

Comparative Pharmacognostic Profiling of Three Major Millets

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ABSTRACT

Background: Millets are small-grained nutri-cereals of the family Poaceae and are increasingly recognized for their nutritional and functional significance. Establishing standardized quality parameters is essential for their authentication and proper utilization. **Objectives:** This study presents a comparative evaluation of the anatomical, physicochemical, and phytochemical characteristics of three major millets-finger millet (*Eleusine coracana* (L.) Gaertn.), pearl millet (*Pennisetum glaucum* (L.) R. Br.), and sorghum (*Sorghum bicolor* (L.) Moench). **Materials and Methods:** Macro- and microscopic analyses, physicochemical assessments, preliminary phytochemical screening, and High-Performance Thin-Layer Chromatography (HPTLC) fingerprinting were performed using standard pharmacognostic procedures. **Results:** Distinct differences were observed in the cellular architecture of the grains. Physicochemical parameters, including moisture content, ash values, and extractive values, exhibited notable intergeneric variations. HPTLC profiling demonstrated characteristic banding patterns at specific wavelengths, reflecting the presence of diverse phytoconstituents across the three millets. **Conclusion:** The anatomical features, physicochemical constants, and HPTLC fingerprints generated in this study serve as reliable reference standards for the identification, authentication, and quality assessment of finger millet, pearl millet, and sorghum.

Keywords: Anatomy, Finger Millet, HPTLC, Pearl Millet, Phytochemical, Sorghum.

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INTRODUCTION

Millets are a group of small-seeded cereals within the family *Poaceae*, recognized for their ability to grow successfully in dry and semi-arid regions. Their resilience to prolonged or intermittent drought sets them apart from most conventional cereal crops. India is currently the leading global producer, cultivating nearly 23 million hectares of millet crops, with small millets contributing about 3.5 million hectares (Michaelraj and Shanmugham, 2013). Their capacity to perform well under low-input conditions-low water requirements, minimal fertilizer dependence, tolerance to ecological variability, and general resistance to biotic and abiotic stress-positions millets as important crops for enhancing global food security (Kole *et al.*, 2015). Furthermore, their natural defence against pests and diseases (Das and Padmaja, 2016) strengthens their suitability for sustainable agricultural systems.

Nutritionally, millets are valued for being non-acidic, gluten-free, fibre-rich cereals that provide substantial amounts of energy, proteins, essential minerals, and antioxidant compounds (Singh *et al.*, 2012). Their notable water-binding capacity and bulking effect help regulate gastrointestinal transit, making them particularly beneficial in diabetic diets (Dayakar Rao *et al.*, 2017). Based on seed size, they are differentiated into major and minor millets. Finger millet, pearl millet, and sorghum constitute the major group and typically require minimal post-harvest treatment, while minor millets-including foxtail, little, kodo, proso, and barnyard millet-generally need additional processing to remove the outer husk.

As global interest in health-promoting foods increases, millets have gained renewed attention both as nutritional staples and as functional ingredients. Traditional Indian medical systems also acknowledge their therapeutic relevance. In Siddha medicine, the nine grains collectively termed *Thonnoo-koolam* or *Navadhanyas* are regarded as optimal dietary grains (Sambasivam, 2006). Their high fibre content supports digestive health and influences gut microbial balance (Kavitha *et al.*, 2024). Ayurveda similarly recommends millets for managing various lifestyle-induced disorders (Neelayathatchi *et al.*, 2023). These attributes underscore the dual role of millets as food crops and as contributors to traditional health practices.



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In this context, the present study undertakes an anatomical and phytochemical comparative evaluation of the major millets- finger millet, pearl millet, and sorghum.

MATERIALS AND METHODS

Organoleptic Characters

Organoleptic characters such as colour, odour and taste were noted.

Anatomical Studies

Hand sections of various parts of the plant material are taken and stained with saffranin and mounted in Glycerin under 10X as well as 40X objective of microscope (Trease and Evans, 2002).

Physico-Chemical Evaluation

Physico-chemical constants like total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, volatile oil and loss on drying at 105°C were determined as per standard protocol (Ayurveda Pharmacopoeia of India, 2006).

Phytochemical Screening

Qualitative screening of various phytochemicals were done as per standard protocol (Ayurveda Pharmacopoeia of India, 2006).

