



Morpho-quantitative assessment and biochemical characterization of grain amaranth (*Amaranthus hypochondriacus*) to determine the nutritional composition

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ABSTRACT

Grain amaranth (*Amaranthus hypochondriacus* L.) has gained increasing attention as a potential nutrient-rich crop with numerous health benefits. The present study was carried out, during the summer (*khari*) season of 2019 and 2020 at College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha to evaluate morpho-quantitative and biochemical parameters of grain amaranth. Four varieties of grain amaranth in five different replications were taken. Morpho-quantitative assessment indicated that, Suvarna (11.23 q/ha) yielded the most, GA 2 flowers opened 50% after 45.68 days, whereas BGA 2 took 51.52 days. The RMA 7 variety matured in 102.5 days, while the Suvarna variety matured in 104.2 days. RMA 7 had the longest panicle (38.9 cm), whereas Suvarna had the smallest (35.68 cm). Suvarna had the most plants (74.3), followed by BGA 2 (73.5). Biochemical analysis revealed that, BGA 2 contained more chlorophyll (1.537 mg/g), and RMA 7 had more total carbohydrate (319 mg/g). Moreover, BGA 2, GA 2, Suvarna and RMA 7 exhibited comparable phenol content. Moreover, Fe (66 mg/100 g), Mg (284.5 mg/100 g), Mn (5.71 mg/100 g), Zn (11.3 mg/100 g), Ca (178.7 mg/100 g), and K (400.50 mg/100 g) were detected by ICP-OES analysis. FTIR and HPTLC analysis indicated more number of functional group present in the varieties. Thus, the present study unveiled that seeds of grain amaranthus varieties are rich source of different essential elements, and other essential biochemical parameters, with higher antioxidant activity. Hence this pseudocereal can be used to provide good food supplements to the infants as well as adults.

Keywords: Biochemical analysis, Crop improvement, Food security, Grain amaranth, Nutrition

Grain Amaranth (*Amaranthus hypochondriacus* L.) (2n=2x=32), has emerged as a significant crop in the domain of sustainable agriculture and human nutrition. This hardy, ancient grain has gained recognition for its potential to contribute substantially to global food security owing to its resilience to adverse environmental conditions, adaptability to diverse agroecosystems, and impressive nutritional attributes (Janmohammadi *et al.* 2022). Rich in proteins, dietary fiber, vitamins, and minerals, this versatile grain holds promise not only as a source of nourishment, but also as a component of sustainable cropping systems (Grubben and Denton 2004).

Amaranthus are indifferent to the type of soil and are drought-resistant. They are grown as a grain crop in countries with a temperate climate (western Europe), as well as in hot-climate countries like Mexico, USA, African countries

and India (Pulvento *et al.* 2022). However, protein extracted from grain amaranth has an amino acid profile that is more equivalent to the protein standard established by the FAO and WHO for attaining adequate nutrition (Balakrishnan and Schneider 2022). The biological value of the leaves of grain amaranth is considerable and is on par with or even higher than that of vegetables that are typically eaten (Zamudio *et al.* 2022). There have been claims made that some species of grain amaranth exhibit traits that might have medicinal use (Arslan-Tontul *et al.* 2022).

The present investigation embarked on a comprehensive assessment of grain amaranth, encompassing both morpho-quantitative and biochemical dimensions, with the primary objective of delineating its nutritional composition. This comprehensive exploration offers a holistic understanding of grain amaranth's nutritional value and its potential contribution to addressing dietary deficiencies and promoting health.

MATERIALS AND METHODS

The present study was carried out during the summer (*khari*) seasons of 2019 and 2020 at College of Agriculture,

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(Odisha University of Agriculture and Technology), Bhubaneswar (20.15°57.4 N; 85.47°34.1 E), Odisha. Seeds of 4 varieties of grain amaranth, viz. GA 2, Suvarna, BGA 2, RMA 7 were obtained from the All India Coordinated Research Network on Potential Crops, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha. For the morphological observations, the plant materials were planted in the field. The seed were sown in lines with three replications with spacing of 45 cm × 15 cm having plot size 5.4 m². All the recommended agronomic practices were followed to raise a good crop.

