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Variation in physical standards of milk yam (*Ipomoea digitata* L.) tubers during its growth and development

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Abstract
Milk yam is an underutilsed medicinal plant with proven functional potential with a wide range of therapeutic benefits and its folkloric use as nutraceutical. This study was aimed on evaluation of physical standards of milk yam tubers during its different growth stages so as to assess its optimum stage of harvest maturity for better drug quality. Milk yam vine cuttings having two nodes were raised in polybags and depotted carefully after tuber formation at tri-monthly intervals for a period of two years and subjected to quantitative phytochemical analysis. Tubers harvested at twenty one months after planting recorded optimum physical standards viz., minimum moisture content (57.80 per cent), optimally less crude fiber (6.19 per cent), high ash values (total ash -4.47 per cent, water soluble ash – 3.22 per cent and acid insoluble ash – 0.60 per cent), starch content – 39.17 per cent and pH value, 5.29. Twenty one months after planting is identified as optimum maturity stage for harvesting the tubers with better quality.

Keywords: harvest, maturity, nutraceutical, phytochemical, ksheervidari, standards

Introduction

Ipomoea digitata L. (2n=30) is a type of morning glory plant commonly called Milk Yam in English, Ksheervidari in Sanskrit and Vidharikand in Hindi. Even though origin of milk yam is unknown it is pantropic, naturalized in moist tropical regions (river banks, marshy areas) of India and many other parts of the world [25]. Functional potential of milk yam has already been proved and it has got a wide range of therapeutic benefits as well as its folkloric use as a nutraceutical [47]. Its tubers are considered as an alternate source of *Vidari* and is widely used to prepare popular Ayurvedic nutraceutical products like Chavanaprasha [44].

Maturity of the officinal part is an important criteria for gaining maximum potency for developing formulations out of it. Only mature (bigger size) tubers of *I. digitata* L. have been used for preparing galactagogues and immune-modulatory herbal medicines by the traditional medical practitioners. Khan *et al*. [17] reported that immature tubers had 4.00-12.50 mm length and 6.00-10.10 mm girth whereas mature tubers had 9.00-12.20 mm and 23-23.10 mm, length and girth respectively and concluded that girth of tubers determined the maturity of the plant. Dried cut pieces of *I. digitata* L. tubers had light brown outer surface transverse warts and ridges on the epidermis. The cut surface was creamy and fleshy with a smooth texture, sweet taste and had no particular smell. Milk yam tuber powder is greyish brown in colour having no characteristic odour and bitter in taste. Vessels have simple and scalariform cross perforation plates, stone cells and starch [3].

Determination of proximate composition plays a significant role for the standardization of crude drugs [11]. The specifications laid out by The Ayurvedic Pharmacopoeia of India [3] for milk yam tuber powder are, foreign matter-not more than two per cent, moisture content-not more than ten per cent, total ash-not more than six per cent, acid insoluble ash-not more than one per cent, alcohol soluble extractive-not less than 20 per cent, water soluble extractive-not less than eight per cent and starch-not less than 14 per cent. The present study is done with the objectives of finding the maturity stage of milk yam with optimum physical standards so that it can be harvested at its best possible quality.

Materials and Methods

The experiments were conducted at the Instructional Farm, College of Agriculture, Vellayani, Thiruvananthapuram, (8^0 25’ 46” N latitude & 76^0 59’ 24” E longitude) from September 2015-September 2017. Milk yam vines of a local ecotype collected from the Instructional Farm, College of Agriculture, Vellayani was used for the study. A herbarium of *I. digitata* L. was prepared in duplicate and submitted to the internationally recognized Janaki Ammal Herbarium (RRLH) [Accession No.-23207] and authenticated from CSIR-Indian Institute of Integrative Medicine, Jammu.

Healthy rooted cuttings were raised in polybags (40x24x24 cm and 600 gauge thickness), filled with potting mixture (soil, sand and farm yard manure in equal proportions). The polybags were then placed in the experimental plot and the vines were trailed in pandal. The polybags are depotted carefully after tuber formation (three months after planting) at tri-monthly intervals for a period of two years and subjected to analysis of physical standards.

