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

ABSTRACT

The unripe fruit of Musa × paradisiaca L., classically known as apakva kadali, belonging to the family Musaceae, is used as pa-thya in atisara (diarrhea), in grahami (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. The studies revealed that unripe banana fruit showed the presence of small outer protrusion of papillae on the epidermis, abundant tannin-containing cells in the epicarp and mesocarp. Elongated air-spaced cells are surrounded by the parenchyma cells in the epicarp, abundant starch grains in the mesocarp portion, and acicular crystals of calcium oxalate in raphide bundles. To detect the location of various constituents of the drug, sections of unripe fruits were treated with various reagents, and studies showed the presence of phenols, protein, calcium oxalate, magnesium, starch, cellulose, calcium pectate, lignin, sulfated mucopolysaccharides, tannin, and silica contents. Phytochemical studies showed the presence of alkaloids, carbohydrates, phenols, proteins, starch, tannins, steroids, and saponins.

Keywords: Antidiabetic, Histochemical, Microscopy, Musa paradisiaca, Phytochemical, Unripe fruit.

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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 rugged 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 stems 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, anti diarrheal, anti allergic, 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); veerya (potency): Sheta (cold); dosha-karma (effect on doshas): Pitta shamaka (pacifies pitta); karma: Sangrahika (blocks the movement of fecal matter), ruchya (imparts taste); rogagnnata: Trut (dyspepsia), raktapitta (bleeding disorders), netraroga (diseases of the eye), prameha (diabetes), rakttisara (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...
speak Thangmi language eat it raw and is used in many rituals by neighboring villages.9 Green fruit paste is used for cuts, diarrhea, and dysentery in Bangladesh.10 The fruit is mixed with Spondias pinnata (L.f.) Kurz. bark juice is used to treat dysentery problems in Odisha (India).11 Unripe fruit and flower is used for the treatment of diarrhea and dysentery in Manipur.12 In Kavrepalanchowk in Nepal, unripe fruit juice is given to treat diarrhea and dysentery; banana powder is applied to treat colic disease; 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 adsorption 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 five-winged, 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.

Table 1: Micrometric details of unripe banana fruit

<table>
<thead>
<tr>
<th>Type of cell</th>
<th>Minimum (measurements in µm)</th>
<th>Maximum (measurements in µm)</th>
<th>Average with standard error (measurements in µm)</th>
<th>Standard deviation (in µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillae</td>
<td>96.05</td>
<td>58.31</td>
<td>74.42</td>
<td>15.09</td>
</tr>
<tr>
<td>Parenchyma cells</td>
<td>83.15</td>
<td>33.24</td>
<td>57.37</td>
<td>12.78</td>
</tr>
<tr>
<td>Tannin cells</td>
<td>434.77</td>
<td>227.85</td>
<td>349.11</td>
<td>93.89</td>
</tr>
<tr>
<td>Air-spaced cells</td>
<td>559.23</td>
<td>56.01</td>
<td>241.39</td>
<td>123.85</td>
</tr>
</tbody>
</table>

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 oxalate</td>
<td>Black in color</td>
<td>All parts</td>
</tr>
<tr>
<td>Silver hydrogen peroxide</td>
<td>Magnesium</td>
<td>Brick red color</td>
<td>Epidermis, parenchyma cells, slerenchyma cells, vascular bundles</td>
</tr>
<tr>
<td>Titian yellow</td>
<td>Silica</td>
<td>Yellowish orange</td>
<td>Epidermal cells, slerenchyma cells, tannin cells, vascular bundles</td>
</tr>
<tr>
<td>Methyl red</td>
<td></td>
<td></td>
<td></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, vascular bundles</td>
</tr>
<tr>
<td>Phlorogluconol 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>

Figs 5A to K: Histochemical studies of Musa × paradisiaca L.: (A) Calcium contents 10×; (B) calcium pectate contents; (C) calcium contents 10×; (D) magnesium contents 10×; (E) magnesium contents in tannin cells 10×; (F) phenol contents 40×; (G) protein contents 10×; (H) silica contents 40×; (I) silica contents 10×; (J) starch contents 10×; and (K) tannin contents 10×
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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REFERENCES

हिन्दी सारांश

फार्म्यूनोर्मिटिकल एंड हिस्टोकोमिकल स्टडीज ऑफ अपक्वा कादली
(अनराइपेड बनाना) — मूसा × पारदिसियाका एल.

शाला तिजुमललकी रामप्रसान,¹ प्रताप रेंकी भारतेश्वर,² पवाईसाह भीमंकरः³ किशोर कुमार रामकृष्णा,⁴
वंदना भारती,⁵ वेंकटेश्वरलाल गड्डम,⁶ देवका पिंडर,⁷ अनुपम के. भंगल,⁸ नायनराम श्रीकांत,⁹
करतार एस. धीरेन्द्र¹⁰

वर्तमान अन्वेषण में मूसेली कुट के बुद्धावत में प्रयोग होने वाले मूसा पैराविहित एल. (केले) के अपक्वा फलों की
फार्म्यूनोर्मिटिकल का अध्ययन किया गया। जिसके अलावा मेटलोसॉफिकल, गाइडरिनोसॉफिकल, पाउडर माइक्रोसॉफिकल, मेटलोरिजेशन
अध्ययन, प्रारंभिक पादप रसायन विश्लेषण, उत्तर पादप रसायन विश्लेषण एवं पादप प्रति कोलोनीफ्राइम (टीएलएस) विम्बिक्रिट आडि
के का अध्ययन का प्रयास किया गया है। अध्ययन में यह पता चला कि जब सूक्ष्मदर्शी द्वारा अपलोकन किया गया तो एमिटरिलिफ
पर पौधेका लग्भग उल्लोकण पाये गए। एवं एपीकार, मेसेकार्म में अधिक टेंफिन कोशिकाओं की मौजूदगी पाई गई। अधिक
बाद में एक वाली कोशिका को एपीकार में पैरैकाइकोण कोशिकाओं द्वारा चारी तरीके द्वारा भेजे गए गए तथा मेसेकार्म भाग में
अधिक वटर वेना, रेफ्रिक बिंगल, बीलियम ऑप्सिरिट्ड के एमिटरिलिफ क्रियाकार, आडि पाये गए। आँधियों के विभिन्न घटकों
की उपस्थिति का पता लगाने हेतु विभिन्न अधिकारिकाओं के साथ अपरिमाण फलों के भाग के साथ परीक्षण किया गया। तबसके
परिणामस्वरूप फिनोल, प्रोटीन, बीलियम ऑप्सिरिट्ड, मेंस्ट्रीशियम, स्टार्ट, सेंडुलोज, बीलियम पेंटेट, लिमनिन, सल्फेड न्यूक्से
पोलीसेक्ट्रोइड्स, टेंफिन एवं शिलिका आडि पाये गए। पादप रसायन अध्ययन द्वारा एक्स्कोइड्स, कार्बीहाइड्रेट्स, फिनोल, प्रोटीन,
स्टार्ट, टेंफिन, रिट्वेड्क्स, सेमिलिन्स की मौजूदगी दर्जा गई।