Morpho-quantitative assessment: Observations were recorded on five randomly selected plants from the middle rows of plot for seven biometric traits, except for days to flowering, days to maturity and 10 ml seed weight (g) (i.e. a lot of seeds was drawn at random from each treatment and each genotype in three replications in a 10 ml beaker, and the weight of 10 ml seed was recorded and expressed in g), which were recorded on a plot basis and from a random sample of the plot respectively.

Biochemical characterization: Chlorophyll content analysis was performed by a standard method (Gylling *et al.* 2014). The absorbance was measured at 645 and 663 nm and compared to the solvent blank (80% acetone) by following a standard calculation method (Gitelson and Merzlyak 1997) as follows;

$$\text{mg chlorophyll A/B tissue} = \frac{12.7 (A_{663}) - 2.69 (A_{645}) \times V}{1000}$$

$$\text{mg chlorophyll B/g tissue} = \frac{20.2 (A_{645}) - 4.68 (A_{663}) \times V}{1000}$$

$$\text{mg chlorophyll B/g tissue} = \frac{20.2 (A_{645}) - 4.68 (A_{663}) \times V}{1000}$$

and,

$$\text{mg chlorophyll C/g tissue} = \frac{20.2 (A_{645}) - 8.02 (A_{663}) \times V}{1000}$$

where A, Absorbance at the specific wavelengths; V, Final volume of chlorophyll extract in 80% acetone and W, Fresh weight of tissue extracted. Working standard solutions were prepared by dilution of the stock standard solutions to desired concentration in 2% HNO₃. The Anthrone and Lowry methods were employed to calculate carbohydrates and proteins, respectively (Zuriaga-Agusti *et al.* 2013). However, the total phenolic content of the plant extracts and the standard antioxidant materials was determined using standard methods (Zuriaga-Agusti *et al.* 2013).

Mineral content analysis was performed by a standard method (Zasoski and Burau 1977). For the determination of P, K, Ca, Mg, S, Fe, Mn, Zn and Cu, aliquots of this solution can be used using the ICP-OES unit. Aliquots of ICP multi element standard solution (10 to 50 mg/litre Merck) containing the elements such as (Zn, P, Fe, Mn, Cr, Mg, Cu, Ca, Na and K) were used in the preparation of calibration solutions.

Plant extract is made by centrifuging 2 gm of ground seed with 20 ml of methanol. The supernatant is used for FTIR analysis. 1 ml of the sample is placed on the detector of FTIR. Grain amaranth samples were placed in the path of an infrared beam, which absorbed and transmitted light and then the light signal penetrated the sample to the detector. The detector measured the intensity of the radiation moving into a sample and the intensity of the radiation transmitting through the sample. Its output as a function of time was converted into a plot of absorption against wavenumber by a computer using a Fourier transform method. The observed spectra were the absorbance of the different paper samples versus the wave number range 4000–400/cm (Zasoski and Burau 1977).

Seed powder of grain amaranth samples was subjected to extraction method (Beltran-Orozco *et al.* 2020). Grain amaranth seeds were grounded to a fine powder. 20 ml of 80% methanol was used for the extraction of ground samples (1.5 g) in a shaker at room temperature overnight. The extracts were then recovered by centrifugation at 8000 × g at 4°C for 15 min. The supernatant was collected in a beaker and was dried after measuring the weight. The dried extract was then made up with methanol at 1 mg/ml. The total flavonoid content was determined using the colorimetric method described by Zhishen *et al.* (1999) with modifications. In accordance with the Shimada (1992) method, the antioxidant analysis was done, by using the following formula,

$$\text{Antioxidant activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}$$