Tubers were made free from aerials parts, rootlets and adhering sand particles by thoroughly washing under running tap water, then chopped into approximately one centimeter cube pieces and dehydrated in a hot-air oven at 45±2 ^{0}C. The dried pieces were coarsely powdered and passed through sieve No. 40 and stored in airtight glass containers under refrigeration for further studies [15].

Determination of Physical Standards

Moisture Content (per cent)

Fresh milk yam tubers were shredded into approximately three millimeter size. A representative sample (10 g) of prepared crude drug was taken into previously weighed moisture cups and dried in hot-air oven at 65 ^{0}C until it gained a constant weight for estimating moisture content using gravimetric method and expressed as percentage [4]. Moisture content (per cent) was calculated using the formula.

$$\text{Moisture content} = \frac{\text{Initial sample weight (g)-Final sample weight (g) x 100}}{\text{Initial sample weight (g)}} \text{ (per cent)}$$

Total Ash Percentage (per cent)

The carbon free residue left after incineration of tuber powder in a muffle furnace at a temperature not exceeding 450 ^{0}C for two-three hours was weighed out and total ash content was calculated using the formula given below and expressed in percentage [2].

$$\text{Total ash (dry basis) per cent by weight} = \frac{(W_2 - W) \times 100}{(W_1 - W)(100 - M)}$$

Where,

W = Weight of empty dish (g)

W_1 = Weight of dish + sample (g)

W_2 = Weight of dish + ash (g)

M = Moisture content of the sample (per cent)

Acid Insoluble Ash (per cent)

The total ash present in the sample when treated with hydrochloric acid reacted with minerals and form soluble salts. The acid insoluble ash, the residue left behind consisting mainly of silica, was calculated using the formula given below and expressed in percentage [2].

$$\text{Acid insoluble ash (per cent)} = \frac{[(W_2 - W_1) \times 100]}{W}$$

Where, W = Weight of the sample (g)

W_1 = Weight of empty dish (g)

W_2 = Weight of dish + ash (g)

Water Soluble Ash (per cent)

The total ash present in the sample when boiled in water and Filtered through an ash less filter paper and the insoluble matter obtained was further ignited at a temperature not exceeding 450 ^{0}C. The difference in weight of total ash and insoluble matter in the sample was considered as water soluble ash and was expressed in percentage [2].

Crude Fiber (per cent)

Crude fiber content in milk yam tuber powder was estimated using the method described by Sadasivam and Manickam [36]. Percentage of crude fiber in the sample was calculated as follows.

$$\text{Crude fiber content (per cent)} = \frac{\text{Weight loss for the sample after ignition (g)}}{\text{Weight of the sample taken (g)}} \times 100$$

Starch Content

After extracting sugars with 80 per cent ethyl alcohol, starch was solubilized with dilute per chloric acid, and determined colourimetrically by sugar-anthrone-sulphuric acid reaction [24].

Ph Value

The pH value of milk yam tuber powder was recorded using electronic pH meter (Model-MK VI) [37].

Results and Discussion

Adulteration and substitution in crude drug is quite common now a days and hence, adequate quality control strategies has to be developed [10]. Analysing the physical standards can give valuable information for assessing the quality and maturity of the crude drugs [41]. Physical standards of milk yam tubers viz., moisture content (per cent), total ash (per cent), water soluble ash (per cent), acid insoluble ash (per cent) crude fiber (per cent), pH value and starch content (per cent) significantly varied during different stages of growth from third MAP to 24th MAP and are shown in Table 1.

Change in Moisture Content (per cent)

It is clear (Table1) that during initial stages of tuber development (third MAP) milk yam tubers possessed very high moisture content, 95.43 per cent. Toyohara *et al.* [42] explained that it might be due to the high moisture holding capacity of young parenchyma tissues. As growth progressed, moisture content decreased and reached a minimum value, 61.60 per cent by 12 MAP when the milk yam plant was in its reproductive stage. Similar reduction in moisture content during early stages of growth was reported in Greater yam (*Dioscorea alata*) by Toyohara *et al.* [42]

Further, a slight increase in moisture content occurred when vegetative phase resumed from 13th MAP onwards and again attained maximum value during 18th MAP (66.20 per cent). Senescence commenced in the aerial parts during 18th MAP onwards and continued

Table 1: Physical standards of milk yam (*Ipomoea digitata* L.) tubers at different maturity stages