Hptlc Fingerprinting

The alcohol extract of 3 millets and its powder ingredients were subjected to HPTLC analysis. The instrument employed CAMAG HPTLC system (Muttentz, Switzerland) which was equipped with a sample applicator TLC autosampler 4 with win CATS software version 1.4.4. Each extract was applied as two tracks of volume 5 μ L and 10 μ L. Respectively. The plate was developed using the solvent system, Toluene: Ethyl acetate (6: 2) in a twin trough chamber. The plate was developed up to 7 cm, removed from the chamber and allowed to dry. The developed plate was scanned using TLC Scanner 3 and analyzed with win CATS software version 1.4.4. at λ_{\max} 254 nm using deuterium light source, at λ_{\max} 366 nm with mercury light source and the slit dimensions were 8.00 mm \times 0.40 mm. Densitometric documentation was done. After scanning, the plate was observed under 254 nm and

366 nm and TLC chromatograms were recorded. Then the plate was dipped in vanillin-sulfuric acid reagent and dried at 105°C on a hot plate till the colour of the bands appears. The plate was visualized under white light and scanned at 575 nm. TLC chromatograms, R_f values and fingerprint data were recorded by win CATS software.

Ethical Statement

The plant materials used (major millets) were collected from authorized sources and all procedures complied with institutional and national guidelines on the ethical use of plant materials.

Statistical Analysis

The experiments were done in triplicates and results were expressed in triplicate values with standard deviation.

RESULTS

Organoleptic Characters

The physical parameters like state, nature, odor, taste, touch, flow, property, and appearance revealed as given in Table 1. The images of three major millets are given in Figure 1.

Anatomy

The cellular structure of grains of finger millet, pearl millet and sorghum was studied. Significant differences were observed in the anatomy of 3 grains. The comparison of the three were tabulated below (Table 2).

Finger millet

Unlike other millet grains, finger millet is a Utricle which thus making the pericarp not to be completely fused with testa. The millet is globular in shape with pericarp membranous, hyaline and fragile and can be easily removed while washing/rubbing. The grain consists of 3 layers the pericarp, testa and endosperm. The unique characteristic of finger millet is pigmented testa composed of 5 layers in a wavy appearance. Aleurone layer is seen below testa as a single layer and cannot be easily detached from testa. The endosperm cells are angular in shape and contains

Table 1: Organoleptic characters of three major millets.

| Sl. No. | Character | Finger millet | Pearl millet | Sorghum |
|---------|---------------|-------------------------------------|--|----------------------|
| 1 | State | Solid | Solid | Solid |
| 2 | Nature | Fine | Rough | Rough |
| 3 | Odor | Characteristic odour | Characteristic odour | Characteristic odour |
| 4 | Touch | Soft | Rough | Rough |
| 5 | Flow Property | Non-Free flowing | Non-Free flowing | Non-Free flowing |
| 6 | Appearance | White in colour with reddish grains | Cream in colour with white and grey grains | Cream in colour |
| 7 | Taste | Characteristic taste | Characteristic taste | Characteristic taste |

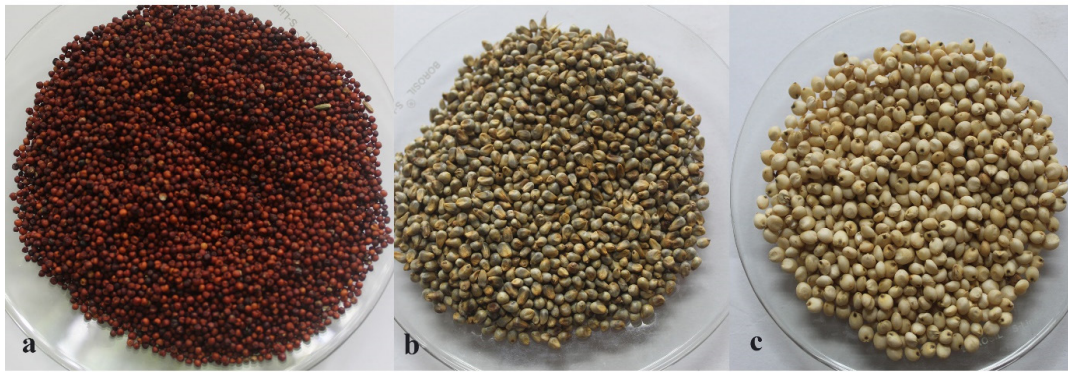


Figure 1: Grains of 3 millets; a. Finger Millet, b. Pearl Millet, c. *Sorghum bicolor*.

simple as well as compound starch grains of varying sizes (Figure 2).