This analysis was carried out by using HPTLC system (Tajner-Czopek *et al.* 2020). Samples (dissolved in HPLC grade methanol) were loaded on pre-coated HPTLC plates (Silica gel 60F 254, Merck, Germany), by using nitrogen as a spraying gas and TLC sample loading instrument (CAMAG LINOMAT 5). TLC plate was developed in solvent system toluene: ethyl acetate: methanol (2:4:13). After development, the plate was observed in UV chamber (CAMAG) and scanned at 254 nm with slit dimension 5 mm × 0.45 mm by using TLC scanner (CAMAG). Results were generated by using HPTLC software WinCATS 1.4.4.6337. Bands were observed on silica plate by Spectroline Model CM-10A Fluorescence analysis cabinet (UV lamp with dark box) by using the UV light at -254 nm and then peak area was measured for calculating absorbance unit (AU) by Manual winCAT scanner 3 (densitometric method). Then absorbance units (AU) were calculated, flavonoid content as mg equivalent per g dry extract from standard curve equation (Souri *et al.* 2022):

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

RESULTS AND DISCUSSION

Morpho-quantitative assessment: The BGA 2 variety yielded 10.86 q/ha, whereas the Suvarna variety yielded

11.23 q/ha. BGA 2 cultivars reached 50% blooming after 51.52 days, whereas in GA 2 variants bloomed after 45.68 days. RMA 7 matured at 102.5 days, while Suvarna matured after 104.2 days, the longest of the four kinds. GA 2 is the tallest and RMA 7 is the smallest i.e. 101.20 cm and 111.72 cm, respectively. RMA 7 had the longest panicle at 38.9 cm, while Suvarna had the smallest at 35.68. The four varieties have similar inflorescence lengths. Suvarna had the most plants (74.3), followed by BGA 2 (73.5). GA 2 has the fewest plants/acre (Table 1).

It is one of the few multipurpose crops (Gimplinger *et al.* 2007) that can produce grains and tasty green vegetables with excellent nutritional value for humans and animals. This study examined the biochemical and nutritional content of four kinds of amaranthus seed flour, as well as its protein quality, prospective usage in functional foods and nutraceuticals, and amino acid profiles. Morpho-quantitative assessment suggested that, GA 2 yielded 11.22 q/ha, Suvarna 11.23 q/ha, BGA 2 10.86 q/ha, and RMA7 10.95 q/ha. However, a trial in Vienna, Austria, found hand-harvested yields of 2200–3000 kg/ha. Both groups had similar genotypes (Gimplinger *et al.* 2007). The qualitative characteristics varied among the varieties taken in the present study. However, these differences offer abundant opportunities for plant breeders to engage in selection when developing plant breeding programmes (Akaneme *et al.* 2013).

Chlorophyll, carbohydrate, protein, and phenol content: Chlorophyll a showed a positive and significant correlation with chlorophyll b (0.764) and chlorophyll a and chlorophyll b had a positive and significant correlation with total chlorophyll i.e. 0.948 and 0.919. The total estimated carbohydrates content of grain amaranth of GA 2, Suvarna, BGA 2 and RMA 7 were 309 mg/100 g, 312 mg/100 g, 315 mg/100 g, and 319 mg/100 g respectively (Table 1). The seed protein content of grain amaranth was evaluated and was found significant in four accessions, namely GA 2, Suvarna, BGA 2, RMA 7, having protein content 14.25 (g/100 g), 13.5 (g/100 g), 17.2 (g/100 g), 14.01 (g/100 g) respectively (Table 2). The present investigation revealed that phenolic compounds were present in high concentration in cells of seeds have been known responsible for the resistance of the young tissues. In the present case, all accessions, namely GA 2, Suvarna, BGA 2 and RMA 7 had phenol content, i.e. 0.76, 0.68, 0.72, and 0.71 mg/g, respectively (Table 1).

