

Morphological characterization, phytochemical investigation and starch isolation of *Amorphophallus bulbifer* Blume. (Wa.u)

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Abstract

Earth vegetables namely *Amorphophallus bulbifer* Blume. grown in Hinthada District was collected, classified and identified. Most of them were growing wild and cultivated for their economic purpose. The macroscopical and microscopical characters of corm were examined. Phytochemical and physicochemical examination were also studied. Nutritive value and elemental components were analyzed. In nutritional studies, *Amorphophallus bulbifer* Blume. was found to have highest Vitamin C content. It was about 16.25 mg. And medicinal and nutritional usefulness of elephant yam starch, konjac starch was isolated from fresh corm of *Amorphophallus bulbifer* Blume. Yield percentage of starch, characterization and gelatinization temperature were analysed. Yield percentage of starch was 2.250% and waste product of starch isolation was 5.21% respectively. Amylose and amylopectin contents of isolated starch were determined by using UV visible spectrophotometric method. Amylose content was 18.00% and amylopectin was 82.00% respectively.

Keywords: *Amorphophallus bulbifer* Blume., Konjac starch, Vitamin C

Introduction

The use of starch as an adhesive as well as food stuff has been known to the early Egyptians since 3500 BC (Radley, 1943). Starch is early end product of photosynthesis which at night is rapidly broken down into source and transported to other organs. It is the most abundant and important of the plant reserve polysaccharide (Wicken, 2001).

Roots and tubers belong to the class of food that basically provides energy for the human diet in the form of carbohydrate (FAO, 1990).

Amorphophallus bulbifer Blume. is found to the Equatorial exotics of rare and beautiful tropical plants of the world (<http://www.equatorialextotic.com/gallery.htm>). The corms are used for edible purpose (curries and pickles) only after long washing and prolong cooking. The tender petioles are also edible (Wealth of India, 1956).

Amorphophallus bulbifer Blume is a perennial plant and a member of the family Araceae. It is a wild growing habitat and also known as Wa-u, Phyan-u in Myanmar. English name is elephant yam or elephant food yam. In China, Japan and Indonesia, it is also called konjac plant.

The original home of the konjac plant is considered to be in Southeast Asia. There are many species of konjac plant in the East and Southeast Asia that belong to the *Amorphophallus*, for example, *A. Konjac* C. Koch (Japan, China, Indonesia), *A. bulbifer* Bl. (Indonesia), *A. oncophyllus* Hook. (Indonesia), *A. variabilis* Bl. (Philippines, Indonesia, Malaysia). They contain Konjac mannan in their tubers.

Konjac glucomannan (KGM) is considered as one of the most viscous and water soluble dietary fibers. It is a high molecular weight polysaccharide mainly at a molar ratio of 1.6:1. Due to its β 1-4 linkage, KGM is resistant to salivary or pancreatic amylase enzyme, it directly passes in an unchanged form into the colon and it is then fermented by colonic bacteria (<http://www.sciencedirect.com>topics>).

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So, isolation of elephant yam starch, konjac starch was carried out from fresh tuber of *Amorphophallus bulbifer* Blume by the method of Bendenhurizen's 1984.

Aims and Objectives are

1. To study the morphological characters
2. To analyze the nutritive value and elemental components of powdered tuber
3. To isolate the elephant yam starch or konjac starch from fresh tuber of *Amorphophallus bulbifer* Blume.

Material and methods

Collection and morphological characterization

The plant specimen was collected in Hinthada District during the flowering and fruiting periods. Identification of the plants specimen was done by using Purse glove, 1972.

Macroscopical and microscopical characterization

The specimen was sectioned by free hand for microscopical studies with special emphasis on starch distribution. Macroscopical characters were studied by the method of Trease and Evans, 1978.

Preliminary phytochemical examination and physicochemical characterization

Phytochemical examination of powdered samples has been conducted with the method of Harbone, 1989. Physicochemical characterization has been examined with the method of W.H.O, 1998.