Months After Planting (MAP)	Parameters						
	Moisture content (per cent)	Total ash (per cent)	Water soluble ash (per cent)	Acid insoluble ash (per cent)	Crude fiber (per cent)	Starch (per cent)	pH value
T1 (3MAP)	95.43	0.39	0.25	0.23	0.95	2.07	5.40
T2 (6MAP)	89.67	2.19	1.45	0.30	1.29	8.47	5.37
T3 (9MAP)	78.93	2.43	1.62	0.33	1.45	18.60	5.34
T4 (12MAP)	61.60	4.32	2.71	0.43	3.95	31.97	5.24
T5 (15MAP)	65.50	3.08	1.99	0.47	5.85	27.27	5.93
T6 (18MAP)	66.20	3.23	2.13	0.47	6.16	27.30	5.33
T7 (21MAP)	57.80	4.74	3.22	0.60	6.19	39.17	5.29
T8 (24MAP)	63.93	3.69	2.17	0.62	7.30	34.10	6.13
S.Em ±	1.27	0.28	0.13	0.04	0.22	1.93	0.12
CD (P=0.05)	3.795	0.823	0.385	0.125	0.660	5.784	0.351

accompanied with enhanced dry matter accumulation in the tubers which was efficiently favoured by hot and dry weather conditions experienced during that period (August 2016-April 2017) (Fig. 1 & Fig. 2). Khatun *et al.* [18] opined that decrease in moisture content with the advancement of maturity was due to the accumulation of solid and mineral matters in the tissues. Analysis of milk yam tubers of 21 MAP also showed higher starch deposition and remarkable reduction in moisture content too. The present finding coincided with the trend followed by moisture content of Greater yam [12] and Potato [20] tubers during its developmental stages.

It is noteworthy here to speculate that a further reduction in moisture content might had occurred during 22nd and 23rd MAP when senescence progressed followed by flowering and fruiting. Nevertheless, the down pours happened during the final harvest (24 MAP) effected further moisture gain by the tubers (63.93 per cent).

Moisture content of several medicinal plants reviewed by Ranade and Acharya [34] had detailed the influence of weather on moisture changes during growth and development. Succinctly, mature milk yam tubers (21-24 MAP) consisted of more than 50 per cent water and could be considered as a highly hydrated storage organ for counteracting stresses during growth as proclaimed for potatoes by Sowokinos [39]. Investigations on moisture content of *Ipomoea batatas* [35], *Ipomoea staphylina* [27], *Psophocarpus tetragonolobus* [30] and wild and edible *Ceropegia sp.* Tubers[8] also recorded such a higher moisture content of 66.00, 66.74, 60.10 and 75.82-78.24 per cent respectively.

Change in Ash Values (per cent)

Total ash content of milk yam tubers represents total mineral matter present within the drug and it can be the cause of several pharmacological properties [41]. Total ash consists of physiological ash and non-physiological ash, the former being mineral matter derived from the plant tissue and the latter represents residue of extraneous matter like

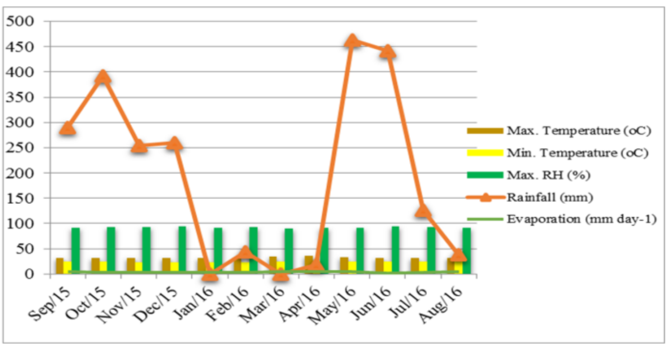

Fig 1: Weather data during Sept. 2015 to Aug. 2016

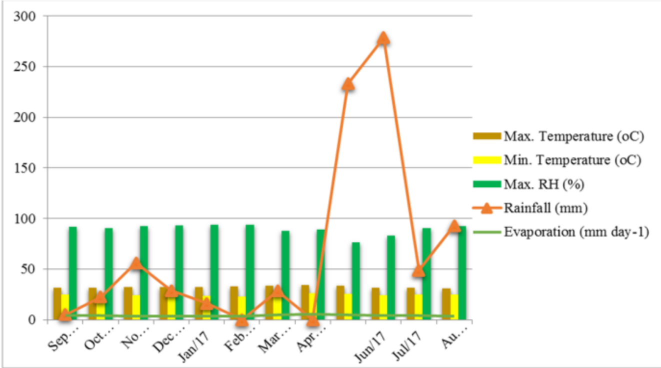

Fig 2: Weather data during Sept. 2016 to Aug. 2017

Tiliaceous or earthy mineral contamination [7]. In a crude drug, mineral matter soluble in water are indicated as water soluble ash and those which are insoluble in acids are called acid-insoluble ash[19].

