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RESEARCH ARTICLE

PHYTOCHEMICAL EVALUATION OF *ELEUSINE CORACANA* IN CORRELATION WITH ITS NUTRITIONAL VALUE

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Abstract

Eleusine coracana, a tropical cereal, commonly known as finger millet found in region of India and Africa contains large source of phytochemicals. The nutrient content of finger millet is high and can be further enhanced by processing, the hypoglycemic, anti-ulcer properties and hypo-cholesterolic effect of finger millet are among its health benefits. Scientific proof of the existence of phenolics and flavonoids in finger millet, however, is lacking. Additionally, analytical methods to calculate the amounts of gallic acid and quercetin have not been developed. The present investigation includes phytochemical screening, estimation of total phenolic and flavonoid content, determination of calcium content, as well as analytical method development for two bio-actives using High-Performance Thin Layer Chromatography (HPTLC). The final method was optimized using Toluene: Ethyl acetate: Methanol: Formic acid (3:3:0.8:0.4 v/v/v/v) and densitometric scanning of the plate was performed in absorption mode at 271 nm. Validation of the developed method was done according to the ICH guidelines. Linearity range was found to be 200-600 ng/spot with R^2 value 0.988 and 0.9976 for quercetin and gallic acid, respectively. Other validation parameters like precision, repeatability, and accuracy were performed and the results were found according the specifications. The quantification of quercetin and gallic acid in the sample was found to be 0.61% and 0.55% w/w, respectively. Despite the widespread use of synthetic antioxidants, calcium determination, estimates of quercetin and gallic acid in the present investigation provided assurance of health benefits & quality and purity of finger millet. However, there is growing evidence that consumers prefer natural antioxidants due to their potential for lower toxicity.

Keywords: Nutrition, HPTLC, Validation, Gallic acid, Quercetin, Finger millet

INTRODUCTION

Health has recently been an issue in terms of malnutrition among new-borns and children under the age of five, particularly in developing nations such as India. A poor diet causes issues, such as deficiency disorders including anaemia, scurvy, cretinism, and stillbirth [1]. Cereals are plants that have traditionally been utilized solely as a source of food and as animal feed. They serve as a source of energy for a typical human diet. More people are gravitating towards gluten-free diets, which use millet prominently [2, 3]. The grain known as millets, which belongs to the grass family, has a higher nutritious value. Millets come in a variety of varieties, including proso, foxtail, pearl, finger, and kodo millet. All varieties of millet are excellent sources of vitamins, minerals, carbohydrates, protein, and lipids. Additionally, millet is an excellent provider of minerals including phosphorus, manganese, magnesium, and calcium [4].

Tropical grain *Eleusine coracana*, sometimes referred to as finger millet, is grown in parts of Africa and India and is a rich source of phytochemicals. Oleic acid (47.17% w/w), linoleic acid (24.78%), and palmitic acid (23.06%) are the primary components of fatty acids, while stearic acid (0.58%) and arachidic acid (0.27%) are present in minor concentrations [5, 6]. Additionally, millet is a strong source of phosphorus, manganese, magnesium, and calcium, minerals that lower the risk of heart disease and are necessary for the metabolism of energy as well as for conditions like osteoporosis and low-density bone disease. Due to its calcium concentration and decreased bone resorption, finger millet has high calcium bioavailability and enhances calcium retention compared to other staple foods, therefore it may have favorable benefits, particularly in children, the elderly, and women, according to the limited published research [7]. According to several previous reports, *E. coracana* contains polyphenols like tannins, flavonoids, and phenolic acid. These substances are consumed by humans and animals and have high nutritional value as well as a variety of pharmacological effects. These include antioxidant, anti-estrogenic, anti-mutagenic, anti-carcinogenic, anti-platelet aggregation inhibitory activity, antiviral, and anti-inflammatory activity. Furthermore, it possesses antibacterial, anti-diabetic, wound-healing, antilithiasis and in cardiac conditions like atherosclerosis qualities [8-11].

The quality parameters need to be set for assuring the standards of *E. coracana* species collected from Gujarat. Scientific validation of crude drug is extremely important prior to their go for pharmacological and clinical applications. As a result, it is essential to adhere to internationally accepted standards for determining their identity, quality, and purity [12,13]. The World Health Organization has described a series of tests

to ensure the quality of raw materials from medicinal plant. The aim of present investigation includes development of quality control parameters with respect to phytochemical screening, physicochemical evaluation; estimation of calcium content and heavy metal screening using atomic absorption spectroscopy (AAS) [13,16]. Because they include phenolic compounds, plant secondary metabolites have the potential to trap free radicals that are present in the human body, making antioxidant activity one of their most valuable qualities. The development of an HPTLC technique for the quantification of gallic acid & quercetin in the various gathered samples was done as a subsequent inquiry, and the method was verified [14]. Total phenolic and flavonoid content analysis will be necessary to demonstrate the finger millet's potential health benefits; calcium content analysis will be useful to demonstrate how important calcium is as an integrative element of the human body and how important it is for quality control; and HPTLC analysis of the plant will guarantee its purity and quality.