Pearl millet

The seed type of pearl millet is caryopsis. Cuticles are remarkably thick with smooth surface. Epicarp is composed of elongated epidermal cells. Mesocarp is composed of 2-3 rows of cells, lost their cellular outline at some places and found to be obliterated. Testa is thin and single layered. Aleurone layer is seen just below the testa as a single layer of regularly oriented cubical cells and rectangular cells. Aleurone cells contain protein bodies. Endosperm contains abundant polygonal starch grains which are closely packed (Figure 3).

Sorghum bicolor

The seed is generally spherical, but may be flattened on one side and is a naked caryopsis. The grain is composed of pericarp, endosperm and germ. Pericarp is white in colour. Cuticle is single layered with rough or uneven outlines. Epicarp is composed of single layer of thick walled cells. Mesocarp of 2-3 rows of relatively thin walled angular cells. Cells of pericarp contains starch grains. Testa is distinct and aleurone layer is seen beneath the testa and contains protein bodies. Endosperm cells contains numerous starch grains of various shapes and sizes. Round, oval or polygonal starch grains with- and without hilum also were seen (Figure 4).

Physico-chemical analyses

The physico-chemical values obtained for 3 major millets are given in Table 3.

Phytochemical screening

The qualitative screening of phytoconstituents is listed in Table 4.

HPTLC Fingerprinting

The HPLC fingerprint pattern of 3 major millets were given as Figure 5. Chromatogram of finger millet shows a band at R_f 0.2 under 254 nm, prominent band at R_f 0.7 under 366 nm and major bands at R_f 0.5, 0.79 and 0.96 under white light. In case of

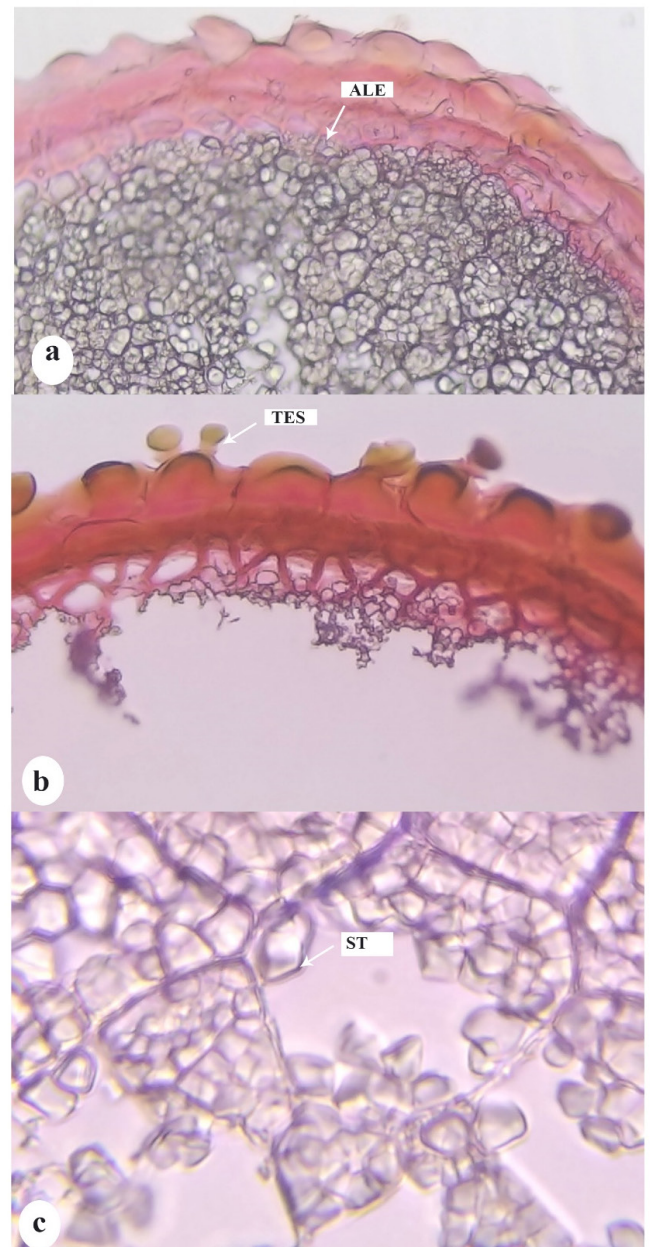


Figure 2: Anatomical structure of grain of finger millet. a. T.S. of grain (a portion enlarged), b. enlarged view of testa, c. enlarged view of endosperm. TES-Testa, ALE-Aleurone layer, ST-Starch grains.

