Pharmacognostical and Histochemical Studies on Apakva Kadali (Unripe Banana Fruit): Musa × paradisiaca L.

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ABSTRACT
The unripe fruit of Musa × paradisiaca L., classically known as apakva kadali, belonging to the family Musaceae, is used as pata-thya in atisara (diarrhea), in grahari (irritable bowel syndrome), and agnimandya (loss of appetite). The unripe fruits are considered to be helpful in the management of diabetes mellitus. Being an important medicinal plant, the present investigation is an attempt to explore the pharmacognostical analysis and thin-layer chromatography fingerprint studies on the widely used Musa × paradisiaca L.


INTRODUCTION
Kadali/apakva kadali (unripe banana fruit), commonly sold in the market as aadige bale kayi/curry bale/bale kayi, is a berry with mucilaginous nature, botanically equated to Musa × paradisiaca L., belonging to the family Musaceae. The plant contains high content of resistant starch and other essential nutrients, which are considered to be helpful in controlling the sugar in diabetic patients. It is a tree-like herb, up to 9 m in height; leaf sheaths are tubular, forming a thick trunk; leaf blade is ca. 1.5 m, oblong, usually ragged in appearance, splitting between the transverse parallel veins; spike ca. 1 m, drooping, peduncle thick, bracts opening in succession, 15 to 20 cm, ovate, concave, dark red, somewhat fleshy, outer tepals 22 to 24 mm, five-toothed, fleshy, tinged pink, inner tepals 19 to 20 mm, ovate, acute, and concave; and stamens 5, fruit oblong, fleshy. In the wild form, 5 to 7 cm with seed, seedless, and longer in the cultivated varieties are found.

The unripe fruit is astringent in taste. It is boiled in mustard oil and made into a paste, and the paste is useful in external application for measles and eczema. The literature revealed that major amount of starch, vitamin A, vitamin C, and potassium are present in the plant. Fruit peels showed the presence of chlorophyll, carotene, xanthophylls, carbohydrates, pectin, crude protein, tannins, flavonoids, terpenoids, alkaloids, glycosides, serotonin, norepinephrine, dopamine, and various macro- and microelements like Na, P, Ca, Mn, K, Fe, Zn, N, and Cu in detectable ranges.

The plant is reported to possess analgesic, antiulcer, antilithiatic, antimicrobial, antihypertensive, antidiarrheal, antiallergic, antioxidant, diuretic, hypolipidemic, antilithiatic, antimicrobial, antihypertensive, antidiarrheal, antiallergic, antioxidant, diuretic, hypolipidemic, hypoglycemic, hair growth promoting, hemostatic, muscle relaxant, mutagenic, wound healing, vasodilatory, and hepatoprotective activities.

Ayurvedic Pharmacodynamics
Rasa (taste): Tikta (bitter), kashaya (astringent); guna (properties): Ruksha (dry); vertya (potency): Sheeta (cold); doshakarma (effect on doshas): Pitta shamaka (pacifies pitta); karma: Sangrahi (blocks the movement of fecal matter), roochya (imparts taste); rogagnata: Trut (dyspepsia), raktapita (bleeding disorders), netraroga (diseases of the eye), prameha (diabetes), raktaitisara (bloody diarrhea).

Folklore/Ethnobotanical Relevance of Kadali Fruits
Musa × paradisiaca L. inner pulp mixed with lemon juice is used for lip blackness in Kashmir. People in Nepal who
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is used to treat dysentery problems in Odisha (India).11 histochemical, and preliminary phytochemical studies. study its macroscopic, microscopic, powder microscopy, (i.e., unripe banana) to undertaken on the apakva kadali and green bananas are cooked as a vegetable.13

The pharmacognostic evaluation is an important part of the drug standardization, more specifically to the natural drugs. The literature scrutiny on the subject reveals that no pharmacognostic study of the unripe fruits has been done so far.14-16 Therefore, the present study was undertaken on the apakva kadali (i.e., unripe banana) to study its macroscopic, microscopic, powder microscopy, histochemical, and preliminary phytochemical studies.