EXPERIMENTAL WORK

Identification, collection and processing of *Eleusine coracana*

Eleusine coracana seed samples have been acquired from three separate regions. First, from the local market of Kachchh, Gujarat, India (ECK), then from Maharashtra, India (ECM), and finally from Ahmedabad, Gujarat, India (ECA). Eleusine coracana seed powder is often referred to as 'Ragi' flour. Collected seeds were milled into flour and then sieved using a 60# sieve for consistent size. Seeds were recognised by their colour, fragrance, size, and shape after powdered samples had their colour, texture, and taste evaluated.

Determination of physico-chemical parameters

Proximate parameters have been performed for evaluation of three samples which includes loss on drying, ash value such as total ash value, acid insoluble, water soluble and alcohol soluble and water-soluble extractive values.

Preparation of crude extracts and Phytochemical screening

Extractive values are used to estimate the amount of soluble components present in each of the three powdered samples. Petroleum ether, ethyl acetate, methanol, and water were used to create the fractions, which were then tested for the presence of phytoconstituents using phytochemical screening. The physical parameters of each produced extract following a further extraction were examined. The presence of numerous phytoconstituents such as saponins, alkaloids, carbohydrates, flavonoids, steroids, and terpenoids, as well as anthraquinone glycosides, coumarins, tannins, phenolic and carotenoids was then determined using the following chemical tests, which were performed on each sample independently [15].

Determination of calcium and heavy metals content by AAS

For determination of metal and mineral composition atomic absorption spectroscopic technique is used. Powdered samples of *E. Coracana* (ECA, ECK and ECM) have been analysed for determination of calcium content and heavy metals using atomic absorption spectroscopy (AAS). According to AOAC official Method 999.11, it was analyzed for the presence of lead (as Pb), cadmium (as Cd), and arsenic (as As), and AOAC official Method 971.21 [16] was used to determine the presence of mercury (as Hg) in plant material. In flame AAS, the burner converts the aerosol/gas mixture created by the spray chamber and nebulizer, into free, ground state atoms. A sample is introduced into the atom cell, where it is desolvated and then atomized. The burner head is aligned so that the light beam passes through the flame, where the light is absorbed. Only a small fraction of the sample reaches the flame, and the atomized. Sample passes quickly through the light path. It is detected by detector and that signal is amplified and read out device converts it to digital form [18].

Estimation of total phenolics

Total Phenolic content of *E. coracana* extract was determined by using folin-ciocalteu method. Gallic acid was used as a reference standard and calibration curve was prepared. After 24 hours of maceration, 0.1 g of ECA, ECK, and ECM samples were extracted using 100 milliliters of methanol and filtered. Methanol was used to reduce the filtrate's final volume to 100 milliliters. For the purpose of estimating the total phenols, 5 milliliters of extract were further diluted with methanol of equal volume. To 1mL of the methanolic remove (500µg/mL) 10 mL of refined water and 1.5 mL of weakened folin-ciocalteu reagent (1:2) were added and the combination was saved aside for 5 min in the wake of adding 4 mL of 20%w/v Na_2CO_3 arrangement the last volume was acclimated to 25 mL utilizing refined water. The reaction mixture was incubated at room temperature with intermittent shaking for color development. The absorbance was measured at 765 nm at an interval of 30 min until 2h using distilled water as a blank. [20, 28] The total phenolic content was determined from linear equation of a standard curve prepared using gallic acid.

Estimation of total Flavonoids

The flavonoids content was determined by aluminum tri-chloride method using quercetin as a reference standard. Quercetin was used as a reference standard and calibration curve was prepared. 0.1 g of dried flour was extracted with 100 mL methanol by maceration for 24 hrs and filtered. Methanol was used to reduce the filtrate's final volume to 100 milliliters. One milliliter of this extract was diluted to 10 milliliters and used to estimate the flavonoids. In the prepared 3 mL of the methanolic extract, 3mL of methanolic $AlCl_3$ was added [21] and after 10 min, the UV absorbance was taken at 430 nm. The

results were expressed as g/100g of dry matter using quercetin as a reference.

