

# Estimation of phenolic acids in different preparations of seeds of finger millet (*Eleusine coracana*): Their possible implications in human health

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## Abstract

HPLC analysis for phenolic acids of seeds of six varieties (Gaza, KM-252, PES-400, Ranichauri, VL-146, VL-149) and a local variety and their different preparations, i. e., dry, water soaked, cooked seeds, flour and bread of *E. coracana* indicated that dry seeds of some cultivars are rich in phenolic acids. Variety Ranichauri (Local varieties) and KM-252 had five and four phenolic acids respectively, in which GA was maximum. Gaza, VL-146 and VL-149 had three phenolic acids in which gallic acid was maximum. Water soaked seeds of the above mentioned cultivars gave similar results in which GA was maximum in most of them followed by TA, VA, Chl-A and Caf-A. Water extract of seeds showed several water-soluble phenolic acids such as TA, GA, P-Cat-A, FA, Chl-A, CA. Among them, P-Cat-A (592.05 µg/g) and FA (147.52 µg/g) were present in good amount. Dry and cooked seeds, extract of boiled seeds, flour and bread also had several phenolic acids in good amount.

## INTRODUCTION

Finger millet (*Eleusine coracana* subsp. *coracana*) and its wild relatives are members of the Chloridoideae, one of the four primary subfamilies of the grass family Poaceae. The Chloridoideae have received much less attention than any other grass lineage. Only finger millet is found among the Chloridoideae as an important cereal crop. It is grown in over 4 million hectares and is the primary food of millions of people in dry-land regions of East Africa, Central Africa and Southern India. The seeds have high nutritional value as they are rich source of calcium, iron and methionine (1). Mugula, and Lyimo (2) evaluated the nutritional quality and acceptability of finger millet-based food (tempe) as potential weaning foods in Tanzania, which became very popular among the rural people. Use of fermented flour of finger millets increases the availability of protein, starch and minerals in human body which inhibits *Salmonella typhimurium* and *Escherichia coli* (3, 4, 5, 6).

Several epidemiological studies have consistently shown that consumption of fruits and vegetables, as well as grains, has been strongly associated with reduced risk of chronic diseases such as cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts and age-related functional decline (7, 8, 9, 10, 11).

The antioxidant characteristics of plant-based bio-product can be attributed to the available amount of polyphenols (12). Until recently most of the nutritional interests in polyphenols was in the deleterious effect caused by the ability of polyphenols to bind and precipitate macromolecules such as dietary protein, carbohydrates and digestive enzymes and thereby reducing food digestibility (13, 14, 15). However, interest in food phenolics has increased because of their antioxidant and free radical scavenging ability (11). Polyphenols are products of secondary metabolism of plants and distributed in plant organs with maximum amount in seeds specially in spices (16). They are synthesized biogenetically from two main pathways, e.g., the shikimate and the acetate pathways (17, 18). Natural polyphenols can range from simple molecules such as phenolic acids, to highly polymerized compounds, such as tannin.

Several bioactive phytochemicals, non-nutrient plant compounds in fruits, vegetables, grains and other plant foods prepared from the grains may have been linked in the reductions of the risks of several major chronic diseases (11, 19). Ravikumar and Seetharam et al. (20) reported that *E. coracana* is resistant against *Pyricularia grisea* due to the presence of preformed structural and biochemical defense. Gowda et al. (21) reported the relationship of protein, phenols

and tannins with blast disease in finger millets. Increasing evidences suggest that the antioxidant activity of phytochemicals (phenolic acids) in fruits and vegetables may have a greater impact on human health and plant diseases. Keeping this in view experiments were conducted to analyze phenolic acids of different preparations of seeds of *E. coracana*. The results are presented here.