Meanwhile, from the biochemical analysis of the present study, it was concluded that total chlorophyll content positively affected grain output/plant because it predicted an increase in grain production per plant. However, the National Botanical Research Institute, Lucknow found similar results in grain amaranth genetic study for biochemical and quantitative traits (Pandey and Singh 2010). Increasing carbohydrate and protein content is an important objective in breeding for high protein varieties in any crop improvement programme. Meanwhile, the results of the present research were found similar to previous research in which, the protein

Table 1 Morpho-quantitative trait evaluation and quantification of different biochemical parameters of different grain amaranth varieties

Variety	Plant height (cm)*	Length of panicle (cm)*	Length of inflorescences (cm)*	50% flowering*	Days of maturity*	Plant population at harvest/plot*	Yield (q/ha) *	Chlorophyll a (mg/g) #	Chlorophyll b (mg/g) #	Total chlorophyll (mg/g) #	Carbohydrate content (mg/g) #	Protein content (g/100g)#	Phenol Content (mg/g)#
GA 2	111.72	37.35	7.81	45.68	103.1	71.54	11.22	1.421	0.876	2.279	309	14.25	0.76
Suvarna	106.43	35.68	7.81	49.32	104.2	74.3	11.23	1.418	0.843	2.254	312	13.5	0.68
BGA 2	106.98	37.1	7.8	51.98	103.6	73.5	10.86	1.537	0.901	2.385	315	17.2	0.72
RMA 7	101.2	38.9	7.86	50.42	102.5	74.1	10.95	1.487	0.894	2.285	319	14.01	0.71
Mean	103.35	7.82	37.26	106.58	73.36	49.35	11.06	1.47	0.88	2.3	313.75	14.74	0.72
SD	0.72	0.03	1.32	4.3	1.26	2.68	0.19	0.06	0.03	0.06	4.27	1.67	0.03
CV	0.72	0.03	1.32	4.3	1.26	2.68	0.19	0.04	0.03	0.03	0.01	0.11	0.05

*Pool of *kharij* 2019 and 2020; # Estimated on fresh weight basis.

Table 2 Scavenging activity of grain amaranth seed extracts

Volume (μl)	Amaranth absorbance	BHT absorbance	Scavenging activity (%) (Sample)	Scavenging activity (%) (Standard)
50	1.373	0.185	17.98	88.9
100	1.34	0.162	19.95	90.3
150	1.202	0.156	28.19	90.6
200	0.939	0.134	43.906	91.9
Mean	1.2135	0.15925	27.50	90.42

content was varied from 6.10–9.00 g/100 g of fresh leaves, and the amount of carbohydrate in fresh leaves varied from 9.75 g–21.29 g (Srivastava 2011). Meanwhile, the results of this study indicate a nutritive potential for the *Amaranthus* leaves, therefore, domestication of this plant is suggested along with assessment of its chemical and nutritional properties (Srivastava 2011). Nevertheless, the host-virus interaction may have caused the increase in phenolic content by triggering phenol production (Vir and Grewal 1975, Jha *et al.* 2022). Phenolic chemicals are widely distributed in higher plants and are involved in fungal host-pathogen interactions. They are crucial to disease resistance (Kazi *et al.* 2022). Phenols and their oxidized metabolites may limit fungal spore germination, spore growth, and inhibiting fungal cell wall enzymes (Pandey and Singh 2010).