Development and validation of HPTLC method

Preparation of standard solution of quercetin and gallic acid:

An accurately weighed quantity (5mg) of gallic acid and quercetin was dissolved in 10 mL of methanol to gel stock solution of 500 µg/ mL from the prepared stock solution, 1 ml of solution was further dilute it up to 10 ml with methanol to get 50 µg/ml of working standard solution of gallic acid and quercetin for further chromatographic analysis.

Preparation of plant extracts

1 g of samples of ECA, ECK, and ECM were extracted with 100mL methanol by reflux and filtered. The filtrate was evaporated to dryness and the dried methanolic was dissolved in 10 mL of methanol. The solution was filtered using membrane filter and sonicated.

Optimized Chromatographic condition

The pre-coated silica gel on aluminium plate $60F_{254}$, (10cm x 10cm), prewashed by methanol and activated at 105 °C for 15 minutes prior to chromatography. Using a CAMAG Linomat 5 applicator with a 100-L syringe and a constant application rate of 0.1 L/s, the solutions were applied to prewashed TLC plates in bands that were 5 mm wide, 10 mm from the bottom edge, 10 mm from the side edge, and 6 mm apart. Chromatograms were run to an 80 mm solvent front by ascending development in the CAMAG twin through chamber pre-saturated for 20 min with toluene, ethyl acetate, methanol, and formic acid (3:3:0.8:0.4, v/v/v/v) as a mobile phase at ambient temperature (25 ± 2°C and 40% RH) [22-27]. The chromatogram run length was approximately 80 mm from the application point. Following development, TLC plates were air-dried using a hair dryer. The plate was densitometrically scanned in absorption mode at 271nm using a CAMAG TLC Scanner 3 with winCATS software.

Validation of developed HPTLC method for quercetin and gallic acid

The established procedure was verified for linearity and range, accuracy, specificity, precision, LOD (limit of detection), and LOQ (limit of quantification) in accordance with the International Council for Harmonisation (ICH) recommendations [29,30].

Linearity and range

From standard solution (50µL/mL) volume of 4, 6, 8, 10, 12 µL were spotted on the TLC plate to obtain final concentration 200 to 600 ng/spot of quercetin and gallic acid. The plate was developed in optimized condition and peak areas were plotted against concentration to get calibration curve. Results were analysed for linearity equation and co-relation coefficient.

Precision (Intraday Precision and Interday Precision)

Repeatability of sample application was determined by spotting the standard solution (200ng/spot) 6 times on the same plate and peak. The intermediate precision was measured by injecting three different days and three times on single day which is for three different concentrations (300, 400, 600ng/spot) that were applied in manner of three replicate. The results were reported in terms of % RSD.

Accuracy

Three different levels (80%, 100% and 120%) of assay concentration were used for the recovery study by standard (500ppm) addition method to check the accuracy of that method. The solutions were applied and analysed through finalized chromatographic condition. % recovery was calculated for each level.

Limit of Detection and Limit of Quantification

LOD/LOQ was determined by linearity range solution in triplicate.

RESULTS AND DISCUSSION

Morphological description of collected samples of *E. coracana*

Morphological studies are conducted to authenticate and identify plant samples, advancing the research into further analysis. By contrasting the morphological descriptions provided with those found in the literature, the gathered plant samples were identified. The seeds of E. coracana were found to be spherical in form, about 1 mm in diameter, and reddish brown in colour with a mucilaginous flavour (Figure 1). The manufactured flour was discovered to be smooth and mucilaginous tasting, with a pink tint (Figure 2).

Figure 1: Seeds of *E. coracana*

Figure 2: Flour of *E. coracana*

Determination of physico-chemical parameters

Table 1 lists the findings from tests on drying loss, ash values, and extractive value of ECA, ECK, and ECM. It was noted from the reported extractive values that the water-soluble extractive value is larger than the alcohol soluble extractive value, possibly as a result of the presence of secondary metabolites such as phenolics and carbohydrates.

Table 1: Physico-chemical parameters of *Eleusine coracana*

Tests	Values	ECA (% w/w)	ECK (% w/w)	ECM (% w/w)
Loss on drying	Moisture content	1.9	1.9	1.25
Ash value	Total Ash value	2.95	3.15	3.26
	Acid insoluble	1.05	0.96	0.91
	Water soluble	0.575	0.498	0.482
Extractive value	Water soluble	5.5	5.8	5.4
	Alcohol soluble	4.64	4.79	4.76
	Pet ether soluble	2	1.7	1.8