## **MATERIALS AND METHODS**

### **EXTRACTION OF PHENOLIC ACIDS FROM RAW AND VARIOUS PREPARATIONS OF**

One gram of dried, water soaked, boiled seeds, flour and bread (vernacularly known as chapati) of *Eleusine coracana* was macerated separately in a pestle-mortar and finely crushed samples were suspended in 5 ml of ethanol-water (80:20; v/v). These samples were collected in screw-capped tubes and the suspension was subjected to ultrasonication (Branson Sonifier, USA) for 15 min at 4 ° C followed by centrifugation at 7,500 rpm for 15 min. The clear greenish supernatant was subjected to charcoal treatment to remove pigments from each sample and was then transferred to glass tubes. The residue was re-extracted twice and supernatant was pooled prior to evaporation under vacuum (Buchi Rotavapor Re Type). Dried samples were re-suspended in 1.0 ml HPLC grade methanol by vortexing and filtered through membrane filter (pore size 0.45 µm, Millipore) before HPLC analysis.

### **EXTRACTION OF PHENOLIC ACIDS FROM AQUEOUS EXTRACTS OF**

Five ml of water soaked/boiled seeds extract of *E. coracana* taken in screw capped tubes and fractionated with ethyl acetate and fractionated materials were evaporated. Dried samples were re-suspended in 1.0 ml HPLC grade methanol by vortexing and filtered through membrane filter (pore size 0.45µm, Millipore) before HPLC analysis.

### **HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) ANALYSIS**

HPLC analysis of fractionated material of seeds of *E. coracana* was performed on HPLC system (Shimadzu Corporation, Japan) equipped with two Shimadzu LC-10 ATVP reciprocating pumps, a variable UV-VIS detector (Shimadzu SPD-10 AVP), an integrator and Winchrom software for data recording and processing (Winchrom, India) (16). Reversed phase chromatographic analysis was carried out in isocratic conditions using C-18 reversed phase HPLC column {(250 x 4.6 mm i.d., particle size 5 µm) Luna

5µ C-18 (2), Phenomenex, USA} at 25°C. Running conditions included mobile phase methanol-0.4 % acetic acid (80:20, v/v), flow rate 1.0 ml/min, injection volume 5 µl and detection at 290 nm. Fractionated material (1mg/ml) and phenolic acids dissolved in HPLC grade methanol were injected thrice in the sample loop and the mean of the peak areas of individual compounds was taken for quantification. Tannic (TA), gallic (GA), protocatechuic (P-cat-A), caffeic (Caf-A), vanillic (VA), ferulic (FA), o-coumaric (o-Cou-A), chlorogenic (Chl-A) and cinnamic acids (CA) were used as internal and external standards. Phenolic compounds present in the sample were identified by comparing retention time (Rt) of standards, e.g., TA (Rt. 2.76 min), GA (Rt. 2.86 min), P-cat-A (3.04 min), Caf-A (Rt. 3.10 min), VA (Rt. 3.26 min), FA (Rt. 3.42 min), o-Cou-A (Rt. 3.58 min), Chl-A (Rt. 4.16 min) and CAs (Rt. 4.45 min). These phenolic acids were identified by co-injection of internal and external standard for their confirmation. Amount of individual compound was calculated by comparing peak areas of reference compounds with those in the samples run under similar elution conditions.

## **RESULTS AND DISCUSSION**

HPLC analysis for phenolic acids of seeds of six varieties (Gaza, KM-252, PES-400, Ranichauri, VL-146, VL-149) and a local varieties of *E. coracana* and their different preparations, i. e., dry, water soaked, cooked seeds, flour and bread of *E. coracana* were taken in this experiments. HPLC analysis of dried seeds indicated that some cultivars were rich in phenolic acids while others were poor. Variety Ranichauri (Local varieties) had TA, GA, VA, Chl-A and CA acids. Among them, GA (11.54 µg/g) was maximum followed by TA (6.07 µg/g) and VA (1.0 µg/g) but Chl-A and CA in traces. KM-252 cultivar had four phenolic acids (GA, Caf-A, O-Cou-A and CA) with a maximum amount of GA (33.67 µg/g), others were in traces. Gaza, VL-146 and VL-149 had three phenolic acids. The GA (23.20 µg/g) was maximum followed by TA (4.73 µg/g) but VA was in trace in VL-149. VL-146 also showed maximum amount of GA (21.03 µg/g) followed by TA (2.52 µg/g) with VA in trace. However, in Gaza cultivar, GA (12.14 µg/g) was maximum followed by VA (1.37 µg/g) with CA in trace. In PES-400 only GA and VA were detected, in which GA (32.92 µg/g) was maximum followed by VA (1.16 µg/g) (Table 1.).

