



## RESEARCH ARTICLE

# 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 *pathya* in *atisara* (diarrhea), in *grahani* (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 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.<sup>1</sup>

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.<sup>1</sup> The literature revealed that major amount of starch, vitamin A, vitamin C, and potassium are present in the plant.<sup>2</sup> 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.<sup>1,3</sup>

The plant is reported to possess analgesic, antiulcer, antilithiatic, antimicrobial, antihypertensive, antidiarrheal, antiallergic, antioxidant, diuretic, hypolipidemic, hypoglycemic, hair growth promoting, hemostatic, muscle relaxant, mutagenic, wound healing, vasodilatory, and hepatoprotective activities.<sup>2,4</sup>

## Ayurvedic Pharmacodynamics

*Rasa* (taste): *Tikta* (bitter), *kashaya* (astringent); *guna* (properties): *Ruksha* (dry); *veerya* (potency): *Sheeta* (cold); *doshakarma* (effect on doshas): *Pitta shamaka* (pacifies pitta); *karma*: *Sangrahika* (blocks the movement of fecal matter), *ruchya* (imparts taste); *rogaghmata*: *Trut* (dyspepsia), *raktapitta* (bleeding disorders), *netraroga* (diseases of the eye), *prameha* (diabetes), *raktatisara* (bloody diarrhea).<sup>5-7</sup>

## Folklore/Ethnobotanical Relevance of *Kadali* Fruits

*Musa* × *paradisiaca* L. inner pulp mixed with lemon juice is used for lip blackness in Kashmir.<sup>8</sup> People in Nepal who

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speak Thangmi language eat it raw and is used in many rituals by neighboring villages.<sup>9</sup> Green fruit paste is used for cuts, diarrhea, and dysentery in Bangladesh.<sup>10</sup> The fruit is mixed with *Spondias pinnata* (L.f.) Kurz. bark juice is used to treat dysentery problems in Odisha (India).<sup>11</sup> Unripe fruit and flower is used for the treatment of diarrhea and dysentery in Manipur.<sup>12</sup> 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.<sup>13</sup>

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.<sup>14-16</sup> 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.<sup>17</sup> Photomicrographs were captured with Catcam camera. Powder and maceration studies were also carried out following the standard methods.<sup>17,18</sup>

### 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.<sup>18</sup>

### Preliminary Phytochemical Studies

Preliminary phytochemical screening was carried out for different extracts by using standard procedures.<sup>19</sup>

### 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.<sup>20</sup>

### Histochemical Study

Histochemical tests were also carried out for different parts with fresh samples as described by Krishanmurthy.<sup>21</sup>