Phytochemical screening

While methanolic extract contains phytosterols, triterpenoids, phenolics, and flavonoids, aqueous extract demonstrated the presence of carbohydrates, saponins, phenolics, and tannins. While petroleum ether includes alkaloids, phytosterols, and triterpenoids, other organic fractions including the ethyl acetate fraction tested positive for alkaloids, phytosterols, triterpenoids, and flavonoids. (Table 2)

Table 2: Preliminary phytochemical screening

Phytoconstituent	Aqueous extract	Methanolic extract	Ethylacetate extract	Pet. Ether extract
Alkaloids	-ve	-ve	+ve	+ve
Carbohydrates	+ve	+ve	-ve	+ve
Phenolic	+ve	+ve	-ve	-ve
Tannins	+ve	+ve	-ve	-ve
Flavonoids	-ve	+ve	+ve	-ve
Phytosterol & triterpenoids	-ve	+ve	+ve	+ve
Saponin	+ve	-ve	-ve	-ve

Quantification of calcium and presence of heavy metals by AAS

Table 3 shows amount of calcium found in all three samples of *E. coracana*. The amount of calcium is found to be more in ECM as compared to other samples. Many species of plants have been successful in absorbing contaminants such as lead, cadmium, chromium, arsenic, and various radionuclides from soils. Heavy metals are also responsible for toxic effects of plants especially lead (Pb), arsenic (As) and mercury (Hg) are poisonous heavy metals, thus it is required to check the presence of heavy metals in raw material before further investigation [31]. It was observed that the collected samples of *E. coracana* **does not contain** any heavy metals (Table 3). This indicates that the samples can be further safely used for estimation and evaluation of phytoconstituents in terms of its therapeutic uses.

Table 3: Detection of calcium by AAS

Sample	**Variety**	**Calcium content per 100 g**
Finger millet flour	ECA	225.12
	ECK	238.18
	ECM	342.28

Estimation of total phenolics and flavonoid content

According to the protocol and standard procedures, phenolics were calculated using the Folin-Ciocalteu reagent (1:2) and flavonoids using the $AlCl_3$ technique. All samples included greater flavonoid concentration, which suggests that finger millet has better health advantages. (Table 4, figure 3). Significant amounts of phenolics and flavonoids in the form of gallic acid and quercetin will be involved in regulating a broad range of cellular signalling pathways to prevent, mitigate, or slow the progression of chronic disorders, including cardiovascular and neurodegenerative diseases; it has an effect on diabetic retinopathy, liver diseases, microbial infections, anti-obesity and anti-diabetic, all types of cancer, promote self-renewal, and reduce blood pressure.

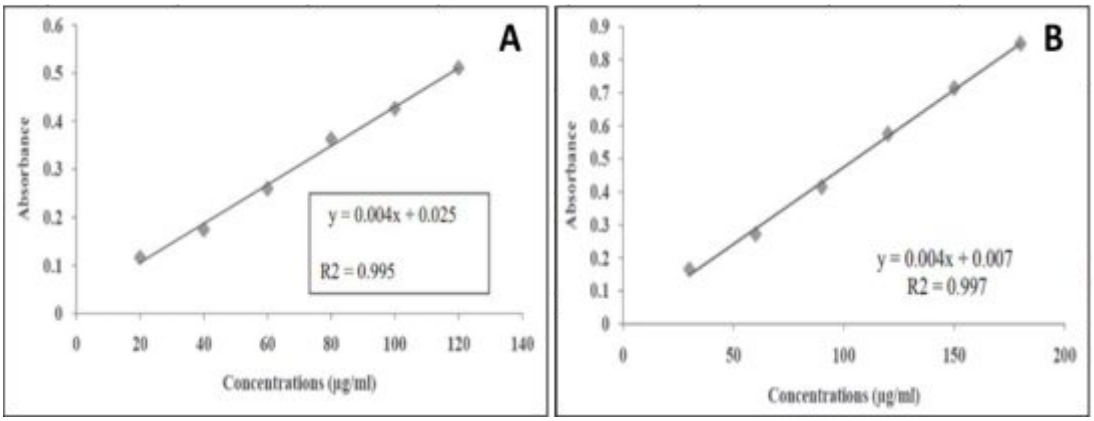

Figure 3: Standard curve of A) gallic acid for phenolics, B) quercetin for flavonoids

TABLE 4: Estimation of phenolics and flavonoids

%w/w Finger millet flour	ECA	ECK	ECM
% Flavonoid content	0.361	0.617	0.591
% Phenolic content	0.334	0.468	0.427

Development of HPTLC method for estimation of quercetin and gallic acid

Along with the aforementioned mobile phase, several mobile phase compositions were examined. The desired resolution of quercetin and gallic acid was accomplished using the mobile phase toluene: ethyl acetate: methanol: formic acid (3:3:0.8:0.4 v/v/v/v). Figure 4 shows a chromatogram of quercetin (1000ng/spot) and gallic acid (1000ng/spot) from an HPTLC plate prepared under optimized conditions.