**Figure 1**

Table 1: HPLC analysis of phenolic acids in the seeds of

Varieties	Phenolic acid (µg/g fresh wt)						
	Tannic acid	Gallic acid	Vanillic acid	Chlorogenic acid	Caffeic acid	o-Coumaric acid	Cinnamic acid
Gaza	ND	12.14±0.15	1.37±0.05	ND	ND	ND	0.005±0.001
Ranichauri	6.07±0.2	11.54±0.95	1.00±0.09	0.12±0.03	ND	ND	0.11±0.03
VL-146	2.52±0.6	21.03±1.14	0.51±0.02	ND	ND	ND	0.02±0.02
VL-149	4.73±0.09	23.20±1.08	0.37±0.09	ND	ND	ND	ND
KM-252	ND	33.67±1.52	ND	ND	0.24±0.6	0.13±0	0.93±0.06
PES-400	ND	32.92±2.51	1.16±0.12	ND	ND	ND	ND

ND-under detection limit, ± Standard error.

Water soaked seeds of the above mentioned cultivars of *E. coracana* gave similar results in which GA was maximum in most of them followed by TA, VA, Chl-A and Caf-A. Among all the cultivars, VL-146 had GA, TA, VA and Caf-As, in which GA (13.14 µg/g) was maximum followed by TA (5.95 µg/g) with VA (0.91 µg/g) and Caf-A (0.10 µg/g) in traces. In KM-252, three phenolic acids were detected in which GA (6.04 µg/g) was maximum followed by TA (4.46 µg/g) and VA (0.02 µg/g) in traces. However, Gaza and VL-149 had two phenolic acids, i.e., GA and VA but in Ranichauri GA and Chl-A were present (Table 2).

**Figure 2**

Table 2: HPLC analysis of phenolic acids of the water soaked seeds of

Variety	Phenolic acid (µg/g fresh wt)				
	Tannic acid	Gallic acid	Vanillic acid	Chlorogenic acid	Caffeic acid
Gaza	ND	12.40±0.98	2.6±0.01	ND	ND
Ranichauri	ND	11.09±0.81	ND	0.50±0.01	ND
VL-146	5.95±0.83	13.14±0.41	0.91±0.01	ND	0.10±0.002
VL-149	ND	9.11±0.05	0.96±0.06	ND	ND
KM-252	4.46±0.56	6.04±0.06	0.02±0.35	ND	ND
PES-400	ND	3.80±0.22	ND	ND	ND

ND-under detection limit, ± Standard error.

Water extract of seeds of the above cultivars of *E. coracana* showed several water-soluble phenolic acids such as TA, GA, P-Cat-A, FA, Chl-A, CA. Among them, P-Cat-A (592.05 µg/g) was maximum followed by FA (147.52 µg/g) and CA (0.006 µg/g) in Gaza cultivar. However, water extract of other cultivars (i. e., VL-146, VL-149, and KM-252) had five phenolic acids (FA, GA, TA, Chl-A and CAs), in which FA acid was maximum followed by GA, TA, Chl-A and CAs. Water extract of PES-400 had FA, GA, TA and CA, in which FA (136.79 µg/g) was maximum followed by GA (26.48 µg/g), TA (19.96 µg/g) and CA (0.03 µg/g). Water extract of seeds of Ranichauri cultivar had FA, P-Cat-A and Chl-A. Among them phenolic acids, FA (18.04 µg/g) was maximum followed by P-cat-A (16.57 µg/g) and Chl-A (0.82 µg/g) (Table 3).

**Figure 3**

Table 3: HPLC analysis of phenolic acids of water extract of seeds of

Varieties	Phenolic acid (µg/g fresh wt)					
	Tannic acid	Protocatechuic acid	Gallic acid	Ferulic acid	Chlorogenic acid	Cinnamic acid
Gaza	ND	592.05±0	ND	147.52±2.03	ND	0.006±0.001
Ranichauri	ND	16.57±0	ND	18.04±1.12	0.82±0.15	ND
VL-146	4.02±0.02	ND	8.90±0.03	77.09±2.32	2.61±0.42	0.21±0.009
VL-149	2.44±0.0	ND	5.77±0.65	56.14±1.51	1.83±0.02	0.41±0.005
KM-252	5.24±0.09	ND	7.8±0.16	71.5±2.86	0.18±0.01	0.05±0.0001
PES-400	19.96±0.16	ND	26.48±0.03	136.79±2.56	ND	0.03±0.0003

ND-under detection limit, ± Standard error.