MATERIALS AND METHODS
Procurement and Processing of Plant Samples
The fresh fruit samples of apakva kadali were collected from three different vegetable markets of Bengaluru, India, namely, Krishna Rajendra Puram and Jayanagar. The fruits were identified with the help of Bengaluru flora and from the survey of medicinal plant unit, Regional Ayurveda Research Institute for Metabolic Disorders, Bengaluru, India, processed with the voucher specimen no 12361, and deposited in Regional Ayurveda Research Institute for Metabolic Disorders, Bengaluru, for future reference.

Macroscopic and Microscopic Analysis
Unripe banana fruits were cut into small pieces, shade dried, and powdered. Powdered material was used to carry out powder microscopy. Dried unripe banana fruits were soaked in 70% alcohol for 24 hours, and free hand sections were taken, cleared with chloral hydrate solution and water, and stained with safranin according to the standard prescribed methods.17 Photomicrographs were captured with Catcam camera. Powder and maceration studies were also carried out following the standard methods.17,18

Physicochemical Analysis
Physicochemical analysis, such as ash values and extractive values was carried out according to the standard procedures prescribed in Ayurvedic Pharmacopoeia of India.18

Preliminary Phytochemical Studies
Preliminary phytochemical screening was carried out for different extracts by using standard procedures.19

Extraction Procedure
The crude powder sample was successively extracted with petroleum ether, chloroform, and ethanol through Soxhlet extraction process and dried under reduced pressure by using rotary evaporator.

Thin-layer Chromatography
Processed fruit powder was extracted with petroleum ether, chloroform, and methanol at room temperature with the help of rotary shaker. Thin-layer chromatography (TLC) studies of these extracts were carried out by using commercially available precoated silica gel 60 F254 (Merck, Germany) plates with standardized adsortion layers. All the solvent systems were selected by trial and error basis. The chromatograms were developed in twin trough glass chambers on 10 × 10 cm plates till the mobile phase traveled up to a distance of 8 cm from starting point. Plates were dried at room temperature for 5 to 10 minutes and observed under ultraviolet (UV)-254; photographs were taken; and retention factor (Rf) values were recorded.20

Histochemical Study
Histochemical tests were also carried out for different parts with fresh samples as described by Krishanmurthy.21

RESULTS AND DISCUSSION
Macroscopic Characters
Fruit berries were up to 30 cm long, outer leathery fivewingd, shiny, greenish and mucilaginous when fresh; rough and black when dry; inner region white in color, hard, powdery, and with less or without seeds (Figs 1A to C).

Microscopical Characters
Transverse section of unripe fruit showed different specific characters. The outer layer epicarp, consisting of a single layer of epidermis made up of rectangular-shaped parenchyma cells covered by a thin cuticle, papillae-like outer protrusion from each epidermal cell, was also observed. Followed by epidermis, thick-walled irregular-shaped parenchyma cells were present, where these cells are compactly arranged and heavily loaded with abundant, oval-shaped starch grains. Sclerenchymatous cells were found in groups, encircled by thin-walled parenchymatous cells, tannin cells, and vascular bundles scattered in this region. Presence of 10 to 14 layers of compactly arranged parenchymatous cells, without air spaces and longitudinally extended, was found. Mesocarp showed loosely arranged tangentially elongated parenchymatous cells consisting of abundant oval starch grains, raphide
bundles with needle-like crystals, and few longitudinally extended parenchymatous cells containing tannin (Figs 2A and B).

**Powder Microscopy**

Powder is light brown to ash in color, rough to touch; smell agreeable with sweet taste. When observed under the microscope, different fragments of tissues were observed. Fragments of epidermal cells with papillae, different shape of parenchyma cells, sclerenchyma cells, reticulate helical vessels in groups, xylem cells in surface view, tannin-containing cells, and abundant starch grains in groups were found as shown in Figs 3A to H.

**Maceration Studies**

Macerate of unripe fruit showed disintegrated tissues of different polygonal type of epidermal cells in surface view, parenchyma cells in groups, and oval-shaped starch grains, single fiber and parenchyma cells, helical xylem vessel, tannin-containing cells, and abundantly scattered oval starch grains (Figs 4A to H).

Main diagnostic characters of the fruits could be summarized in the form of presence of small outer protrusion of papillae on the epidermis, presence of abundant tannin-containing cells in the epicarp, mesocarp, and in the powder of the drug. There are long air-spaced cells surrounded by the parenchyma cells in the epicarp. Presence of abundant starch grains and acicular crystals of calcium oxalate in the mesocarp portion, and the presence of helical xylem vessels, pitted xylem vessels, and acicular crystals in the powder microscopy were observed.

