe fruit pulp of *Borassus flabellifer* L.

Material and Methods

Botanical Aspect

The specimens used in this research were collected from Dalla Township, Yangon Region. The ripe fruits of *Borassus flabellifer* L. were collected from June to September in 2013 and flowers were collected from December, 2013 to January, 2014. Fresh specimens were used for taxonomic identification with the help of available literatures such as Backer (1968), Dassanayake (2000), Hooker (1894).

The ripe fruits of *Borassus flabellifer* L. were washed, peeled and pulped. The pulp was extracted manually with a sieve. At the same day, fruit pulp (180 g) was extracted with various solvents and their solubility were observed. Some fruit pulp was dried in incubator at 30°C for one week. The dried samples were pulverized and stored in air-tight containers.

Chemical Aspect

Preparation of dessert by using natural colourants from ripe fruit pulp of *Borassus flabellifer* L.

Fresh fruit pulp (5 g) was mixed with rice powder (10 g) and 200 ml of water. The mixture was heated on stove. When the mixture become thick, it was poured into another container and heated with steam. Fresh fruit pulp (5 g) was mixed with agar powder (10 g) and added 200 ml of water. The mixture was heated on stove. When the mixture was become thick, it was poured into another container and let it cool. Powdered fruit pulp (1 g) was mixed with (2 g) rice powder and 40 ml of water. The mixture was heated on stove. When the mixture was become thick, it was poured into another container and heated with steam. Powdered fruit pulp (1 g) was mixed with (2 g) agar powder and 40 ml of water. The mixture was heated on stove. When the mixture was thick, it was poured into another container and let it cool.

Preliminary Phytochemical investigation of powdered samples of *Borassus flabellifer* L.

Preliminary phytochemical tests were made according to the method of Central Council for Research in Unani Medicine (1987) and Trease and Evans (2009).

Physico-chemical examination of ripe fruit pulp of *Borassus flabellifer* L.

Physico-chemical properties which includes moisture content, total ash, acid insoluble ash, water soluble ash and solubility in non polar and polar solvents such as pet-ether, ethyl acetate, acetone, ethanol, methanol and water soluble matter contents of powdered samples were carried out by the methods of British Pharmacopoeia (1968) and World Health Organization (1998).

Determination of some elements (EDXRF)

The elements were analyzed by using Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometer at Department of physics, University of Mandalay.

Quantitative determination of some elements by AAS

The total ash samples were used to study the constituents of elements by using Atomic Absorption Spectrophotometer (AAS) at Universities' Research Centre (URC).

Nutritional values of ripe fruit pulp

Protein, fibre, fat, carbohydrate and sugar contents have been determined at Food Industries Development Supporting Laboratory (FIDSL) by the method of A.O.A.C (Horwitz,1980).Vitamin C contents have been determined at Myanmar Scientific and Technological Research Department, according to the procedures given in the method of A.O.A.C (Horwitz, 1980).

Results

Botanical Aspect

Morphological characters of *Borassus flabellifer* L.

Figure 1. Habit

Figure 2. Fruits

Figure 3. Male flower

Figure 4. Pistilate flower

Habit of *Borassus flabellifer* L. are very tall, dioecious palms, trunk stout, unarmed. Leaves are terminal, fan-shaped, multifid; petiole deeply chanelled, armed with coarse irregular teeth. Spadices are very larges interfoliar, peduncle sheathed with open spathes; male spadix branched, rachillae large, cackin-like, closely imbricated bracts enclosed spikelets of flowers, sunken in cavity. Staminate flowers are minute. Sepals 3, connate; petals 3, connate; stamen 6, filament short, anther bilobed. Female spadix sparingly branched, bearing few scattered solitary flowers. Pistillate flowers are large, sepals 3,reniform, imbricate; petals 3; staminode 6, gynoecium rounded, tricarpellate, central basal placentation; stigma low and knob-like. Fruits are large, bearing 1 to 3 seeds, perianths persistent, epicarp smooth, mesocarp fibrous, endocarp composed of 3 pyrenes, seeds bilobed (Fig.1 to 4).

Chemical Aspect

Preparation of desserts by using natural colourants from ripe fruit pulp of *Borassus flabellifer* L.

