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Original Article

HPTLC, Physico-chemical, Phytochemical, Macroscopic, Microscopic Analysis of Seeds of a Nutri Cereal-Finger Millet/Ragi [(*Eleusine coracana* (L.) Gaertn.]

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ABSTRACT

Background: Finger Millet/Ragi [(*Eleusine coracana* (L.) Gaertn.], an important nutri cereal/millet is being cultivated in various regions across the globe. The largest cultivating continents are Asia and Africa. In this scenario of mounting importance to millets/nutri cereals as the U.N General Assembly announced 'The International Year of Millets 2023', there was an urgent need for analysing this drug scientifically, for the purpose of standardisation and quality control. **Objectives:** HPTLC, physico-chemical, phytochemical, macroscopic, microscopic analysis of seeds of Finger Millet were conducted for standardization. **Materials and Methods:** Macroscopical and microscopic analysis (Anatomical and Powder Microscopy), and physico-chemical analysis of powder of seeds/grains of Finger millet were done. For, phytochemical analysis, alcoholic extraction (reflux) was performed in the powder of the drug. For HPTLC analysis, the best fingerprint was obtained in with Toluene: Ethyl acetate: Formic acid (5: 3: 0.1) as mobile phase. **Results:** In anatomical study, unique 5-layered testa, starchy endosperm, and single aleurone layer were identified. Physicochemical analysis revealed identity and purity of Ragi. Terpenoids, quinines, alkaloids, carbohydrates, proteins and glycosides were identified as the constituent phytochemicals. The fingerprint profile of HPTLC exhibited many peaks which correspond to the various phytoconstituents present in Ragi. **Conclusion:** The results of the study will be beneficial in identifying Finger millet as the whole drug or in powder form, and thus standardising the raw drug.

Keywords: Finger Millet, HPTLC, Physicochemical, Phytochemical, Standardization.

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INTRODUCTION

The Year 2023 has been observed as 'The International Year of Millets'. The General Assembly of U.N, in a conference conducted in 2021 proclaimed it. The greatest advantage of Millets is that they can thrive even on terrains where there is scarcity of water. As a result, they are a good option for nations to eradicate scarcity of food and thus the dependency on imported cereal grains will be reduced. There will be opportunities in this year to increase public knowledge about the health and nutritional benefits of millets. Additionally, there will be augmentation in the demand of millets in markets.[1]

Finger Millet/Ragi [(*Eleusine coracana* (L.) Gaertn., Poaceae family], an important nutri cereal/millet is being cultivated in various regions across the globe. The largest cultivating continents are Asia and Africa. In the United States of America, it is considered a super cereal.[2] In Ayurveda, its therapeutic benefits are also acclaimed. It is called Nrityakundala, Nartaka, or Ragi in various Ayurvedic texts. The plant is an erect annual grass 60-150 cm in height with tillering tufted stems. Leaves are 5-7 mm broad with sheath and ligule of hairs far overtopping the stem, spikes 4-7, 3-6 flowered, each spikelet containing 4-7 grains

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(the useful part) which are globose or flattened, varying in colour from dark reddish brown to nearly white.[3]

As per Ayurveda, the grains are sweet in taste, with cold potency and is easy to digest. The therapeutic properties include nourishing the body, increasing strength and immunity of the body, balances vata, pitta, kapha humours.[4] In Ashtanga Hridaya, it is described as powder of ragi taken along with honey and goat's milk for a period of 7 days will remove renal stones.[5]

From the nutritional point of view, it provides around 7% protein, 1.5% ether extractives, 80% carbs, 18% dietary fibre and 3% minerals. Among all cereals, Ragi is richest in calcium, around (340 mg/100 g). It contains tannins (0.60%), phytates (0.48%), polyphenols. It is also known to provide a number of health benefits, including anti-diabetic, antioxidant, hypocholesterolemic and anti-microbial action.[6]

This millet has a fairly good market all over the world and is easily accessible from health food stores or even local markets without prescriptions. As a result, its quality control is a major public health concern owing to the problems of adulteration and substitution. So far, standardisation study of the drug has not been reported. Hence, the HPTLC, physico-chemical, phytochemical, microscopic and macroscopic analysis (anatomical analysis of the seeds and powder microscopy) were undertaken for the purpose of standardizing the drug.