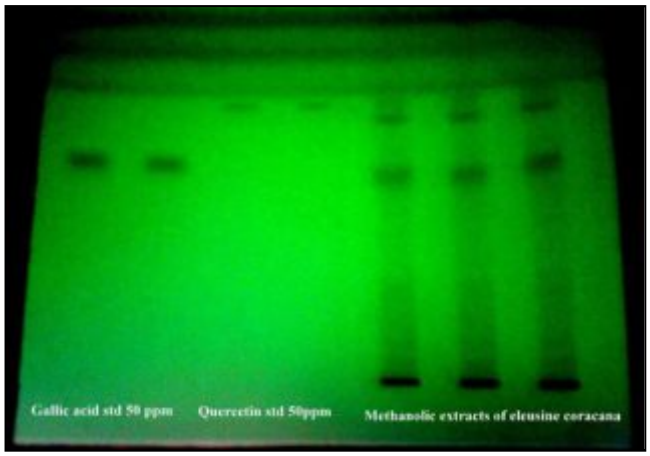

Figure 4: HPTLC chromatogram of Gallic acid at R_f 0.55 and quercetin at R_f 0.72

Analytical method validation of developed HPTLC method

Linearity

The calibration equation and correlation coefficient were derived by applying least square regression analysis to the discovered peak area and concentrations in order to ascertain their linearity. The observed linearity suggests that the system follows Beer's Law. Linearity was determined by adding various amounts of the standard quercetin to the plate. Linearity was found for quercetin at Rf 0.55 and gallic acid at Rf 0.72 (Figure 5) over a concentration range of 200-600 ng/spot with correlation coefficient (r^2) = 0.9939 and calibration curve equation y = 14.867x + 192.96 correlation coefficient (r^2) = 0.9976 and calibration curve equation Y=7.2344x + 828.9 (Table 5), respectively (Figures 5, 6).

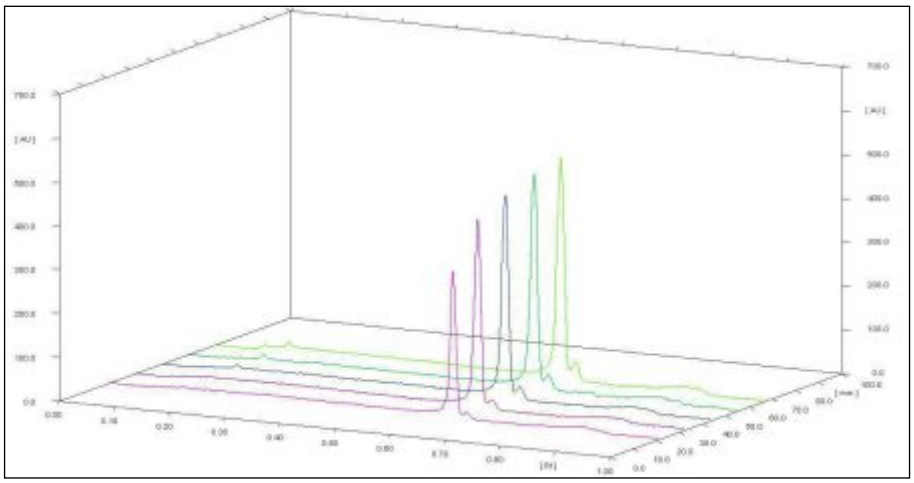

Figure 5: HPTLC Chromatogram of linearity of quercetin (200-600ng/spot)