Table 2: Anatomical comparison of grains of three major millets.

| Sl. No. | Parts | Finger millet | Pearl millet | Sorghum |
|---------|----------------|-----------------------------------|-----------------------------|--|
| 1 | Seed type | Utricle | Caryopsis | Caryopsis |
| 2 | Pericarp | Membraneous and fragile | Single layer of round cells | Single layer of thick-walled cells |
| 3 | Testa | 5 layered | Single layered | Single layered |
| 4 | Aleurone layer | Irregular rectangular cells | Cubical cells | Elongated cells |
| 5 | Endosperm | Angular cells | Angular cells | Angular cells |
| 6 | Starch grains | Simple and compound starch grains | Polygonal starch grains | Round, oval or polygonal starch grains |

Table 3: Physico-chemical comparison of three major millets.

| Sl. No. | Parameter | Finger millet | Pearl millet | Sorghum |
|---------|----------------------------|---------------|--------------|------------|
| 1. | Loss on Drying at 105°C | 12.11±0.01 | 8.22±0.23 | 10.24±0.32 |
| 2. | Total Ash Content | 2.01±0.08 | 1.37±0.21 | 1.53±0.11 |
| 3. | Acid Insoluble Ash | Nil | Nil | Nil |
| 4. | Alcohol Soluble Extractive | 1.91±0.12 | 5.36±0.24 | 5.01±0.03 |
| 5. | Water Soluble Extractive | 3.21±0.34 | 5.45±0.21 | 3.54±0.15 |
| 6. | Volatile Oil | Nil | Nil | Nil |
| 7. | pH | 6.91±0.34 | 6.13±0.23 | 5.86±0.12 |

Table 4: Preliminary phytochemical comparison of three major millets.

| Sl. No. | Phytoconstituents | Finger millet | Pearl millet | Sorghum |
|---------|-------------------|---------------|--------------|---------|
| 1 | Saponins | + | - | - |
| 2 | Tannins | + | + | - |
| 3 | Phenols | - | - | - |
| 4 | Terpenoids | + | + | + |
| 5 | Alkaloids | + | + | + |
| 6 | Flavanoids | - | + | + |
| 7 | Steroids | - | - | - |
| 8 | Glycosides | - | + | + |
| 9 | Carbohydrates | + | + | + |
| 10 | Quinones | + | - | - |
| 11 | Proteins | + | + | + |

+ - presence, - -absence

Pearl millet, the chromatogram showed prominent bands at R_f values of 0.1, 0.62 and 0.76 under 254 nm. Under 366 nm, a single prominent band were observed at R_f of 0.62. After derivatization with sulphuric acid, 4 bands were observed at R_f positions 0.42, 0.58, 0.73 and 0.84. Where as in the case of Sorghum, no distinct bands were observed under 254 and 366 nm. Under 575 nm, 4 bands were observed at R_f positions 0.34, 0.5, 0.59 and 0.87. The peaks in the HPTLC fingerprint profile correlate to its different phytoconstituents and can be utilized to standardize the drug.

DISCUSSION

The present work offers a comparative examination of the anatomical features and phytochemical attributes of three principal millets-finger millet, pearl millet, and sorghum. Millet grains consist of three primary components: the pericarp, the testa, and the endosperm. Within the endosperm, the storage tissue is organized into peripheral, corneous, and central floury zones. The peripheral zone, situated directly beneath the aleurone, contains compactly arranged cells enriched in proteins and