**Micrometric Details**

Micrometric details of unripe fruit were studied as per the standard procedures and the measurements were recorded with the help of Catcam software. The details of the same are presented in Table 1.

**Histochemical Analysis**

Histochemical studies were carried out by treating the fresh sections with different chemical reagents for the location of different phytochemicals in different regions. The details of the results are presented in Figures 5A to K and Table 2.

**Physicochemical Analysis**

Detailed results of physicochemical analysis and extractive values are presented in Tables 3 and 4.
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Preliminary Phytochemical Analysis

Preliminary phytochemical screening of different extracts of unripe fruit showed the presence of various secondary metabolites, viz., alkaloids, carbohydrates, proteins, phenols, saponins, steroids, and tannins. The details are presented in Table 5.

Thin-layer Chromatography

Different mobile phases were used for TLC studies, viz., toluene:hexane:ethyl acetate (6:3.5:0.5) for petroleum ether extract; toluene:hexane:ethyl acetate:chloroform: n-butanol (5:3.5:0.5:0.5:0.5) for chloroform extract; toluene:ethyl acetate (9.3:0.7) for methanol extract.
Table 2: List of stains and reagents used for the analysis of various histochemicals in different regions of unripe fruit

<table>
<thead>
<tr>
<th>Stain/reagent</th>
<th>Histochemical</th>
<th>Results</th>
<th>Locality of the cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluidine blue O</td>
<td>Polyphenols</td>
<td>Cell contents turn turquoise green to blue green color</td>
<td>Sclerenchyma cells and vascular bundles</td>
</tr>
<tr>
<td>Fast green</td>
<td>Protein</td>
<td>Proteins appear bright green</td>
<td>Sclerenchyma cells and vascular bundles</td>
</tr>
<tr>
<td>Sudan III</td>
<td>Lipids</td>
<td>Lipids/oils turn red color</td>
<td>Tannin cells and vascular bundle</td>
</tr>
<tr>
<td>Alkaline pyrogallol method</td>
<td>Calcium</td>
<td>Yellowish brown color</td>
<td>All parts</td>
</tr>
<tr>
<td>Silver hydrogen peroxide</td>
<td>Calcium oxalate</td>
<td>Black in color</td>
<td>–</td>
</tr>
<tr>
<td>Titan yellow</td>
<td>Magnesium</td>
<td>Brick red color</td>
<td>Epidermis, parenchyma cells, sclerenchyma cells, vascular bundles</td>
</tr>
<tr>
<td>Methyl red</td>
<td>Silica</td>
<td>Yellowish orange</td>
<td>Epidermal cells, sclerenchyma cells, tannin cells, vascular bundles</td>
</tr>
<tr>
<td>Iodine-potassium iodide reaction</td>
<td>Starch</td>
<td>Blue to black in color</td>
<td>Starch grains in the epicarp and mesocarp</td>
</tr>
<tr>
<td>Tannic acid–ferric chloride method</td>
<td>Calcium pectate</td>
<td>Blue to black in color</td>
<td>Epidermal cells, parenchyma cells, tannin cells, sclerenchyma cells, vascular bundles</td>
</tr>
<tr>
<td>Phloroglucinol method</td>
<td>Lignin</td>
<td>Red in color</td>
<td>–</td>
</tr>
<tr>
<td>Ferric chloride method</td>
<td>Tannin</td>
<td>Bluish black or grayish black</td>
<td>Starch grains, tannin cells, vascular bundles</td>
</tr>
<tr>
<td>Toluidine blue O</td>
<td>Sulfated mucopolysaccharides</td>
<td>Pink to reddish purple color</td>
<td>All the cell wall of phenolic content cells changes to reddish purple color</td>
</tr>
</tbody>
</table>
The Rf values for different extracts were as follows: Petroleum ether extract (UV-254), after spraying with anisaldehyde sulfuric acid – 0.22, 0.30, 0.38, 0.64, 0.975; chloroform extract (UV-254) 0.45, after spraying with anisaldehyde sulfuric acid – 0.45, 0.725, 0.94; methanol extract (after spraying with anisaldehyde sulfuric acid) – 0.175, 0.275, 0.35, 0.41, 0.69 (Figs 6A to E).