Among all these cultivars, VL-149 had maximum phenolic acids after cooking, in which GA acid (4.41 µg/g) was maximum followed by VA, Chl-A and CA in traces. VL-146 showed only GA, VA and CA in which GA (5.50 µg/g) was maximum followed by VA and Chl-A in traces. However, Ranichauri also had three phenolic acids, in which GA (2.59 µg/g) was maximum but Chl-A and O-Cou-A were in traces. Gaza, KM-252 and PES-400 cultivars had only two phenolic acids, in which GA was maximum in all these cultivars but other phenolic acids were in traces (Table 4.).

**Figure 4**

Table 4: HPLC analysis of phenolic acids in the boiled seeds of

Variety	Phenolic acid (µg/g fresh wt)					
	Gallic acid	Vanillic acid	Ferulic acid	Chlorogenic acid	o-Coumeric acid	Cinnamic acid
Gaza	2.34±0.56	ND	0.04±0.01	ND	ND	ND
Ranichauri	2.59±0.23	ND	ND	0.15±0.002	0.02±0.007	ND
VL-146	5.50±0.12	ND	0.58±0.01	ND	ND	0.03±0.041
VL-149	4.41±0.013	0.82±0.34	ND	0.13±0.042	ND	0.04±0.006
KM-252	5.12±0.65	0.07±0.51	ND	ND	ND	ND
PES-400	5.98±0.31	ND	ND	ND	ND	0.01±0.009

ND-under detection limit, ± Standard error.

Several phenolic acids were detected in water extract of boiled seeds in HPLC analysis. Among them, GA and FA were present in most of the extracts. Extract of Ranichauri cultivar had a maximum of five phenolic acids (P-Cat-A, FA, GA, Caf-A and O-Cou-A). Among them, P-cat-A (85.05µg/g) was maximum followed by FA (35.9 µg/g) and GA (7.19 µg/g) but Caf-A and O-Cou-A were in traces. Gaza and KM-252 cultivars also had five phenolic acids but their amount was in traces. Extract of Gaza was rich in FA (6.33 µg/g) followed by GA (4.85 µg/g) but Chl-A, Caf-A, and O-Cou-A in traces. However, in KM-252, GA (3.07 µg/g) was maximum but other phenolic acids like FA, Chl-A, O-Cou-A and CA were in traces. PES-400 and VL-149 had four phenolic acids, in which former was rich in FA (32.46 µg/g) followed by GA (12.36 µg/g) and VA (2.51 µg/g) but CA (0.13 µg/g) was in traces. However, VL-149 cultivar had maximum FA (67.9 µg/g) followed by GA (6.25 µg/g) but Chl-A and CA in traces (Table 5.).

**Figure 5**

Table 5: HPLC analysis of phenolic acids in the boiled water extract of seeds of

Varieties	Phenolic acid ( $\mu\text{g/g}$ fresh wt)								
	Tannic acid	Gallic acid	Protocatechic acid	Caffeic acid	Vanillic acid	Ferulic acid	Chlorogenic acid	o-Coumaric acid	Cinnamic acid
Gaza	ND	4.85 $\pm$ 0.6	ND	0.03 $\pm$ 0.02	ND	6.33 $\pm$ 1.2	0.03 $\pm$ 0.01	0.32 $\pm$ 0.01	ND
Ranchur	ND	7.19 $\pm$ 0.7	84.05 $\pm$ 2.3	1.87 $\pm$ 0.01	ND	35.9 $\pm$ 1.9	ND	0.02 $\pm$ 0.06	ND
VL-146	1.33 $\pm$ 0.6	13.39 $\pm$ 1	ND	ND	7.23 $\pm$ 0.03	1.09 $\pm$ 0.02	ND	ND	0.04 $\pm$ 0.01
VL-149	ND	6.25 $\pm$ 0.6	ND	ND	ND	67.9 $\pm$ 2.3	0.06 $\pm$ 0.06	ND	0.04 $\pm$ 0.03
KM-252	ND	3.07 $\pm$ 0.5	ND	0.02 $\pm$ 0.02	ND	0.38 $\pm$ 0.02	ND	0.01 $\pm$ 0.05	0.92 $\pm$ 0.04
PES-400	ND	12.36 $\pm$ 0.2	ND	ND	2.51 $\pm$ 0.01	32.5 $\pm$ 1.3	ND	ND	0.13 $\pm$ 0.04