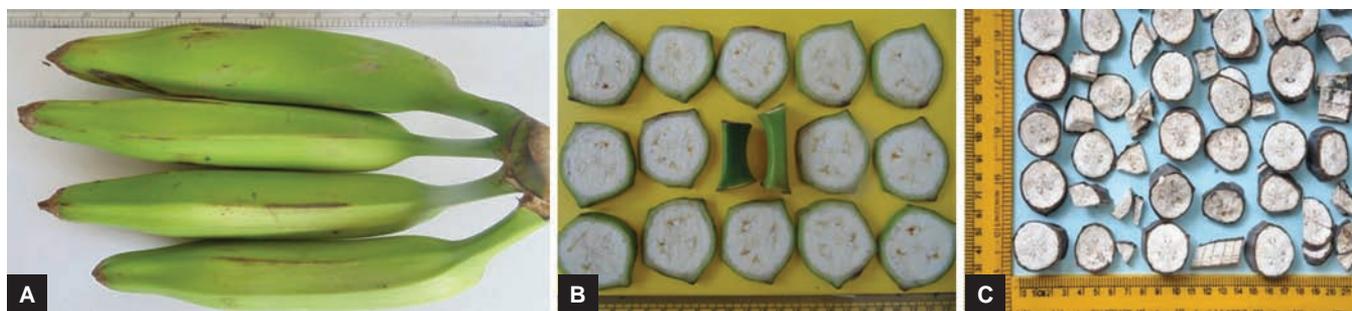
## RESULTS AND DISCUSSION

### Macroscopic Characters

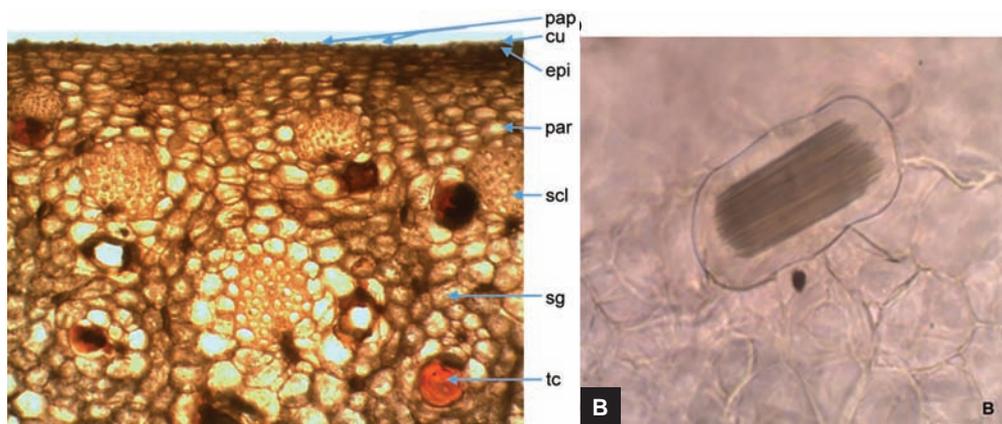
Fruit berries were up to 30 cm long, outer leathery five-winged, shiny, greenish and mucilaginous when fresh; rough and black when dry; inner region white in color, hard, powdery, and with less or without seeds (Figs 1A to C).

### 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



**Figs 1A to C:** (A) Fresh unripe fruit of *Musa x paradisiaca* L.; (B) fresh unripe fruit in pieces of *Musa x paradisiaca* L.; (C) dry unripe fruit in pieces of *Musa x paradisiaca* L.



**Figs 2A and B:** (A) Transverse section of unripe fruit of *Musa x paradisiaca* L. (cu: Cuticle, epi: Epidermis, par: Parenchyma, pap: Papillae, scl: Sclerenchyma, sg: Starch grains, tc: Tannin cells); (B) Raphide bundle showing acicular crystals

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

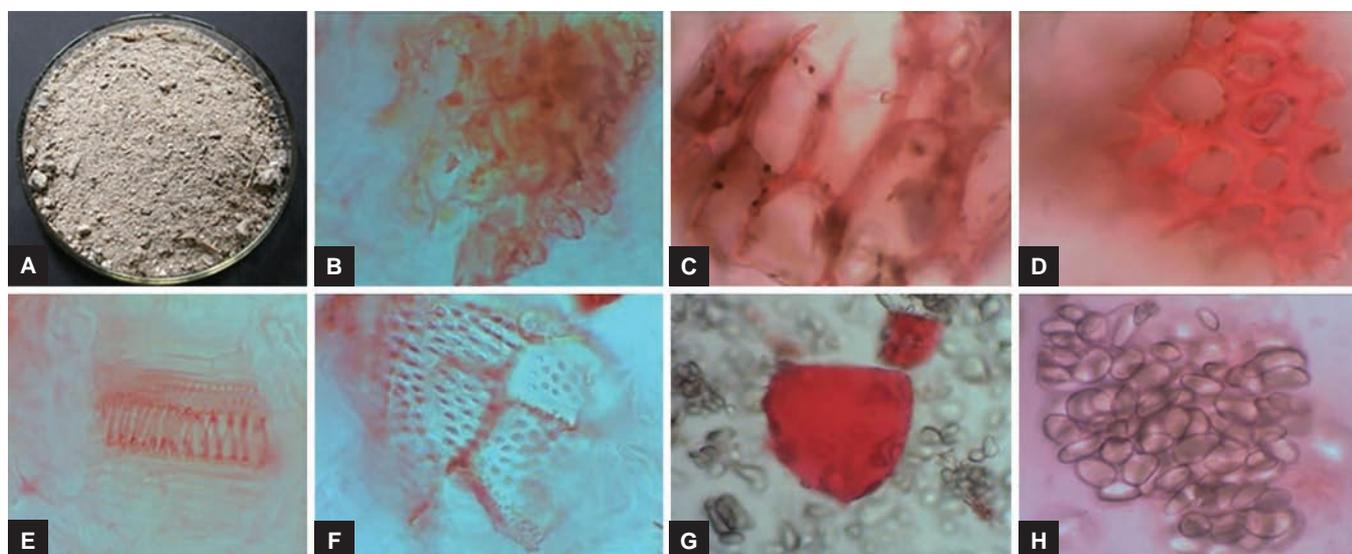
Micrometric details of unripe fruit were studied as per the standard procedures and the measurements were recorded with the help of Catcam software. The details of the same are presented in Table 1.