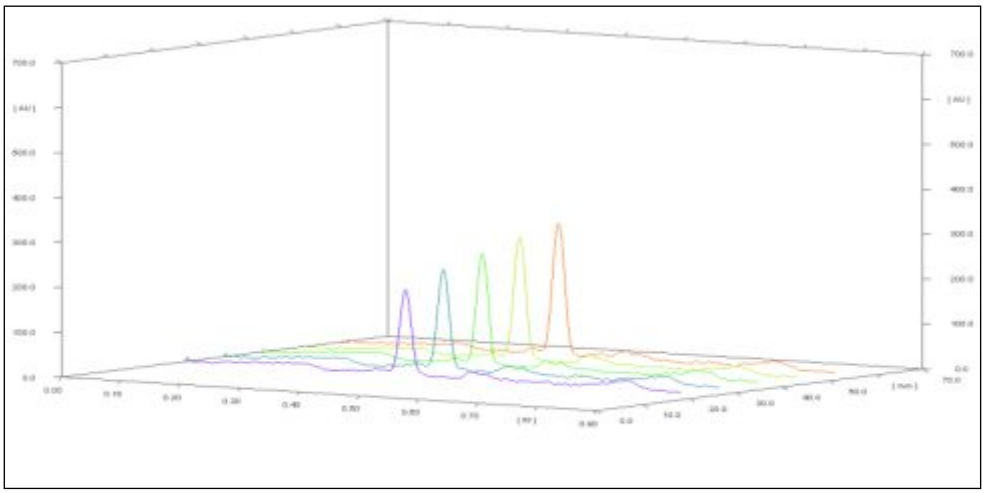

Figure 6: HPTLC Chromatogram for linearity of Gallic acid (200-600ng/spot)

Interday and Intraday precision

All the samples of quercetin and gallic acid were checked for Intra-day and inter-day variation and found to have % RSD values less than 2 during analysis mentioned in table 5. This low value of RSD indicates that the developed method is quite precise for the estimation of quercetin and gallic acid.

Repeatability Instrument Precision

The precision of sample application was demonstrated by the findings that the repeatability of sample application (percent RSD) for the peak areas of gallic acid and quercetin was 1.509 and 1.525, respectively.

Limit of Detection (LOD) and limit of quantification (LOQ)

LOD was 15.386 ng/spot for quercetin and 20.51 ng/spot for gallic acid, found by using equation LOD= 3.3 σ/ S (σ= standard deviation of intercept S =Slope of the linearity curve). The calibration curve was performed six times, and the standard deviation of intercepts was determined. LOQ was 46.625 ng/spot for quercetin and 62.177 ng/spot for gallic acid, found by using equation LOQ= 10 σ/ S.

Table 5: Summary of validation parameters

Sr. No.	Parameters	Result (Quercetin)	Result (Gallic acid)
1	Linearity Range	200-600ng/spot	200-600ng/spot
2	Regression Equation	y=14.867x+192.96	y=7.2344x+828.9
3	Correlation Coefficient (R^2)	0.988	0.9976
4	Intra-day Precision (%RSD)	0.8013	0.8727
	Inter-day Precision (% RSD)	1.3958	0.8026
	Repeatability (% RSD)	1.509	1.5257
5	Limit of Detection (LOD)	15.386 ng/spot	20.51 ng/spot
6	Limit of Quantification (LOQ)	46.625 ng/spot	62.17 ng/spot
7	Specificity	Specific	Specific

Accuracy

The other matrix components' positive and negative effects on the quantification parameters are revealed by accuracy studies. The recovery data presented indicates that the accuracy of quantification of quercetin and gallic acid respectively in presence of other flour components, was found between 99.1 – 100.9 % and 99.0 -101.4 % (Table 6).

Table 6: Accuracy data of Quercetin

Level of recovery	Sample conc (ng/spot	Amount of std added	Total content	Amount recovered	% Recovery	Amount Recovered	% Recovery
				Quercetin		Gallic acid	
80	200	160	360	361.97	100.5	356.7	99.0
	200	160	360	359.02	99.7	361.7	100.4
	200	160	360	358.99	99.7	361.4	100.4
100	200	200	400	399.50	99.8	392.0	98.0
	200	200	400	397.04	99.2	407.8	101.9
	200	200	400	403.45	100.8	400.1	100.0
120	200	240	440	439.64	99.9	442.4	100.5
	200	240	440	436.16	99.1	436.4	99.1
	200	240	440	444.19	100.9	441.1	100.2

Quantification of quercetin and gallic acid in different samples

The developed and validated HPTLC technique was effectively employed to quantify quercetin and gallic acid from a plant species, *Eleusine coracana*. Many mobile phases were applied to separate additional phytoconstituents from collected plants and their constituents were identified. The mobile phase stated here demonstrated excellent separation with minimal interference from other peaks during the development of the HPTLC technology. The quantity of quercetin and gallic acid contained in the plant sample was found to be 0.61% w/w in *Eleusine coracana*, compared to Gallic acid at 0.55% w/w.