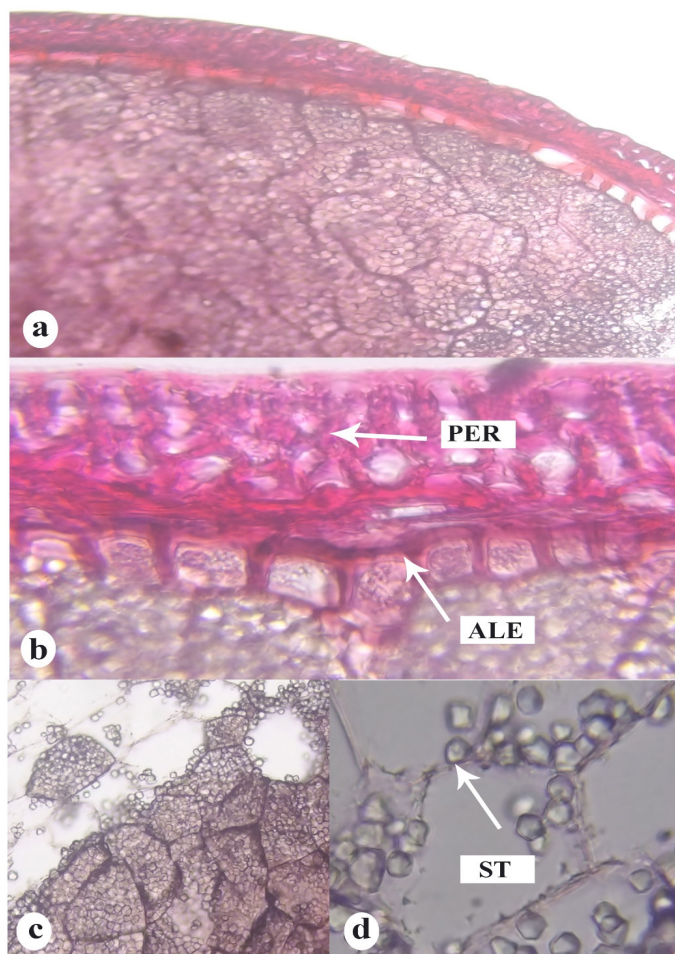


Figure 3: Anatomical structure of grain of pearl millet. a. T.S. of grain (a portion enlarged), b. enlarged view of pericarp and testa, c,d. Endosperm cells. PER-pericarp, TES-Testa, ALE-Aleurone layer, ST- Starch grains.

bearing small starch granules (Zelezna and Varriano-Marston, 1982). Variations in the proportions of the corneous and floury tissues largely influence the hardness and texture of the kernel (Hassan *et al.*, 2021).

Structurally, finger millet differs from the other two major millets in being a utricle, where the pericarp is only loosely associated with the testa. Pearl millet and sorghum, on the other hand, are typical caryopses with a pericarp tightly fused to the seed coat (Hassan *et al.*, 2021). Finger millet is notable for its multilayered testa-comprising five distinct layers-which contributes to the grain's substantial fibre content (Shobana *et al.*, 2013). Its hyaline pericarp can be removed with ease during washing. Pearl millet shows a prominently thick cuticle on the pericarp, with a clearly distinguishable boundary between the testa and endosperm (Maiti and Bisen, 1979). Sorghum grains tend to be round or slightly flattened and are exceptional among nutricereals in containing starch granules within the pericarp tissue (Abebe *et al.*, 2023).

The physicochemical assessment revealed measurable variation across the three millets chosen in the study. Pearl millet exhibited