MATERIALS AND METHODS

Authentic coarsely powdered grains of Ragi were purchased from local Ayurvedic drug dealer, Poojappura, Pin-695012, Kerala, India. The voucher specimens (J/Fr/056) were deposited in the Dravyaguna Vijnan Museum, Regional Ayurveda Research Institute, Poojappura, Thiruvananthapuram, Kerala, India for future reference. HPTLC was done with TLC plates coated with silica gel $60F_{254}$ from Merck, Germany. Analytical Research graded solvents, chemicals, reagents were used.

Macro-microscopy

Organoleptic characters: Noted color, odour, taste. (Figure 1).

Powder microscopy: 500 mg finely pulverized Ragi seeds were mounted in Glycerin at room temperature. Seeds were mounted for 2 hr. Using 10X and 40X objective of bright field microscope (Meswox, India), visualisation was done. The distinguishing features were photographed with camera present in microscope.

Anatomical study: Hand sections of seed of Ragi are taken and stained with safranin and mounted in Glycerin under 10X as well as 40X objective of microscope.

Physico-chemical and Phytochemical analysis

Quantitative analysis for pH, total ash, acid insoluble ash, water soluble ash, loss on drying at 105°C, water-soluble and alcohol soluble extractive were done in duplicate in accordance with International Council for Harmonisation guidelines.[7,8] The physico-chemical and phytochemical analysis for quality control of the raw material was also done as per standard guidelines.[9]

HPTLC

HPTLC finger printing for Ragi was done using CAMAG (Switzerland) HPTLC. 0.5 g of Ragi was weighed out and dissolved in 10 mL ethanol. Heated in a water bath for 10 min, kept for 24 hr and filtered. In Track 1, 5 µL extract applied on silica coated TLC plate (60 F_{254}) by CAMAG ATS4 applicator. Similarly in Track 2, 10 µL applied. Developed upto 85 mm from the bottom in Twin trough chamber (CAMAG) (10 × 10 cm). Mobile phase was toluene: ethyl acetate: formic acid (5:3:0.1 V/V/V). CAMAG visualizer was used to take photographs in short ultraviolet (254 nanometres), long ultraviolet (366 nanometres). Vanillin sulphuric acid was used for derivatising the plates after

Figure 1: Grains of Finger Millet/Ragi [(*Eleusine coracana* (L.) Gaertn].

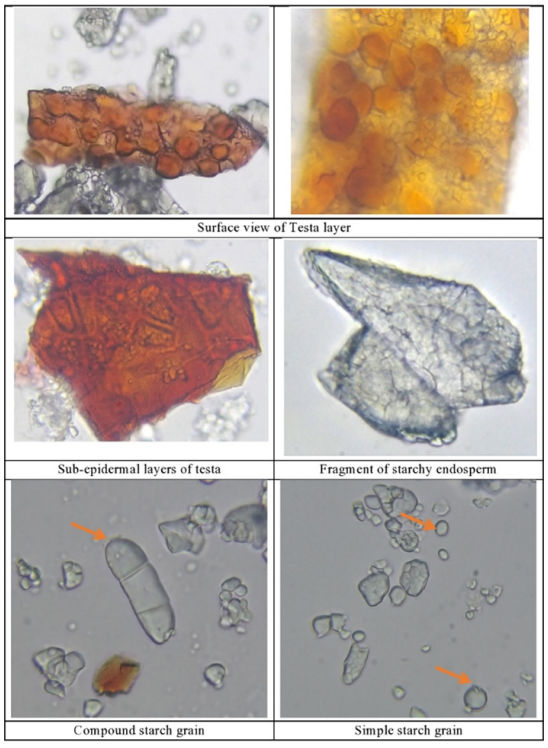

Figure 2: Powder characteristics of *E. coracana* (seed).

scanning, followed by 130°C heating for better visibility of bands. Photographs were taken and further scanned at λ 575 nm.