ND-under detection limit,  $\pm$  Standard error.

Phenolic acid estimation in flour and bread of local cultivar of *E. coracana* indicated that flour had five phenolic acids in which GA (8.80  $\mu\text{g/g}$ ) was maximum but VA (1.99  $\mu\text{g/g}$ ), CA (1.60  $\mu\text{g/g}$ ), O-Cou-A (1.59  $\mu\text{g/g}$ ) and FA (1.05  $\mu\text{g/g}$ ) were in traces. However, after the preparation of bread of the flour the number and amount of these phenolic acids were reduced variable in which GA (35.2  $\mu\text{g/g}$ ) was maximum followed by O-Cou-A (2.95  $\mu\text{g/g}$ ) and CA (0.315  $\mu\text{g/g}$ ) (Table 6).

**Figure 6**

Table 6: Phenolic acids estimation in flour and bread of

Varieties	Phenolic acid ( $\mu\text{g/g}$ fresh wt)				
	Gallic acid	Vanillic acid	Ferulic acid	o-Coumaric acid	Cinnamic acid
Flour	8.801 $\pm$ 0.59	1.99 $\pm$ 0.001	1.053 $\pm$ 0.003	1.59 $\pm$ 0.01	1.602 $\pm$ 0.01
Bread	35.19 $\pm$ 1.3	ND	ND	2.946 $\pm$ 0.56	0.3146 $\pm$ 0.21

ND-under detection limit,  $\pm$  Standard error

HPLC analysis of different preparations of seeds of *E. coracana*, e.g., dry, soaked and boiled seeds and their water extracts as well as flour and bread of *E. coracana* indicated that dry seeds were rich in phenolic acids which play a role in higher concentrations inhibiting their germination just after maturity. Soaking the seeds in water, may result in the adsorption of water on the macro-molecules of the seeds which may activate and break the enzyme conjugates specially gibberellic acid and cytokinins complex responsible for the breakdown of macromolecules (carbohydrates and proteins) in simpler forms, i.e., monosaccharides and amino acid. These simple molecules may be utilized in respiration as substrates for the production of phenolic acids (22, 23, 24). Abundant amount of phenolic acids in the water extract of soaked seeds as indicated in our results may account for the increased concentration of water-soluble phenolic acids, which diffuse in water when the seeds are soaked (22, 23). The high amount of phenolic acids in dry seeds is leached out in water when the seeds are dipped in water. Even after preparing flour of the seeds and bread from the flour, which is commonly consumed by human beings, had higher amount of phenolic acids.

Knowledge of the localization of phenolic compounds in dry, soaked and boiled seeds and their water extracts, flour and bread may contribute in utilizing their immuno-stimulatory activities when used in regular dietary system of human food. If these phenolic acids are isolated in bulk through fraction collector and formulated, they can be exploited for treatment of human and plant diseases (16). Various preparations of *E. coracana* seeds are very commonly consumed by human beings since time immemorial in rural parts of India and African countries. Abundant phenolic acids in water extract of mature seeds has tremendous potential to be realized for formulation to replace synthetic anti-oxidants, as the former are safe alternative. The use of seeds and their preparations may form practical strategy in reducing a number of human diseases, as they are cost-effective and readily available (25). Since most of the cultivars contain gallic acid in high amount, which is highly anti-inflammatory (26), it is advisable to for consumption to those who are suffering from joint pains or inflammation or any other kinds in the body. This is the first report of a complete phenolic acid profile of mature seeds, water-soaked and boiled seeds and water extracts as well as flour and bread of *E. coracana* through C-18 reverse phase column of HPLC system.

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