Mineral element profiling, antioxidant activity, FTIR and HPTLC analysis: The proximate composition of flour of BGA 2 a variety of grain amaranth studied under ICP-OES revealed the presence of a significant amount of different mineral content. The study of the mineral content profile of BGA 2 has shown that amount of Iron contents 66 mg/100 g,

Magnesium (284.50 mg/100 g), Manganese (5.71 mg/100 g), Zinc (11.30 mg/100 g), Calcium (178.7 mg/100 g) and Potassium (400.50 mg/100 g). For antioxidant scavenging activity, different concentrations of grain amaranth seed extracts were used in DPPH assay and compared with BHT (a synthetic antioxidant). The activities as follows for 50 μl (17.98%), 100 μl (19.95%), 150 μl (28.19%) and 200 μl (43.906%). Maximum activity was found when 200 μL of grain amaranth seed extract was used (Table 2). The present study results revealed that a wider range of functional groups were present in the seed extract of grain amaranth Alkynes, Alcohols, Amides, Alkanes, Alkyles, Carboxylic acids Alkenes, Aromatics groups, Aliphatic amines, Esters, Ethers and Alkyl halides. The FTIR analysis also revealed the symmetry of the bond (Table 3, Fig. 1). However, peak 2 of catechol is in R_f range of 0.73 and 0.98, a peak in the grain amaranth seed sample in the range of 0.85 and 0.98 which comes in the range of Catechol. So, it can be concluded that the phenols are present in the grain amaranth seed extracts [the peak 4 in the grain amaranth sample is considered as the catechol (Fig. 2)].

The mineral content profile of the present study was found similar with the results of some research (Esan *et al.* 2018, Kazi *et al.* 2022). Nevertheless, there are scarce reports regarding the mineral composition of leaves, and there is a complete absence of information concerning the qualitative enhancement of foliage yield, especially in relation to minerals in *Amaranthus*, however, some studies showed that vegetable amaranth is a rich source of minerals like calcium, iron, and zinc (Shukla *et al.* 2006). Therefore, the present study would be of use in enhancement of selected minerals in different regions according to local preferences