RESULTS

Organoleptic characters

Described in Table 1.

Powder microscopy

The following cellular characters were observed in the *E. coracana*. Starch grains were the observed in the sample in abundance. Starch grains of two morphotypes viz. Simple and compound type also were seen in isolated and clumped forms as well. Angular cells of endosperm were seen, tightly packed with starch grains. As well as, fragments of testa were also observed viz. epidermal and sub-epidermal layers were observed (Figure 2).

Anatomical study

The pericarp was a fragile, membranous layer that was easily removed by rubbing or washing prior to use. Kernel comprises ofseed coat, embryo,endosperm. Seed coat with five layers is the major distinguishing character of Ragi. The seed type of Finger Millet is Utricle and the shape of the grain is globose. The testa of the grain is pigmented with thick five-cell layers. The starchy endosperm comprised most of the weight of the finger millet utricle. The aleurone layer is single and it lies just below the seed coat and testa. The testa is strongly fused to the aleurone layer and cannot be easily removed. The endosperm is angular in shape and contains simple as well as compound starch grains. The starch grains appear in various sizes (Figure 3).

Table 1: Organoleptic characters of *E. coracana*.

Sl. No.	Specification	Character
1	State	Solid
2	Nature	Fine
3	Odour	No characteristic odour
4	Touch	Soft
5	Flow Property	Non-Free flowing
6	Appearance	White in colour with reddish grains
7	Taste	No characteristic taste

Table 2: Physico-chemical analysis.

Sl. No.	Parameters	Results
1.	LOD at 105°C	12.04
2.	Total Ash	2.00
3.	Acid insoluble ash	Nil
4.	Water soluble ash	0.43
5.	pH of water extract (4% aqueous solution)	6.90
6.	Alcohol soluble extractives	1.89
7.	Water soluble extractives	3.17
8.	Volatile oil	Nil

Physico-chemical Analysis

Tabulated in Table 2.

Phytochemical analysis

Described in Table 3.

HPTLC

The HPTLC analysis of *Eleusine coracana* L. Gaertn. powder is carried out and the results are shown in Figure 4, Supplementary Figures 1, 2, 3. HPTLC study revealed the presence of various phytochemicals as illustrated in the Figures. The chromatograms (Figure 4, Supplementary Figures 1, 2, 3) were obtained upon scanning at UV 254 nm, UV 366 nm and 575 nm and peak tables were generated. The R_f values, peak height, peak area, and percent area of the unknown substances are depicted in the tables in Supplementary Figures 1-3.

DISCUSSION

Standardization and quality assurance of botanicals are major challenges faced by herbal drug industry. Due to variances in the soil and the nutritional quality of the medicinal plants, batch-to-batch variations in their efficacies may occur. Natural, genetic and seasonal variations are other causes of the variation. In order to ensure uniform biological activity, chemical profiling and a quality assurance programme for the manufacturing of herbal medications, standardisation is critical.

Macroscopic features are solid state, fine nature, no characteristic odour and taste, soft touch, not free flowing and are white in

Table 3: Preliminary Phytochemical Analysis.

Eleusine coracana (Ragi)	
Tests	Result
Saponins	-
Tannins	-
Phenols	-
Terpenoids	+
Alkaloids	+
Flavanoids	-
Steroids	-
Glycosides	+
Carbohydrates	+
Quinones	+
Proteins	+