Table 1. compile the data on ash values of milk yam tubers described that nearly one third of the mineral matter was water soluble and the rest would therefore be acid insoluble. In milk yam plants, during first year of growth ash values were found to be increasing from three MAP to 12 MAP. Subsequently a remarkable reduction in total ash and water soluble ash content was observed during 15 MAP. Data on ash values of *Dioscorea rotundata* (Oshei cultivar) and *Dioscorea dumetorum* (Jakiri cultivar) [43] as well as *Dioscorea alata* tubers [40, 12] also exhibited such a reduction in total ash content during its growth and development. Remobilisation of minerals from the tubers and utilizing it for the successive vegetative growth effected it [20]. Likewise, remobilisation of minerals from the senescing shoots to the tubers might be the reason for highest total ash and water soluble ash content recorded over 12 MAP (4.32 and 2.71 per cent respectively) and 21 MAP (4.74 and 3.22 per cent respectively)[31]. Hot and dry weather conditions prevailed during the second year of growth of tubers might have resulted in higher water soluble ash during the period. Data collected on *Calotropis gigantia* [45] and *Sesbania grandiflora* [28] also supported this conclusion. Howbeit, ash values of second year growth was found to be higher than first year. Studies on medicinal plants like *Desmodium gangeticum* [14], *Moringa oleifera* [18] etc. supported this observation.

On the other hand, acid insoluble ash kept on increasing during entire growth period and reached its maximum (0.62 per cent) during final harvest (24 MAP) which was on par with 0.60 per cent recorded during 21 MAP, both values satisfying the standards put forward by AYUSH [3]. Shinkar [38] expressed his views on high acid insoluble ash owing to more calcium oxalate crystals in plants. High calcium oxalate content represents high acid insoluble ash which is not good for milk yam tubers according to AYUSH [3].

On the contrary, total ash and water soluble ash increased up to 21 MAP followed by a notable reduction during final harvest (24 MAP). Total ash content of *Ipomoea staphylina* (2.75 per cent) also supported the ash value of milk yam tubers. However, the amount of total ash estimated from 21 MAP to final harvest could meet the standards put forward by AYUSH [3] which suggests its potential drug action. Total ash content of mature and immature milk yam tubers reported by Khan *et al*. [17] is not in agreement with the present finding since, factors like geographical location, stage of maturity, watering regimes, nutrient status of the soil, weather conditions etc. also influence the total ash content [5].

Change in Crude Fiber (per cent)

Fiber and protein content are considered as two essential parameters for determining the nature of foods [9]. Fiber rich foods had boundless health benefits by virtue of its competence in alleviating bowel problems like constipation than any other food constituent [1]. Furthermore, it is benign for reducing blood cholesterol, reducing cancer risk etc. [32].

An increase in crude fiber content of milk yam tubers all along the stages of its growth and development is recorded in Table 1. During early stages of tuber development, up to 9 MAP (0.95-1.45 per cent) any phenomenal increase in fiber content was recorded. Fiber content cropped up during 12 MAP (3.95 per cent) coinciding with the shoot regrowth preceded by flowering and shoot senescence. As vegetative phase, resumed fiber content increased significantly from 15 MAP (5.85 per cent) and remained on par up to 21 MAP (6.19 per cent). Such an increase in crude fiber content of milk yam tubers recorded during different stages in the two years growth period could be ascribed to the dilution of fibers during the accumulation of starch [43].