Nutritive value and elemental determination

Carbohydrate, protein and fat contents have been determined in the Laboratory of Pharmaceutical Research Department, Myanmar Scientific and Technological Research Centre. Fiber content has been carried out in Cottage Industries Department, Ministry of Co-operative. Vitamin B1 and Vitamin C contents have been determined in National Nutrition Centre. EDXRF spectrophotometry has been analyzed at Universities' Research Centre, University of Yangon.

Starch Isolation

Identification and characterization of isolated starch

Isolated starch 1 gm was mixed with 15 times its weight of water and boiled. When the solution was cooled, a translucent viscous fluid was obtained. When iodine reagent solution was added, a deep blue colour was observed. The blue colour disappeared on boiling and reappeared on cooling. Microscopic examination of isolated starch was made by using light microscope.

Gelatinization Temperature and Amylose and Amylopectin determination

The gelatinization temperature of isolated starch was carried out by Kerr's, 1950. The amylose of isolated starch was determined by the method of Kerr and Turbell, 1993.

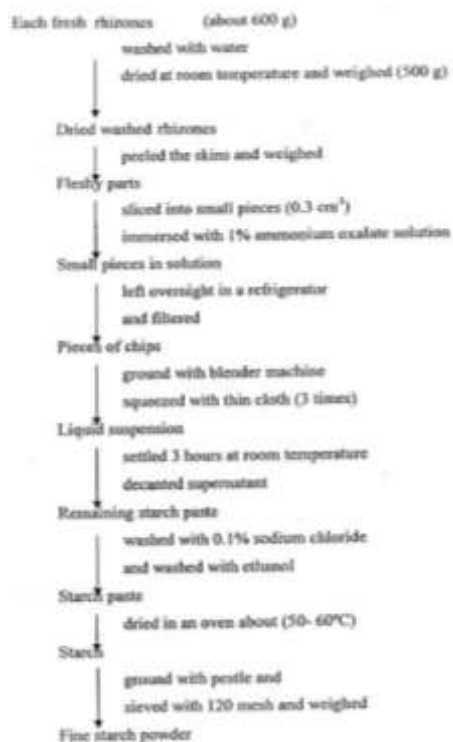


Figure (1) Flow chart for starch isolation

Table (1) Absorbance measured at 680 nm for iodine-stained mixtures of amylose-amylopectin

Composition		Wave Length (nm)	Absorbance measured
% Amylose	% Amylopectin		
0	100	680 nm	0.022
20	80	680 nm	0.102
40	60	680 nm	0.184
60	40	680 nm	0.265
80	20	680 nm	0.345
100	0	680 nm	0.428

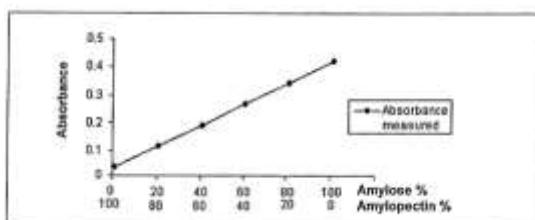


Figure (2) Standard curve of light absorbances of 680 nm against composition of iodine-stained amylose-amylopectin

Light absorbance of isolated starch solution was measured of 680 nm in using UV-visible spectrophotometer. A standard curve was drawn between light absorbances and composition of amylose-amylopectin.

Results

- Scientific name - *Amorphophallus bulbifer* Blume.
- English name - Elephant yam, Elephant food yam
- China, Japan name - Konjac
- Myanmar name - Wa-u, Phyan-u
- Family - Araceae

Morphological outstanding characters

Annual herbs; tuber depress globose, dark brown in color. Solitary, incised with bulbils of the intersection and on the rachises; petiole dark green with pink molting. Spadix; peduncle dark green with pink, appendage ovoid. Pistillate flower lower, reddish brown in colour; neutral flower absent, stamen stout, asymmetrical. Bicarpellary, one to two ovules in each locale, ovaries depress globose, basal placentation, the style sessile, the stigma discoid fruits, Berries, ellipsoid, red in colour. Seeds obovoid, grey in colour.

Morphological characters of *Amorphophallous bulbifer* Blume.