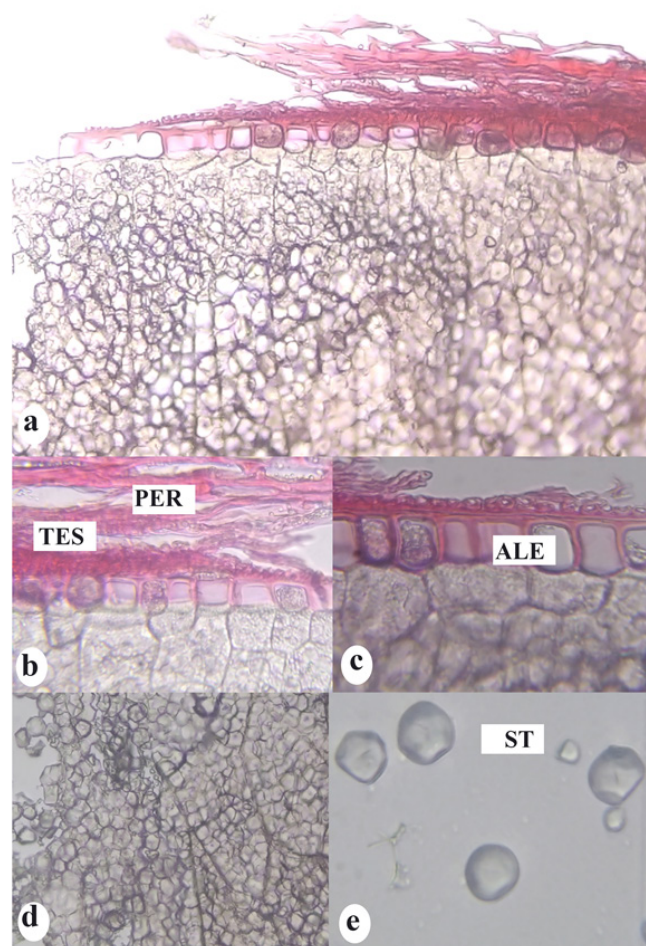


Figure 4: Anatomical structure of grain of sorghum. a. T.S. of grain (a portion enlarged), b,c. enlarged view of pericarp and testa, d,e. Endosperm cells. PER-pericarp, TES-Testa, ALE-Aleurone layer, ST-Starch grains.

the lowest loss on drying, suggesting reduced moisture retention and, consequently, better storage stability (Alozie *et al.*, 2009; Owheruo *et al.*, 2019). The pH values of the three grains-6.91 for finger millet, 6.13 for pearl millet, and 5.86 for sorghum-fell within the expected range for raw millets, though processing methods such as germination may alter these values (Owheruo *et al.*, 2019). Ash values, indicative of total inorganic matter, were within acceptable limits. Extractive profiles varied among the three millets, with finger millet showing comparatively lower alcohol-soluble constituents. Pearl millet exhibited higher water-soluble extractives. Both volatile oils and acid-insoluble ash were absent, suggesting minimal contamination with extraneous mineral matter.

Preliminary phytochemical screening identified a diverse set of secondary metabolites. Tannins were detected in finger millet and pearl millet but were absent in the non-pigmented Type I sorghum evaluated, aligning with previous findings that tannins primarily accumulate in pigmented sorghum varieties (Dykes and Rooney, 2006). Condensed tannins, which influence grain colour, are frequently abundant in coloured millet varieties