### Histochemical Analysis

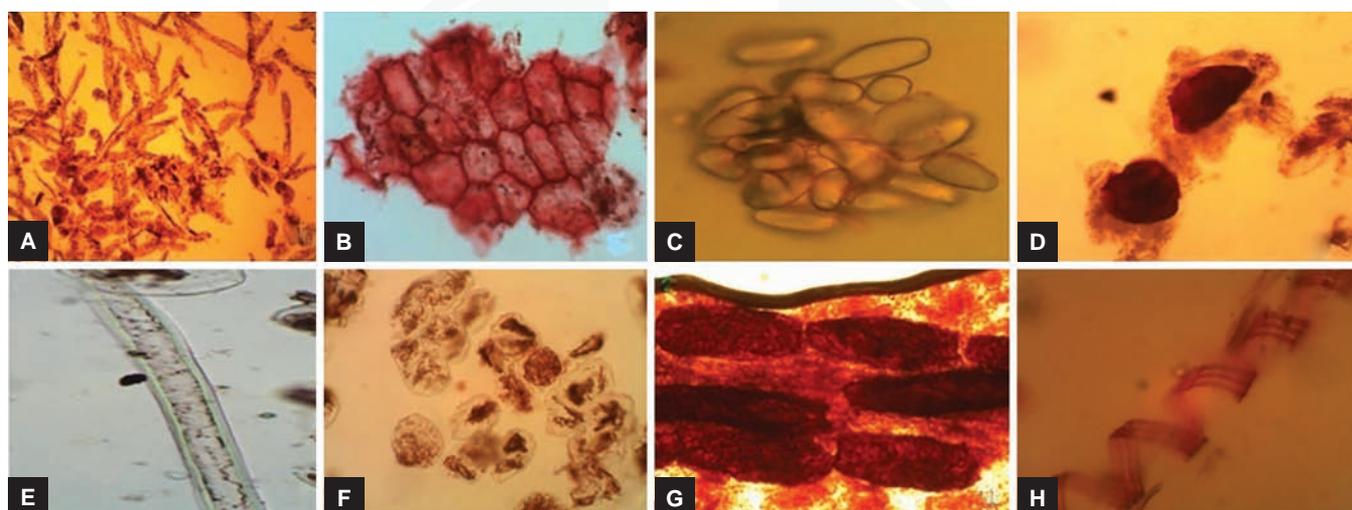
Histochemical studies were carried out by treating the fresh sections with different chemical reagents for the location of different phytochemicals in different regions. The details of the results are presented in Figures 5A to K and Table 2.