Fig. (3) view of wild plants in natural habit



Fig. (4) close-up view of a plant habit



Fig. (5) close-up view of a leaf



Fig. (6) close-up view of an inflorescence



Fig. (7) close-up view of male and female flowers

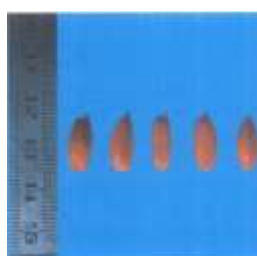


Fig. (8) close-up view of fruits



Fig. (9) close-up view of corms

Table (2) The macroscopical characters of *Amorphophallus bulbifer* Blume.

S.N	Sample	Part used	size	shape	surface characters	character in T.S
1	<i>Amorphophallus bulbifer</i> Blume.	Corm	L=7-11 cm B=8-14 cm	Subglobose depress	Dark brown with warty surface and small root hairs	Creamish white and fleshy

Microscopical characters of *Amorphophallus bulbifer* Blume.

In transverse section, the corm is circular in outline. Epiblema is composed of 4-5 layers in starch free parenchymatous. Cell walls are thick and brown in color. Under the layers of epiblema, ground tissues are found as two zones (outer and inner). The outer ground tissues are composed of 20-26 layers of small, compact parenchymatous cells with starch grains. The inner ground tissues consist of the thin wall, relatively large parenchymatous cells which are loosely packed with abundant starch grains. Vascular bundles are found as collateral types; small, rounded and oval in outline and irregularly scattered in ground tissues. Bundles of needle shape calcium oxalate crystals (raphides) are also found in the ground tissues. The distribution of starch grains is more abundant in the inner zone of the ground tissues.



Fig. (10) *Amorphophallus bulbifer* Blume.



Fig. (11) Inner regions of corm (x200)



Fig. (12) Outer regions of corm (x 100)



Fig. (13) Raphides (x 400)



Fig. (14) Close-up view of cells with starch grains (x 400)

Table (3) Sensory characters of powdered corm

S.N	Sample	Colour	Texture	Odour	Taste
1	<i>Amorphophallus bulbifer</i> Blume.	Dark brown	Granular	Slightly pungent	Irritant, tasteless

Table (4) The microscopical diagnostic characters of powdered corm

S.N	Sample	Fragment of epidermal cell	Starch grains	Fragment of fiber	Vessel element
1	<i>Amorphophallus bulbifer</i> Blume.	50-70µ	abundant	L=100-230µ B=5.0-7.5µ	L=70-230µ B=12-25µ (Scalariform)



Fig. (15) Fragment of epidermal cell (x 400)



Fig. (16) Starch grains (x 400)



Fig. (17) Fiber (x 400)



Fig. (18) Vessel element (x 400)

Preliminary phytochemical examination and physicochemical characterization

Table (5) Preliminary phytochemical examination

No	Types of Product	Test reagent	Observation	Results
1	Starch	I ₂ solution	Blue black ppt	+
2	Carbohydrate	10% α Naphthol, Conc: S/A	Red ring	+
3	Saponin	Distilled water	Frothing	+
4	Reducing sugar	Benedict's solution	Brick-red ppt	+
5	α amino acid	Ninhydrin reagent	Pink purple	+
6	Glycoside	10% lead acetate	White ppt	+
7	Alkaloid	Mayer's reagent Dragendroff's reagent	White ppt Orange ppt	+
8	Steroid	Benzene, Acetic anhydride and Con: S/A	Green ppt	+
9	Phenolic compound	FeCL ₃ solution	No coloration	-
10	Cyanogenic glycoside	Con: S/A & sodium picrate paper	No coloration	-
11	Tannin	Ferric chloride test	No coloration	-

+ Present, - Absent

Table (6) Physicochemical characterization

S.N	Physicochemical characters	Percentage
1	Water content (fresh tuber)	77.53
2	Moisture content of powdered sample	8.94
3	Total ash content	11.35
4	Water soluble matter content	11.43
5	Ethanol soluble matter content	3.20
6	Petroleum soluble matter content	1.62