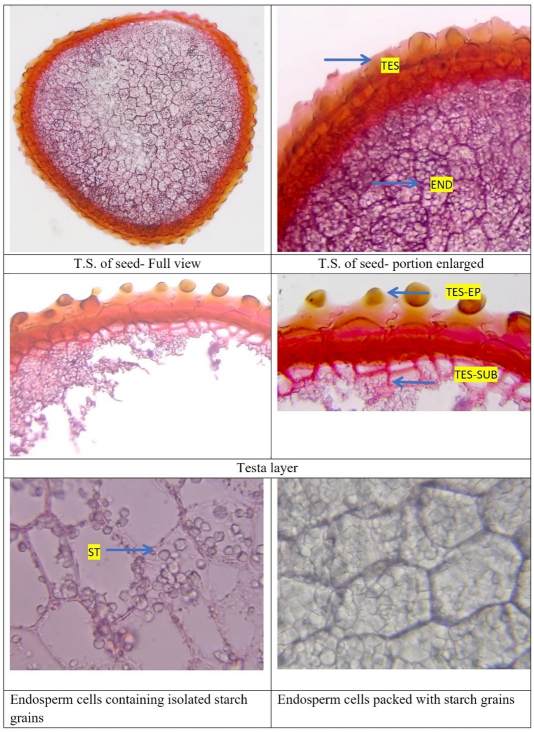

Figure 3: Anatomical characteristics of *E. coracana* (seed).

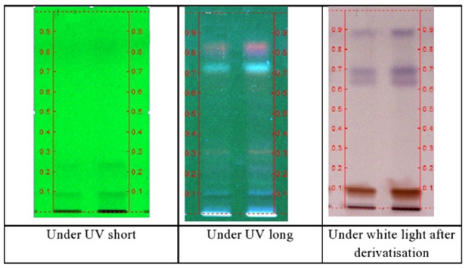

Figure 4: HPTLC fingerprint of *Eleusine coracana* under UV short, UV long and white light after derivatisation.

colour with red grains. Powder microscopy revealed fragments of testa viz. epidermal and sub-epidermal layers, angular cells of endosperm with tightly packed simple and compound type of starch grains. In Anatomical study, it was identified that kernel comprises ofseed coat, embryo,endosperm. Seed coat with five layers is the major distinguishing character of Ragi. Fragile pericarp, unique 5 layered testa, starch endosperm, single aleurone layer were also seen.

In physico-chemical analysis, volatile oil and acid insoluble ash were absent. It also revealed the identity and purity of Ragi. Moisture content of Ragi was 12% pertaining to a little hygroscopic nature. Total ash value gives an idea about the percentage of inorganic content in them and was calculated as 2%. The water-soluble salts which were accounted by water soluble ash value was calculated as 0.43%. The acid insoluble ash, found to be nil which reflects the absence of heavy metals and silicious matter. The water and ethanol soluble extractive value were estimated to be 3.17% and 1.89%, respectively pertaining to less dissolution.

Terpenoids, quinines, alkaloids, carbohydrates, proteins and glycosides were identified as the constituent phytochemicals. The presence of phytochemicals was further confirmed by HPTLC analysis.

HPTLC is a standardization tool which escalate quality control of a drug one step ahead. The fingerprint of a drug will be the same under identical conditions which makes chromatographic fingerprinting a perfect method for identification and authentication of herbal drugs. The Photo documentation of ethanol extract of the sample was done under UV chamber at 254 nanometres initially. It was followed by visualization in 366 nanometres and after derivatisation at 575 nm, which is depicted in Figure 4. Track 1 and 2 represents 5 µL and 10 µL of sample application. Ethanol extract of Ragi exhibited bands with R_f 0.15, 0.25, 0.29, 0.38, 0.62, 0.84 in short Ultraviolet, nine bands having R_f 0.1, 0.21, 0.26, 0.29, 0.43, 0.50, 0.56, 0.60, 0.83 in long Ultraviolet and six bands of R_f 0.41, 0.50, 0.59, 0.79, 0.87, 0.99 in white light after derivatising with vanillin sulphuric acid. The 3 chromatograms for representative track are displayed in Supplementary Figures 1-3.

The peaks observed can be attributed to the terpenoids, alkaloids, glycosides, carbohydrates, quinones and proteins present as major constituents in Ragi. The HPTLC photographs and chromatogram recorded holds to be the authentic finger print for Ragi.