### Physicochemical Analysis

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



**Figs 3A to H:** Powder characteristics of unripe fruits of *Musa × paradisiaca* L.: (A) Unripe fruit powder; (B) epidermal cells with papillae 40x; (C) parenchymatous cells 40x; (D) sclerenchymatous cells 40x; (E) helical xylem vessel 10x; (F) pitted xylem vessel 40x; (G) tannin cells 40x; and (H) starch grains 40x



**Figs 4A to H:** Maceration studies of *Musa × paradisiaca* L.: (A) Elongated parenchyma cells 40x; (B) epidermal cells in surface view; (C) starch grains; (D) tannin cells; (E) single fiber 40x; (F) parenchyma cells 40x; (G) tannin cells in groups 40x; and (H) spiral xylem vessel

**Table 1:** Micrometric details of unripe banana fruit

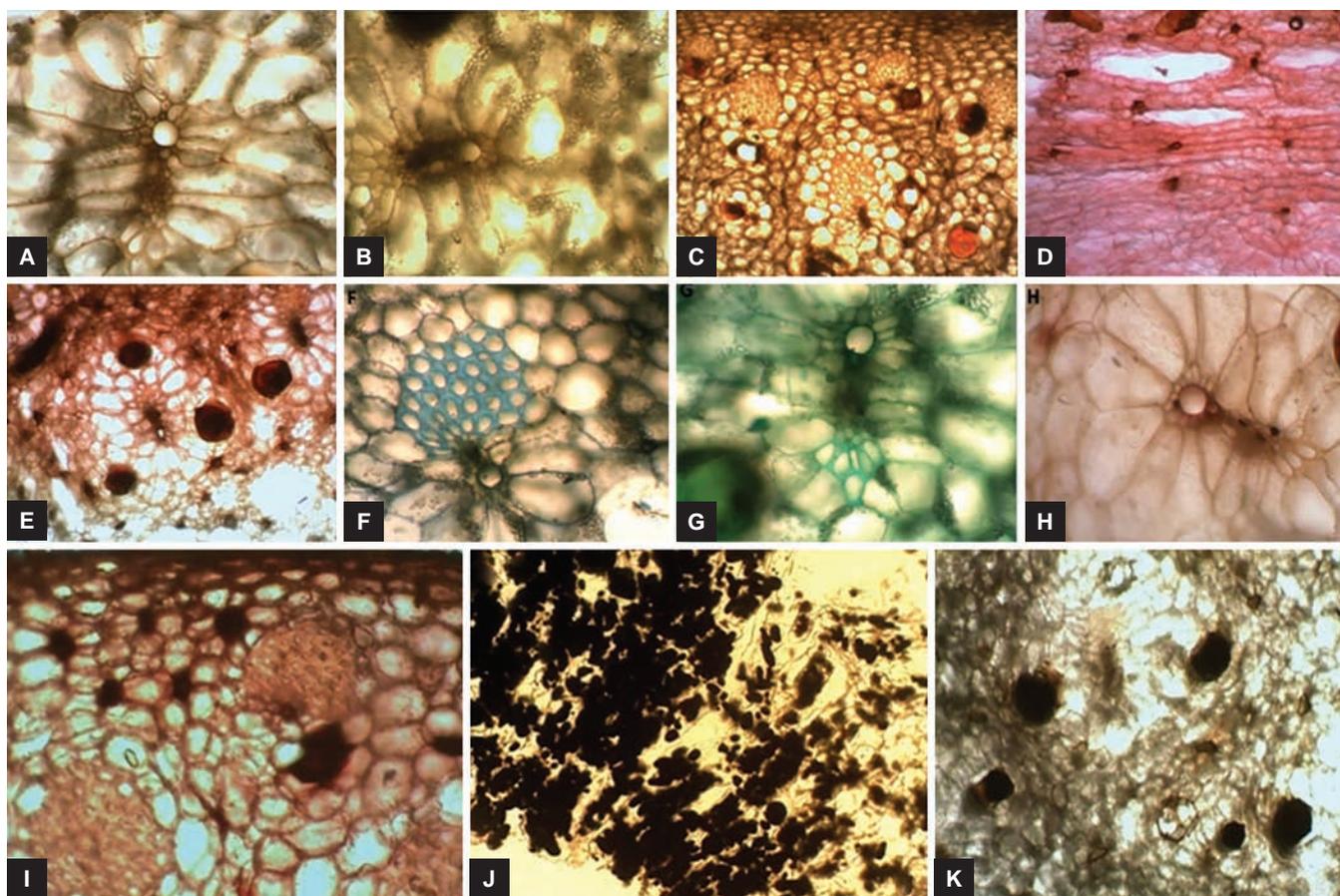
Type of cell	Minimum (measurements in $\mu\text{m}$ )	Maximum (measurements in $\mu\text{m}$ )	Average with standard error (measurements in $\mu\text{m}$ )	Standard deviation (in $\mu\text{m}$ )
Papillae	96.05	58.31	74.42	15.09
Parenchyma cells	83.15	33.24	57.37	12.78
Tannin cells	434.77	227.85	349.11	93.89
Air-spaced cells	559.23	56.01	241.39	123.85