Nutritive values and elemental determination

Table (7) Nutritive values

S.N	Nutritional components	percentage
1	Carbohydrate	18.92
2	Protein	1.2
3	Fat	0.1
4	Fiber	2.325
5	Vitamin B1	0.13mg
6	Vitamin C	16.25mg

Table (8) Elemental determination

S.N	Nutritional components	percentage
1	K	0.935%
2	Fe	0.009%
3	Ca	N.D
4	Zn	0.005%
5	Mn	N.D
6	CH balance	99.051%



Figure (19) Elemental analysis of *Amorphophallus bulbifer* Blume by EDXRF spectroscopy

Starch isolation from fresh corms of *Amorphophallus bulbifer* Blume.

Starch was isolated from fresh corms, by using Badenhuizen’s 1984. Yield percentage of isolated starch was found as 2.250%. Percentage of waste product was 5.212% respectively.

Identification and characterization of isolated starch

Isolated starch has been identified by using 2% iodine solution B.P. Iodine solution test indicated that sample was starch.



Fig. (20) *Amorphophallus bulbifer* Blume

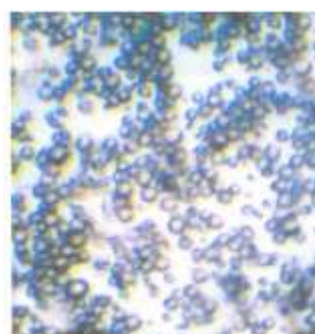


Fig. (21) Starch grains of *Amorphophallus bulbifer* Blume. (x 400)

Table (9) Characterization of isolated starch grains

S.N	Sample	characters	size	shape	Position of hilum	striation
1	<i>Amorphophallus bulbifer</i> Blume.	Simple and compound grains	2-6 μ	Rounded to oval in shape	A small point central hilum	No striation

Table (10) Amylose and amylopectin content of isolated starch by using UV visible spectrophotometric method.

S.N	Sample	Absorbance measured	Amylose content %	Amylopectin content %
1	<i>Amorphophallus bulbifer</i> Blume.	0.090	18.00	82.00

Gelatinization Temperature and Amylose and Amylopectin content determination

Gelatinization temperature of isolated starch was found as 65.48°C.

Discussion and Conclusion

Amorphophallus bulbifer Blume. was found as growing wild and cultivated for economic purpose.

In microscopical studies, epiblema was found to have 4-5 layers with thick wall parenchymatous. Vascular bundles were irregularly scattered in outer and inner ground tissues. Bundles of Calcium oxalate crystals were found in ground tissues. The distribution of starch was more abundant in inner ground tissues. Starch grains were simple and compound grains, rounded to oval, a small point hilum. These characters were in accordance with those mentioned by Santa (1999) and Wicken (2001).

Table 9 Characterization of isolated starch grains

In photochemical study, phenolic compound, cyanogenic glycoside and tannin were found to be absent. Starch, carbohydrate, saponin, reducing sugar, α amino acid, glycoside, alkaloid and steroid were found to be present. In nutritional studies, Vitamin C content was found to be 16.25mg%. Fiber content was found to be 2.325%. It was good source for Vitamin C and fiber diet in human nutrition. In elemental studies, potassium content was found to be the highest. It was also useful for elemental nutrition.

Isolated starch was found to be 2.250% and percentage of waste product was 5.212%. Konjac starch or elephant yam starch is used as medical and various kinds of food and cosmetic application. The main carbohydrate, mannose, glucomannan of konjac starch is a soluble dietary fiber that is not absorbed by humans. Due to its β -1-4. linkage, konjac glucomannan is resistant to salivary or pancreatic amylase enzyme. It is also used as a health food to reduce blood cholesterol and triglyceride level in humans in Japan (Cui et.al, 2013).

At present, dried tuber chips of some Myanmar *Amorphophallus* species have proved to be a good source for foreign exchange in the Hinthada District.

According to this research, *Amorphophallus* species should be widely cultivated with the organic fertilizers. Organic konjac starch and their food products should be produced and

participated the starch and their products of world trade level to earn foreign exchange and upgrad the socio-economic standard of local people in Myanmar.

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