Moreover, crude fiber content of mature milk yam tubers harvested during 18, 24 and 21 MAP [6.16, 6.19 and 7.30 per cent respectively] were comparatively higher than several edible tubers [29]. The appreciable increase in crude fiber content during 24 MAP remained unconvincing since, it might be on account of the regrowth favoured by rains during August 2017. Inquiry on crude fiber content of winged yam tuber (*Psophocarpus tetragonolobus*) revealed it as 2.76 per cent [30] and for wild and edible *Ceropegia sp*. tubers as 8.70-9.10 per cent [8]. Hence, mature milk yam tubers harvested on or after 18 MAP can be regarded as a wholesome competitor of other fiber rich tubers.

Change in Starch Content (per cent) and pH value

Starch is the main insoluble storage carbohydrate in the tubers [22]. By examining Fig. 3. It is easy to narrate the trend in starch content and pH value of milk yam tubers during its growth and development. At all stages of maturity, pH value of milk yam tubers remained acidic, besides, starch content and pH value were inversely related. It is seen that up to 12 MAP starch content steadily increased (2.07-31.97 per cent) and pH value decreased (5.40-5.24). During 15 MAP, though plant regrowth significantly affected starch accumulation (27.27 per cent) an upsurge occurred during 21 MAP (39.17 per cent). Finally, resumption of vegetative growth over 24 MAP caused a slight but insignificant decrease in starch content. A corresponding increase in pH value also occurred during these period.

During the aforementioned starch accumulation periods in milk yam tubers, efficiency of starch bio-synthesis enzyme machinery might be superior to the degrading enzymes [21]. A thorough study on the enzyme starch synthase on Cassava variety F01 revealed 1.40 times higher activity during tuber bulking stage than that at tuber maturity. For potatoes, maximum starch content was recorded during shoot senescence [13], milk yam tubers also followed a similar trend. Conversely, starch degrading enzymes were active during crop regrowth and hence resulted in higher pH value. Higher pH value recorded during 15 and 24 MAP were attributed to the degradation of starch to sucrose, glucose or fructose [26]. Since, it was likewise reported in storage of potato tubers that total soluble solids increased with increase in storage time. The higher pH value could also be assignable to declining organic acid content [6] or polyphenol content [46] as these were used up for respiration or converted to sugars.

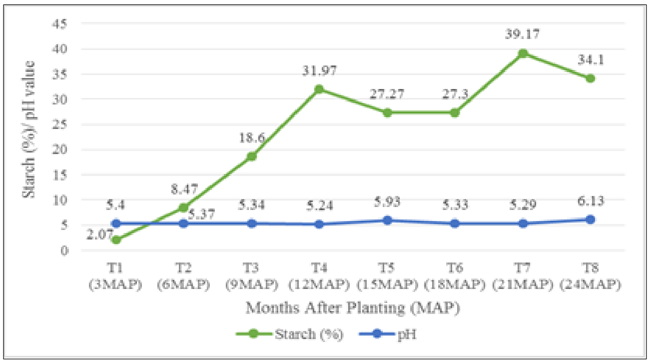

Fig 3: Change in starch content and pH value of milk yam (*Ipomoea digitata* L.) tubers during different maturity stages

Even with a few ebb and flow in starch content of milk yam tubers (Fig. 3.) an overall increment was noticed during the second year of growth. Several studies conducted in different tubers like Cassava [21], Yams [16, 43] and Potato [23, 20, 12] on starch content during it growth and development stages also recorded higher starch accumulation with growth advancement. All along the growth and development stages of milk yam tubers, a positive relationship between starch accumulation and dry matter content as well as negative relationship between starch accumulation, moisture content and pH value could be derived (Table 1. & Fig. 3). As regards the starch content investigated herein, optimum harvesting stage for milk yam tubers could be 21-24 MAP. Hence, it may be suggested that pH value of 5.29 (21 MAP) or above is an indication of maturity of milk yam tubers. Milk yam tubers are a good contestant to sweet potatoes as its starch content is comparable with starch content of *Ipomoea batatas* (14.20-34.10 per cent) [33].

Conclusion

Proximate composition of milk yam tubers including physical standards could judge the quality of tubers. It could be understood that physical standards vary with weather conditions. Milk yam tubers having appropriate physical standards viz., total ash (4.74 per cent), acid insoluble ash (0.60 per cent), water soluble ash (3.22 per cent), crude fiber (7.30 per cent) and pH value (5.29) as per AYUSH (2006) could be noticed when the tubers were in 21 months after planting and hence can be harvested at this stage with maximum possible quality.

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