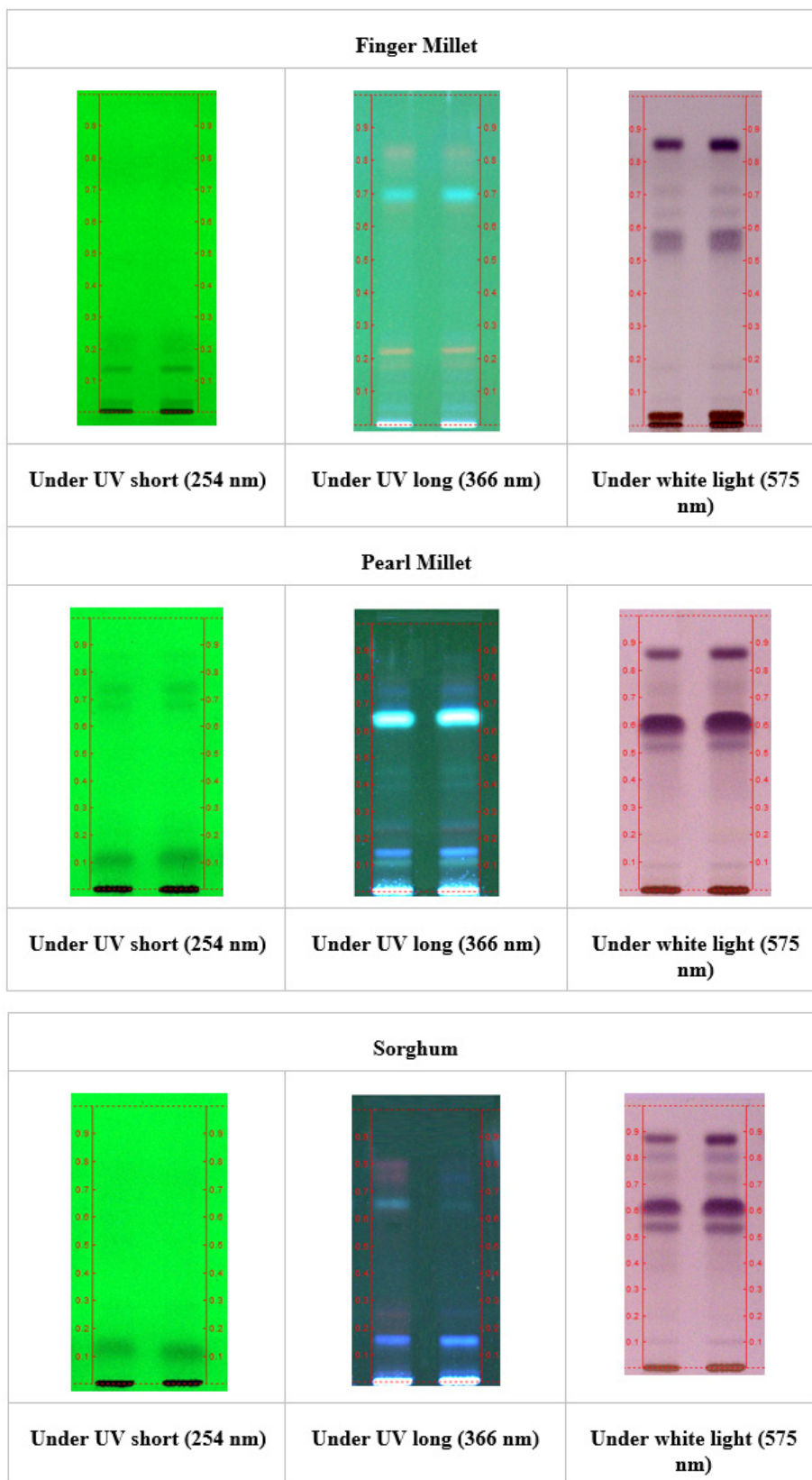


Figure 5: HPTLC fingerprint of three major millets under UV short, UV long and white light after derivatisation.

(McDonough, 2000). Although tannins can impair mineral absorption (Palanisamy *et al.*, 2012), processing methods such as fermentation, malting, decortication, and steaming are known to improve nutrient bioavailability (Sood *et al.*, 2017).

All three millets tested positive for terpenoids and alkaloids, compounds that, together with other phytoactives, enhance their antioxidant potential (Dykes and Rooney, 2006; Viswanath *et al.*, 2009). The rich phytochemical diversity underscores the nutritional and functional food potential of these grains. The qualitative findings from the phytochemical assays were further supported by HPTLC profiling.

The HPTLC chromatograms displayed distinct banding patterns (Figure 5) corresponding to different classes of phytoconstituents. These profiles serve as authentic fingerprints for finger millet, pearl millet, and sorghum, supporting their identity, purity assessment, and quality control.

CONCLUSION

Macro- and microscopic evaluations, physicochemical profiling, preliminary phytochemical screening, and HPTLC fingerprinting collectively provide robust reference parameters for the quality assessment of the three major millets—finger millet, pearl millet, and sorghum. The results generated in this study facilitate the accurate identification and authentication of these grains. Establishing standardized benchmarks based on diagnostic microscopic features, physicochemical values, and the qualitative distribution of key phytoconstituents is therefore essential to ensure the quality, purity, and consistency of these millets for food, nutraceutical, and medicinal applications.

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ABBREVIATIONS

HPTLC: High-Performance Thin-Layer Chromatography; **CCRS:** Central Council for Research in Siddha; **TLC:** Thin-Layer Chromatography; **T.S:** Transverse Section; **R_j:** Retardation factor; **UV:** Ultraviolet; **TES:** Testa; **ALE:** Aleurone layer; **ST:** Starch grains; **PER:** Pericarp.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHORS' CONTRIBUTION

Neethu Kannan B.: Conceptualization, data analysis, writing, review and editing. **Ghanthikumar S:** Data collection, **Natarajan M.:** Data analysis, **Reena V L:** Data analysis

SUMMARY

Coarse powders of 3 major millets were subjected to physicochemical, phytochemical screening and HPTLC fingerprinting. Anatomical studies of seeds were done by hand sectioning. Cellular structure of seeds showed pericarp, testa and starch grains. HPTLC revealed many peaks which correspond to the various phytochemicals present in these major millets. Therefore, the study effectively establishes the identity of the three major millets both in their whole form and as powdered drug.

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