CONCLUSION

Quality control criteria for three samples *Eleusine coracana* were invented in the current investigation. The development of the physicochemical parameters and phytochemical screening revealed the presence of carbohydrates, saponins, phenolics, and tannins, as well as phytosterols, triterpenoids, phenolics, and flavonoids. Various samples collected from different regions of India to study the quality control parameters and to analysis the content of phytoconstituents in order to see the potential effect of the Ragi. The samples were also examined for their total phenolic and flavonoid contents as well as the amount of calcium in the finger millet. It is observed that *Eleusine coracana* contain good flavonoids and phenolic content is higher in sample of ECK which can be taken further for analysis and estimation of bioactive. The absence of heavy metals demonstrated that finger millet has no adverse effects on the body. Consuming heavy metal-contaminated meals can severely deprive the body of important nutrients, causing to weakened immune systems, uterine development retardation, malnutrition-related impairments, and an increased risk of upper gastrointestinal cancer. Lack of calcium during the growth of a child leads to bone related issues later in life, mainly in the form of osteoporosis and osteopenia. Finger millet has the potential to naturally correct calcium deficiency. Improved dietary intake of Calcium in the form of millet may be the most cost-effective way to correct such deficiencies. Estimated total phenolics and flavonoid benefits for human health promotion and disease curing and prevention include antioxidant effects, antibacterial effects, cardioprotective effects, anticancer effects, immune system promoting, anti-inflammatory effects, and skin protection from UV radiation. The HPTLC method was developed and validated for simultaneous estimation of quercetin and gallic acid. The developed HPTLC method can also be implemented to estimate gallic acid and quercetin from other plant samples. The present research work can be utilized for the evaluation of different pharmacological activities and also for the further isolation and estimation of other constituents present in *Eleusine coracana* sample.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

REFERENCES

[1] Anagha, K. K. (2023). Millets: Nutritional importance, health benefits, and bioavailability: A review. *Energy*, *329* (328), 361.

[2] Shobana, S., K. Krishnaswamy, V. Sudha, N. G. Malleshi, R. M. Anjana, L. Palaniappan, and V. Mohan. (2013). "Finger millet (Ragi, Eleusine coracana L.): a review of its nutritional properties, processing, and plausible health benefits." *Advances in food and nutrition research* 69, 1-39.

[3] Chandra, Dinesh, Satish Chandra, and A. K. Sharma. (2016). "Review of Finger millet (Eleusine coracana (L.) Gaertn): A power house of health benefiting nutrients." *Food Science and Human Wellness* 5(3), 149-155.

[4] Kumar, Anil, Mamta Metwal, Sanveen Kaur, Atul K. Gupta, Swati Puranik, Sadhna Singh, Manoj Singh et al. (2016) "Nutraceutical value of finger millet [Eleusine coracana (L.) Gaertn.], and their improvement using omics approaches." *Frontiers in plant science* 7, 934.

[5] Bello, O. M., Ogbesejana, A. B., Balkisu, A., Osibemhe, M., Musa, B., & Oguntoye, S. O. (2022). Polyphenolic fractions from three millet types (Fonio, Finger millet, and Pearl millet): Their characterization and biological importance. *Clinical Complementary Medicine and Pharmacology*, *2*(1), 100020.

[6] Maharajan, T., Ceasar, S. A., & Ajeesh Krishna, T. P. (2022). Finger millet (Eleusine coracana (L.) Gaertn): Nutritional importance and nutrient transporters. *Critical Reviews in Plant Sciences*, *41*(1), 1-31.

[7] Devi, Palanisamy Bruntha, Rajendran Vijayabharathi, Sathyaseelan Sathyabama, Nagappa Gurusiddappa Malleshi, and Venkatesan Brindha Priyadarisini. (2014) "Health benefits of finger millet (Eleusine coracana L.) polyphenols and dietary fiber: a review." *Journal of food science and technology* 51, 1021-1040.

[8] Shashi, B., Sunanda, S., Shailaja, H., Shankar, G., and Nagarathna, K. (2007). Micronutrient composition, antinutritional factors and bioaccessibility of iron in different finger millet *(Eleusine coracana*) genotypes, *Karnataka Journal of Agricultural Sciences,* 20(3): 583-585.

[9] Viswanath, Varsha, Asna Urooj, and N. G. Malleshi. (2009). "Evaluation of antioxidant and antimicrobial properties of finger millet polyphenols (Eleusine coracana)." *Food Chemistry* 114 (1), 340-346.

[10] Nakarani, Udit M., Diwakar Singh, Kiran P. Suthar, Nilima Karmakar, Priti Faldu, and Harshal E. Patil. (2021). "Nutritional and phytochemical profiling of nutracereal finger millet (Eleusine coracana L.) genotypes." *Food Chemistry* 341, 128271.

[11] Anitha, S., Givens, D. I., Botha, R., Kane-Potaka, J., Sulaiman, N. L. B., Tsusaka, T. W., & Bhandari, R. K. (2021). Calcium from finger millet—a systematic review and meta-analysis on calcium retention, bone resorption, and in vitro bioavailability. *Sustainability*, *13*(16), 8677.