CONCLUSION

This study results can be utilised for standardization of *Eleusine coracana* grains as the study sets a well-defined specific protocol. The physicochemical study revealed the purity of the sample. The preliminary phytochemical analysis along with the HPTLC study performed in ethanolic extract of Ragi showed the presence of various therapeutically important chemical constituents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HPTLC: High Performance Thin Layer Chromatography; **U.N:** United Nations; **mm:** Millimetres; **mg:** Milligram; **pH:** Potential of Hydrogen; **µL:** Microlitre; **TLC:** Thin Layer Chromatography; **V/V/V:** Volume/volume/volume; **UV:** Ultraviolet; **λ:** Lambda.

AUTHORS' CONTRIBUTION

Sinimol T P: Conceptualization, data analysis, writing, review and editing. **Soumya M C:** Conceptualization, literature search, data collection. **Sree Deepthi G N:** Literature search, data collection, **Meghna P P:** Conceptualization, literature search, data collection. **Reena V L:** Data collection, data analysis. **Neethu Kannan B:** Data collection, data analysis. **Ghanthikumar S:** Data collection, data analysis.

SUMMARY

Authentic coarsely powdered grains of Ragi were subjected to HPTLC evaluation, preliminary phytochemical, Macroscopic and Microscopic analysis. In anatomical study, unique 5-layered testa, starchy endosperm, and single aleurone layer were identified. Physico-chemical analysis revealed terpenoids, quinines, alkaloids, carbohydrates, proteins and glycosides as the constituent phytochemicals. The fingerprint profile of HPTLC exhibited many peaks which correspond to the various phytoconstituents present in Ragi. The results of the study will be beneficial in identifying Finger millet as the whole drug or in powder form, and thus standardising the raw drug.

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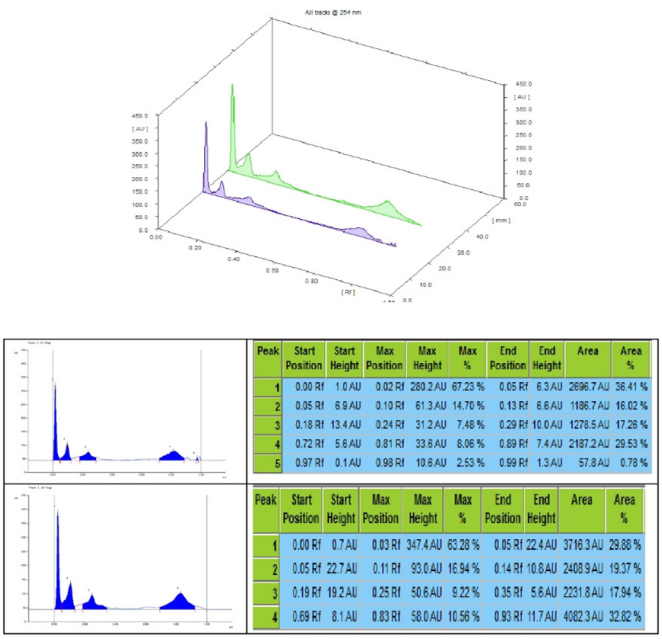
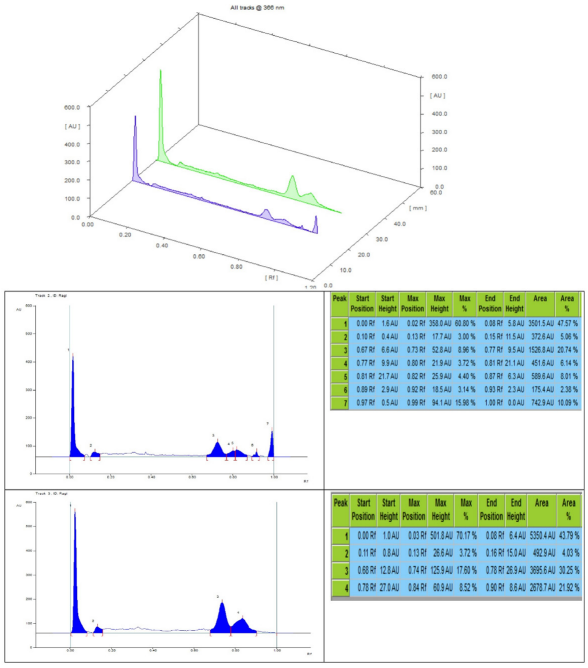