The yellow coloured rice cakes and agar dessert was obtained by applying fresh ripe fruit pulp and pale yellow coloured desserts by applying powdered ripe fruit pulp (Fig.5-13).

Preparation of desserts from ripe fruit pulp of *Borassus flabellifer* L.

Figure 5. Ripe fruit

Figure 6. Ripe fruit pulp

Figure 7. Samples in incubator (air dry condition)

Figure 8. Semidried fruit pulp

Figure9. Powdered fruit pulp

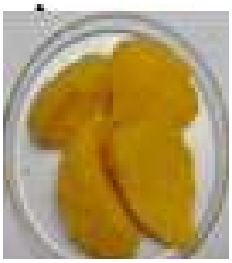

Figure 10. Fresh fruit Pulp rice cake

Figure 11. Fresh fruit pulp agar dessert

Figure 12. Powdered fruit pulp rice cake

Figure13. Powdered Fruit pulp agar dessert

Preliminary phytochemical investigation

In preliminary phytochemical investigation, the presence of alkaloid, glycoside, reducing sugar, saponin, phenolic glycoside, carbohydrate, flavonoid, terpenoid were observed but α-amino acid, tannin and cyanogenic glycoside were absent in ripe fruit pulp of *Borassus flabellifer* L. The results were shown in Table (1).

Table 1. Preliminary phytochemical tests on ripe fruit pulp of *Borassus flabellifer* L

No	Constituents	Extracted solvent	Test reagents	Observation	Results
1.	Alkaloid	3%HCL	1. Mayer's reagent	Whiteppt	+
			2. Dragendroff's reagent	Orangeppt	+
			3. Wagner's reagent	Reddish brownppt	+
2.	Glycoside	DW	10% lead acetate solution	White ppt	+
3.	Reducing sugar	DW	Fehling's solution	Brick red ppt	+
4.	Saponin	DW		Marked frosting	+
5.	Cyanogenic glycoside	DW	Sodium picrate, Conc. H_2SO_4	No colouration	-
6.	Phenolic compounds	DW	1% $FeCl_3$ solution	Deep blue colour	+
7.	α-amino acid	DW	Ninhydrin reagent	No colourchange	-
8.	Carbohydrate	DW	10% α-naphthol and conc. H_2SO_4	Pink ring	+
9.	Flavonoid	EtOH	Mg and conc. HCl	Pinkcolour	+
10.	Steroid	Benzene	Acetic anhydrate and conc. H_2SO_4	Pink colour	+
11.	Tannin	DW	0.1% $FeCl_3$ test solution	Green colour	-
12.	Terpenoid	Pet-ether	Acetic anhydrate and conc. H_2SO_4	Yellowish green	+

(+) = present (-) = absent ppt = precipitate

Physico-chemical investigation

Table 2. Physico-chemical properties of powdered ripe fruit pulp of *Borassus flabellifer* L.

No.	**Physico-chemical characters**	**Content (%)**
1.	Moisture content	15.74
2.	Total ash	5.63
3.	Acid insoluble ash	23.20
4.	Water soluble ash	5.67
5.	Water soluble matters	34.19
6.	EtOH soluble matters	37.10
7.	MeOH soluble matters	40.16
8	Acetone soluble matters	1.95
9.	EtOAc soluble matters	0.38
10.	Pet-ether soluble matters	0.11
11.	N-hexame soluble matters	0.62

Determination of some elements (EDXRF)

Table 3. Elemental analysis of ripe fruit pulp of *Borassus flabellifer* L. by using Energy Dispersive X-Ray Fluorescence spectrometer

No.	Elements	Concentration value (%)
1.	Cl (chlorine)	1.114
2.	K (Potassium)	0.917
3.	P (Phosphorus)	0.046
4.	Ca (calcium)	0.029
5.	S (Sulphur)	0.014
6.	Fe (Iron)	0.006
7.	Cu (Copper)	0.001
8.	Zn (Zinc)	0.001
9.	Rb (Rubidium)	0.001

Quantitative determination of some elements (AAS)

Table 4. Relative concentration of elements in ripe fruit pulp of *Borassus flabellifer* L. by using AAS

No.	**Elements**	**Concentration value (mg/L)**
1.	Cd (Cadmium)	0.089
2.	Cr (Chromium)	0.062
3.	Pb (Lead)	Not detected

Nutritional value of resource plant part

Table 5. Nutritional values of the ripe fruit pulp of *Borassus flabellifer* L.