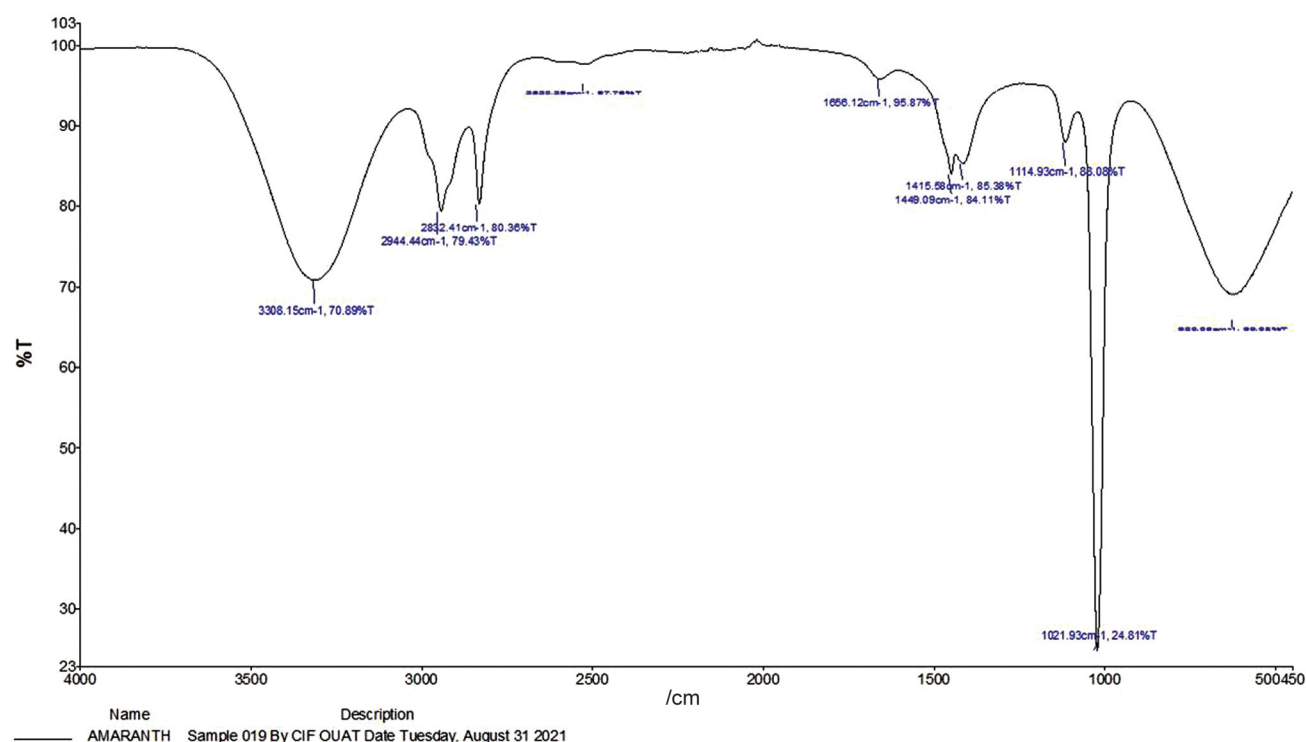


Fig. 1 Spectral graph of functional group analysis of grain amaranth seed extract.

Table 3 FTIR spectra of grain amaranth and their corresponding annotations

Wave no.	Transmittance (%)	Functional Group	Bond	Intensity
3308.15	70.89	Alkynes, Alcohols, Amides	C-H Stretch, O-H Stretch, N-H Symmetric	s, sharp; s, broad; w-m
2944.44	79.43	Alkanes and Alkyls, Carboxylic acids	C-H Stretch, O-H Stretch	s; s, broad
2832.41	80.36	Carboxylic acids	O-H Stretch	s, broad
2520.29	97.76	Carboxylic acids	O-H Stretch	s, broad
1656.12	95.87	Alkenes, Amides	C-C Stretch, C-O Stretch	vw-m; s, broad
1449.09	84.11	Aromatics	C-C Stretch	m
1415.58	85.38	Aromatics	C-C Stretch	m
1114.93	83.08	Aliphatic amines, Alcohols, Carboxylic acids, Esters, Ethers	C-N Stretch, C-O Stretch	m; s
1021.93	24.81	Alcohols, Carboxylic acids, Esters, Ethers, Aliphatic amines	C-O Stretch, C-N Stretch	s; m
620.05	69.08	Alkyl halides, Alkynes	C-Cl Stretch, C-Br Stretch, C-H Bend	m; broad, s

m, Medium; w, Weak; s, Strong; n, Narrow; b, Broad; sh, Sharp.

and nutrient deficiency prevalent among the populations. The study found that, grain amaranth can include minerals, phytochemicals, and antioxidants. Most phytochemicals and antioxidants are higher in grain amaranth. Antioxidant activity was 17.98, 19.95, 28.19, and 43.906% at 50, 100, 150, and 200 l, respectively. These results were similar to grain amaranth (Kazi *et al.* 2022) but greater than turmeric

(31.5%), wheat (10.7%), maize (13%), soybean (12.7%), muesli (2.3%), and linseed (5.7%). The results of the FTIR and HPTLC analysis in the present study was found similar to a previous study (Grundy *et al.* 2020). However, the results observed in physicochemical properties of amaranth provided a crucial basis for its potential applications on industrial scale (Siwatch *et al.* 2017).