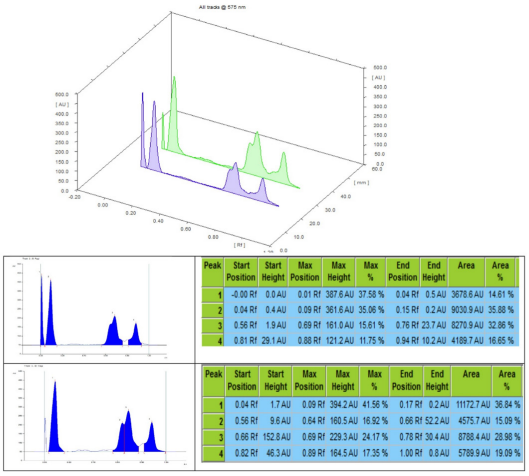
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	1.0 AU	0.02 Rf	280.2 AU	67.23 %	0.05 Rf	6.3 AU	2696.7 AU	36.41 %
2	0.05 Rf	6.9 AU	0.10 Rf	61.3 AU	14.70 %	0.13 Rf	6.6 AU	1186.7 AU	16.02 %
3	0.18 Rf	13.4 AU	0.24 Rf	31.2 AU	7.48 %	0.29 Rf	10.0 AU	1278.5 AU	17.26 %
4	0.72 Rf	5.6 AU	0.81 Rf	33.8 AU	8.06 %	0.89 Rf	7.4 AU	2187.2 AU	29.53 %
5	0.97 Rf	0.1 AU	0.98 Rf	10.8 AU	2.53 %	0.99 Rf	1.3 AU	57.8 AU	0.78 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.7 AU	0.03 Rf	347.4 AU	63.28 %	0.05 Rf	22.4 AU	3716.3 AU	29.88 %
2	0.05 Rf	22.7 AU	0.11 Rf	93.0 AU	16.94 %	0.14 Rf	10.8 AU	2408.9 AU	19.37 %
3	0.19 Rf	19.2 AU	0.25 Rf	50.6 AU	9.22 %	0.35 Rf	5.6 AU	2231.8 AU	17.94 %
4	0.69 Rf	8.1 AU	0.83 Rf	58.0 AU	10.56 %	0.93 Rf	11.7 AU	4082.3 AU	32.82 %

Supp Figure 1: HPTLC chromatographs of Eleusine coracana powder at 254 nm.



Supp Figure 2: HPTLC chromatographs of *Eleusine coracana* powder at 366 nm.



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	0.0 AU	0.01 Rf	387.6 AU	37.58 %	0.04 Rf	0.5 AU	3678.6 AU	14.61 %
2	0.04 Rf	0.4 AU	0.09 Rf	361.6 AU	35.06 %	0.15 Rf	0.2 AU	9030.9 AU	35.88 %
3	0.56 Rf	1.9 AU	0.69 Rf	161.0 AU	15.61 %	0.76 Rf	23.7 AU	8270.9 AU	32.86 %
4	0.81 Rf	29.1 AU	0.88 Rf	121.2 AU	11.75 %	0.94 Rf	10.2 AU	4189.7 AU	16.65 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	1.7 AU	0.09 Rf	394.2 AU	41.56 %	0.17 Rf	0.2 AU	11172.7 AU	36.84 %
2	0.56 Rf	9.6 AU	0.64 Rf	160.5 AU	16.92 %	0.66 Rf	52.2 AU	4575.7 AU	15.09 %
3	0.66 Rf	152.8 AU	0.69 Rf	229.3 AU	24.17 %	0.78 Rf	30.4 AU	8788.4 AU	28.98 %
4	0.82 Rf	46.3 AU	0.89 Rf	164.5 AU	17.35 %	1.00 Rf	0.8 AU	5789.9 AU	19.09 %

Supp Figure 3: HPTLC chromatographs of *Eleusine coracana* powder at 575 nm.