### 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.



**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×

**Table 2:** List of stains and reagents used for the analysis of various histochemicals in different regions of unripe fruit

Stain/reagent	Histochemical	Results	Locality of the cells
Toluidine blue O	Polyphenols	Cell contents turn turquoise green to blue green color	Sclerenchyma cells and vascular bundles
Fast green	Protein	Proteins appear bright green	Sclerenchyma cells and vascular bundles
Sudan III	Lipids	Lipids/oils turn red color	Tannin cells and vascular bundle
Alkaline pyrogallol method	Calcium	Yellowish brown color	All parts
Silver hydrogen peroxide	Calcium oxalate	Black in color	–
Titian yellow	Magnesium	Brick red color	Epidermis, parenchyma cells, sclerenchyma cells, vascular bundles
Methyl red	Silica	Yellowish orange	Epidermal cells, sclerenchyma cells, tannin cells, vascular bundles
Iodine-potassium iodide reaction	Starch	Blue to black in color	Starch grains in the epicarp and mesocarp
Tannic acid–ferric chloride method	Calcium pectate	Blue to black in color	Epidermal cells, parenchyma cells, tannin cells, sclerenchyma cells, vascular bundles
Phloroglucinol method	Lignin	Red in color	–
Ferric chloride method	Tannin	Bluish black or grayish black	Starch grains, tannin cells, vascular bundles
Toluidine blue O	Sulfated mucopolysaccharides	Pink to reddish purple color	All the cell wall of phenolic content cells changes to reddish purple color

**Table 3:** Physicochemical analysis

Name of the parameter	Values (%) w/w
Description	Light brown to ash in coarse powder
Foreign matter	Less than 0.5%
pH (5% w/v aq. solution)	6.95
Loss on drying at 105°C	11.25
Total ash	3.64
Acid-insoluble ash	0.16
Water-soluble extractive	7.1
Alcohol-soluble extractive	1.67

**Table 4:** Extractive values by Soxhlet extraction

Solvent	Values (%) w/w
Petroleum ether (40–60°C)	0.94
Chloroform	0.70
Ethanol	1.86

The R<sub>f</sub> 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

**Table 5:** Preliminary phytochemical tests of *Musa x paradisiaca* L

Natural product group	Test for natural products	Presence (+)/absence (-)
Alkaloids	Dragendorff's test	++ve
	Hager's test	+ve
	Mayers's test	+ve
	Wagner's test	+ve
	Carbohydrates	Anthrone test
	Benedict's test	+ve
	Fehling's test	+ve
	Molisch's test	+ve
Flavonoids		-ve
Phenols	Ferric chloride test	+ve
	Lead acetate test	+ve
Proteins	Biuret's test	++ve
	Millon's test	+ve
Saponins		+ve
Starch		+ve
Steroids	Salkowski reaction	++ve
	Ferric chloride test	+ve
Tannins	Lead acetate test	++ve
	Resins	