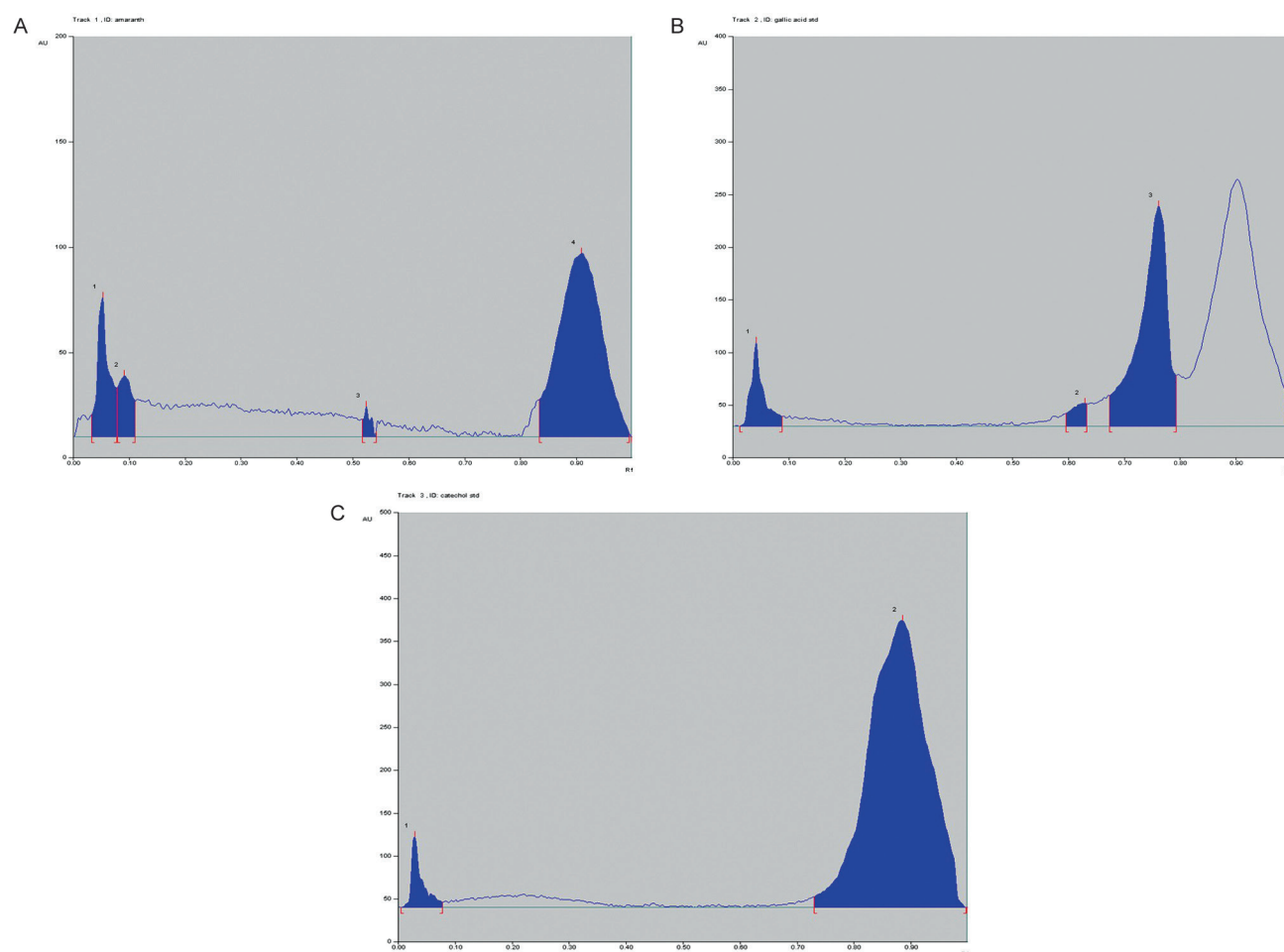


Fig. 2 HPTLC analysis; (A) Graph depicting peaks of Gallic acid and catechol in grain amaranth seed extract; (B) Graph depicting peaks of Gallic acid; (C) Graph depicting peaks of catechol.

In conclusion, the morpho-quantitative assessment and biochemical characterization of grain amaranth indicates, that it is a promising crop with a rich nutritional composition. Its high protein, fiber, and mineral content make it a valuable addition to the diet, especially in regions where nutritional deficiencies are a concern. Further research and promotion of grain amaranth cultivation and consumption could contribute to improve global nutritional and food security. However, genetic transformation and genome editing may benefit from the expected discoveries in the grain amaranth research.

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