DISCUSSION

The present study revealed that apakva kadali has highest potentiality and demand in the local vegetable market since it has rich nutritional values and is rich in resistant starch. Resistant starch helps diabetics as well as nondiabetics. Comprehensive studies showed the presence of some important diagnostic characters like of papillae on the epidermis, abundant tannin content cells in the epicarp, mesocarp region, abundant starch grains and acicular crystals of calcium oxalate in the mesocarp portion, helical and pitted xylem vessels, and acicular crystals in the powder microscopy studies. Histochemically, unripe fruit showed the presence of different histochemicals like phenols, protein, calcium oxalate, magnesium, starch, cellulose, calcium pectate, lignin, sulfated mucopolysaccharides, tannin, and silica contents. Phytochemical studies of the unripe fruits of banana showed the presence of alkaloids, carbohydrates, phenols, proteins, starch, tannins, steroids, and saponins.

CONCLUSION

It is concluded that further studies are required to evaluate and quantify the presence of different phytochemicals in the plant. For the same, high-performance TLC and high-performance liquid chromatography studies could be done. Plant is supposed to possess antidiabetic properties; however, further studies are required to scientifically validate the therapeutic effects and active compounds that are responsible for various therapeutic properties. The fibrous fruits are not only very good for the digestive purposes but also used in different ailments. Furthermore, there is need to expedite the knowledge regarding the uses and beneficial effect of the plant to the common people so that optimum utilization could be done.

ACKNOWLEDGMENTS

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हिंदी सारांश
फार्माकोनॉमिस्टिकल एंड हिस्टोकोमिस्टिकल स्टडीज़ ऑफ़ अपक्व कादली (अनराईफ़ड बनाना) — मूसा × पारदिसिअका एल.

शांता तिमाल्लली रामसेन,¹ प्रताप रेंटी मारवेल्ली,² पच्चाइमाल पीच्चाली,³ किसोर कुमार रामफुण्ड़ण,⁴ वंदना भारती,⁵ वैंकेतेवराल्लु गड्डम,⁶ देवेश तिवारी,⁷ अनुमम के. भंगर,⁸ नायोमान श्रीकांत,⁹ कर्तार एस. धीमान¹⁰

दर्दनार्य औषधियों में मूसेली कुटे के बुद्धावात्र में प्रयोग किये गए औषधीय वेदितियों के आयामों को काफी प्रमाणित किया गया है। जिसके साथ अन्य न्युक्टोक्रोपिक, माइक्रोक्रोपिक, नाउडर माइक्रोक्रोपिक, मेटानियन अयाम, प्रायोगिक पात्र रसायन विलोकन, चुंचक रसायन विलोकन एवं पतली पतल कोलेट्राप्राफी (टीएलएचसी) विशेषज्ञ आयाम को अनुसरण किया गया है। अयाम के फैले पतल के बाद, पूरी तरह से बाहर उत्तरायण पाए गए एवं एथरिक, मेटेक्सायर के अधिक टेंडिन कोशिकाओं की मौजूदगी पाई गई। अधिक बाद में अधिक रात्रिक कोशिका जो एथरिक में पैरिनिकाइन कोशिकाओं द्वारा चार तक के सीधे हुए पाए गए एवं मेटेक्सायर मामले में अधिक रात्रिक शेयर, राकेक्ट बूढ़ा, क्रिलियॉम ऑफेश्टेट के किस्मतिक क्रिप्टलस, आयाम पाए गए। ऑप्पेशन यों चिकित्सक परियोजनाओं का उपलब्धित किया गया है तब विभिन्न अभिज्ञानों के साथ अयामक कणों के साथ साथ परियोजना किया गया की जिसके परियोजनात्राव मुक्त, प्रॉटीन, क्रिलियॉम ऑफेश्टेट, मेक्सियम, स्टार्ट, मेक्सियम, क्रिलियॉम पेठेट, दिल्रिम, साल्फर न्युक्स कॉलेसाइड्स, टेंडिन एवं शिलीक आयाम पाए गए। पात्र रसायन अयाम द्वारा एक्टियोइड्स, कार्बिहाइड्रेट्स, फिनोल, प्रॉटीन, रात्रिक, टेंडिन, स्टार्ट, मेक्सियम, क्रिलियॉम से मौजूदगी वस्तुईं गई।