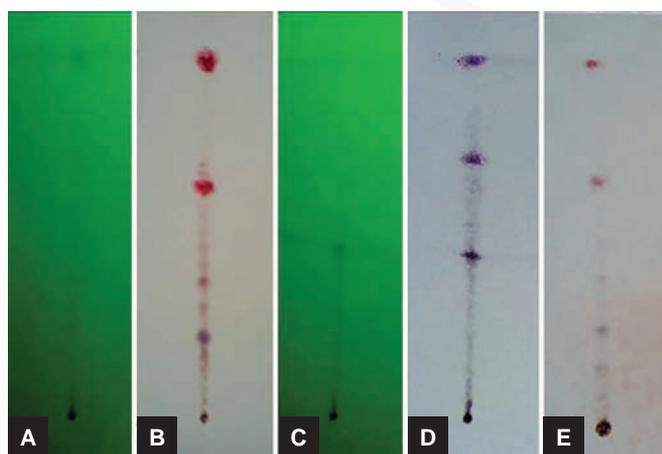
mesocarp portion, helical and pitted xylem vessels, and acicular crystals in the powder microscopy studies. Histochemically, unripe fruit showed the presence of different histochemicals like phenols, protein, calcium oxalate, magnesium, starch, cellulose, calcium pectate, lignin, sulfated mucopolysaccharides, 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.

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**Figs 6A to E:** Thin-layer chromatography fingerprint studies: (A and B) Petroleum ether extract UV-254, after spraying; (C and D) chloroform extract UV-254, after spraying; (E) methanol extract after spraying

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## हिन्दी सारांश

### फार्माकोग्नॉस्टिकल एंड हिस्टोकेमिकल स्टडीस ऑफ अपक्व कदली (अनरार्इपड बनाना) – मूसा × पारदिसीयका एल.

शांता तिरुमाल्लयी रामाशेसन,<sup>1</sup> प्रताप रेड्डी मारमरेड्डी,<sup>2</sup> पच्चाईमाल पीच्चयी,<sup>3</sup> किशोर कुमार रामाकृष्णा,<sup>4</sup>  
वंदना भारती,<sup>5</sup> वेंकटेशवरुलु गड्डुम,<sup>6</sup> देवेश तिवारी,<sup>7</sup> अनुपम के. मंगल,<sup>8</sup> नारायणम श्रीकांत,<sup>9</sup>  
करतार एस. धीमान<sup>10</sup>

वर्तमान अन्वेषण में म्यूसेसी कुल के बहुतायत में प्रयोग होने वाले म्यूसा पैराडिसिएक एल., (केले) के अपक्व फलों की फार्माकोग्नोसी का अध्ययन किया गया जिसके अन्तर्गत मेक्रोस्कोपिकल, माइक्रोस्कोपिकल, पाउडर माइक्रोस्कोपिकल, मेसीरेशन अध्ययन, प्रारंभिक पादप रसायन विश्लेषण, ऊतक रसायन विश्लेषण एवं पतली परत क्रोमेटोग्राफी (टीएलसी) फिंगरप्रिंट आदि के अध्ययन का प्रयास किया गया है। अध्ययन से यह पता चला कि जब सूक्ष्मदर्शी द्वारा अवलोकन किया गया तो एपिडरमिस पर पपीला के लघु बाहरी उत्क्षेपण पाए गए एवं एपीकार्प, मेसोकार्प में अधिक टेनिन कोशिकाओं की मौजूदगी पाई गई। अधिक वायु अधर वाली कोशिका जो एपीकार्प में पेरिनकाइमा कोशिकाओं द्वारा चारों तरफ फैले हुए पाए गए तथा मेसोकार्प भाग में अधिक स्टार्च ग्रेन्स, रेफिड बंडल्स, कैल्शियम ऑक्जलेट के एसिकुलर क्रिस्टल्स, आदि पाए गए। औषधियों के विभिन्न घटकों की उपस्थिति का पता लगाने हेतु विभिन्न अभिकर्मकों के साथ अपरिपक्व फलों के भाग के साथ परीक्षण किया गया जिसके परिणामस्वरूप फिनोल, प्रोटीन, कैल्शियम ऑक्जलेट, मेग्नीशियम, स्टार्च, सेलूलोज, कैल्शियम पेक्टेट, लिगनिन, सल्फेट न्यूको पोलिसैक्राइड्स, टेनिन एवं सिलिका आदि पाए गए। पादप रसायन अध्ययन द्वारा एल्कलोइड्स, कार्बोहाइड्रेट, फिनोल, प्रोटीन, स्टार्च, टेनिन, स्टिरोइड्स, सेपोनिन्स की मौजूदगी दर्शाई गई।

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