[12] Chatli, Manish Kumar, Pavan Kumar, Nitin Mehta, Akhilesh K. Verma, Devendra Kumar, and Om Prakash Malav. (2015). "Quality characteristics and storage stability of emu meat nuggets formulated with finger millet (Eleusine coracana) flour." *Nutrition & food science* 45 (5), 740-752.

[13] Panwar, Preety, Manoj Nath, Vijay Kumar Yadav, and Anil Kumar. (2010). Comparative evaluation of genetic diversity using RAPD, SSR and cytochrome P450 gene based markers with respect to calcium content in finger millet (Eleusine coracana L. Gaertn.). *Journal of Genetics* 89, 121-133.

[14] Patel P.M., Patel, N.M., and Goyal, R.K. (2006). Quality control of herbal products. *The Indian Pharmacist,* 5(45): 26-30.

[15] Norman R. Farnsworth (1966) Biological and Phytochemical Screening of Plants, Journal of Pharmaceutical Sciences, 55(3), 225-276

[16] Singh, U.M., Chandra, M., Shankhdhar, S.C., Kumar, A. (2014). Transcriptome wide identification and validation of calcium sensor gene family in the developing spikes of finger millet genotypes for elucidating its role in grain calcium accumulation. *PLoS ONE*, 9(8)

[17] Nelson, J., Jones, C., Heckle, S., & Anderson, L. (2022). Determination of Heavy Metals in a Variety of Cannabis and Cannabis-Derived Products, First Action 2021.03. *Journal of AOAC International*, *105*(6), 1640-1651.

[18] Chemists, A. O. A. (2002). Official methods of analysis of the AOAC International.

[19] Khandelwal, K. (2008). *Practical Pharmacognosy*. Pragati Books Pvt. Ltd.

[20] Genwali, G. R., Acharya, P. P., Rajbhandari, M. (2013). Isolation of Gallic Acid and Estimation of Total Phenolic Content in Some Medicinal Plants and Their Antioxidant Activity. *Nepal Journal of Science and Technology*, *14*(1), 95–102.

[21] Sharma, V., & Janmeda, P. (2017). Extraction, isolation and identification of flavonoid from Euphorbia neriifolia leaves. *Arabian Journal of Chemistry*, *10*(4), 509–514.

[22] Shen, M. R., He, Y., & Shi, S. M. (2021). Development of chromatographic technologies for the quality control of Traditional Chinese Medicine in the Chinese Pharmacopoeia. In *Journal of Pharmaceutical Analysis*, 11(2), 155–162.

[23] Gulati, G., and Baghe, S. (2013). Quantification of quercetin from alcoholic extract of *Emblica officinalis* by HPLC method. *International Journal of Green Chemistry and Bioprocess*, 3(1), 1-2.

[24] Kumar A., Lakhshaman, K., Jayaveera, K.N., Tripathi S.N.M. and Satish K.V. (2010) Estimation of Gallic Acid, Rutin and Quercetin in *Terminalia chebula* by HPTLC. *Jordan Journal of Pharmaceutical Sciences*, 3(1) 63-68.

[25] Thakkar Y., and Shah V. (2011) Simultaneous estimation of Gallic acid, Curcumin and Quercetin by HPTLC method. *Journal of Advanced Pharmacy Education & Research* 1:70-80.

[26] Tiwari, P., Kumar, B., and Kaur, M. (2011). Phytochemical Screening and Extraction: A Review. *Internationale Pharmaceuticasciencia*, (1)1: 98-106.

[27] Yadav, M., Chatterji, S., Gupta, S.K., and Watal, G. (2014) Preliminary phytochemical screening of six medicinal plants used in traditional medicine. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(5): 539-542.

[28] Siddique, N.A., Mujeeb, M., Najmi, A.K., and Akram, M. (2010). Evaluation of antioxidant activity, quantitative estimation of phenols and flavonoids in different parts of *Aegle marmelos*. *African Journal of Plant Science,* 4(1): 1-5.

[29] Gandhi, D.M., Mehta, P.J. 2013. Validated High-Performance Thin-Layer Chromatographic Method for the Quantification of Betulinic Acid from Two Indian Plants of the Species Dillenia and Ziziphus. *JPC-J Planar Chromat*, 26, 331–335

[30] Gandhi, D. M., Kapadia, N. S., Shah, S. K., Verma, P. D., Shah, K. N., & Shah, M. B. (2012). Pharmacognostical Study and

Development of Quality Control Parameters for *Sarcostemma brevistigma* Wight and Arn. *Journal of Natural Remedies*, *12*(1), 77-86.

[31] Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. (2011). A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *International Journal of Chemical Engineering*.