No.	**Types of nutrients**	**Content (%)**
1.	Carbohydrate	**14.01**
2.	Sugar	**8.60**
3.	Protein	**0.73**
4.	Fat	**0.11**
5.	Fibre	**0.60**
6.	Vitamin C	**0.027**

Discussion

In this research, Morphological characters of *Borassus flabellifer* L. were in agreement with the character mentioned by Dassanayake (2000), Hooker (1894), Kirtikar and Basu (1957).The rice and agar dessert was prepared by using natural colourants of *Borassus flabellifer* L. The fresh natural colourants produced yellow coloured dessert and the powdered fruit pulp produced pale yellow coloured dessert. It was suggested that more interesting colour were obtained by using fresh fruit pulp.

In phytochemical test, the ripe fruit pulp of *Borassus flabellifer* L. indicated the presence of carbohydrate, reducing sugar, glycoside, alkaloids,

flavonoids, saponin, steroid and terpenoid except α amino acid, tannin and cyanogenic glycoside.

According to physico-chemical properties, the solubility of the powdered ripe fruit pulp of *Borassus flabellifer* L. was most soluble in acetone and moderately soluble in methanol and ethanol.These were in agreement with the findings of Vengalah *et.al*. (2015).

In ripe fruit pulp of *Borassus flabellifer* L., chlorine, potassium and phosphorus were found to be principal elements and the other elements such as calcium, sulphur, iron, chromium, copper, zinc and rubidium were trace elements.Minerals are important for the body to stay healthy. Minerals keep bones, muscles, heart and brain to work properly. Minerals which need for human body are calcium, phosphorus, magnesium, sodium, potassium, chloride and sulphur. Ripe fruit pulp of *Borassus flabellifer* L. contain chlorine 1.11 % and potassium 0.91%.

Chlorine is one of the most important electrolytes in the blood, along with sodium, potassium and calcium. Chloride is a highly important, vital mineral required for both human and animal life. Chloride is related to chlorine compounds in common salt NaCl. Chloride is a by-product of the reaction between chlorine and an electrolyte such as potassium, magnesium, or sodium, which are essential for human metabolism (Website 1).

Epidermiological and clinical studies show that a high potassium intake reduces cardiovascular disease mortality. This is mainly attributable to the blood pressure-lowering effects of potassium on the cardiovascular system. A high potassium diet may also prevent or atleast slow the progression of renal disease. The best way to increase potassium intake is to increase the consumption of fruits and vegetables (He, 2008).

The constituents of cadmium and chromium were determined as 0.089 % and 0.062% in ripe fruit pulp of *Borassus flabellifer* L. and lead is not detected.The poisonous level of cadmium and chromium were 0.3mg/ kg. Thus the constituents of Cadmium and chromium in ripe fruit pulp were not harmful for human health. The nutritional values of fresh ripe fruit pulp of *Borassus flabellifer* L. were determined as 14.01% carbohydrate, 8.6% sugar,0.73% protein, 0.11% fat and 0.60% fibre. The contents of carbohydrate were greater than others.

Carbohydrate is the most important food energy provider among the macronutrients, accounting for between 40 and 80 percent of total energy intake. The energy balance be maintained by consuming a diet containing at least 55 percent total energy from various sources of carbohydrates. It is engaging in regular physical activity. Although high- carbohydrate foods provide the full range of vitamin and mineral nutrients, some are also particularly rich in phytochemicals, many of which are antioxidants (FAO/WHO, 1998).

Conclusion

According to the present work is to distribute the apply knowledge of selection of natural colourants and preparation of desserts by using natural colourants with different vehicles (rice and agar) to local people in very simple methods. It is expected that the results of present research will contribute to useful information of natural colourants for applying in pharmaceuticals and food preparations as less toxic and more stable colourants for human.

This study is preliminary investigation of food colourants from plant sources. Further study for isolation and identification of compounds and shelf life of colourants will be needed to produce food colourants for health benefit.

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