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Quantitative analysis of sinigrin from deoiled cake in different *Brassica* species genotypes

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Abstract

Oil seeds play a great role in human and animal nutrition. *Brassica* is an agriculturally important genus containing species with highly diverse morphology and wide ranging utility. Rapeseed-Mustard meal is highly regarded as a component of animal feed with high protein content and a desirable amino acid profile. The presence of some components, in particular glucosinolates (sinigrin) affects the value of the meal and reduces the amount that can be used in animal feed formulations. Quantitative analysis of sinigrin was done in thirty genotypes of *Brassica* species. All the genotypes were collected from the Oilseeds Research Centre of S. D. Agricultural University, Sardarkrushinagar for High performance liquid chromatography (HPLC) analysis. Significant variation was found among the genotypes. Concentration range of sinigrin in deoiled cake of thirty genotypes was from 2.92 $\mu\text{mol/g}$ DM (RTM314) to 173.14 $\mu\text{mol/g}$ DM (NRCG35). Genotypes of *Brassica juncea* were all genotypes sowed <30 $\mu\text{mol/g}$ while *Brassica napus* NRCG35 genotype sowed highest sinigrin content 173.14 $\mu\text{mol/g}$. The *Brassica juncea* species and other species genotypes which had low content of sinigrin where all genotypes good for animal feed and used for the double zero varieties by breeding program. The glucosinolates have also been applied in the prevention of bacterial and fungal spoilage of food products in advanced atmospheric packaging technology which improves the shelf-life of these products. These issues are important priorities for rapeseed mustard breeders.

Keywords: *Brassica*, glucosinolate, sinigrin, antinutritional factor, HPLC

Introduction

Oil seeds play a great role in human and animal nutrition. *Brassica* is an agriculturally important genus containing species with highly diverse morphology and wide ranging utility. The rapeseed-mustard, a major edible Rabi oilseed crop. The genus *Brassica*, belongs to *Brassicaceae* (Cruciferae) family and includes six cultivated species. Among them, *Brassica nigra* (n=8), *B. oleracea* (n=9) and *B. Rapa* (n=10) are diploids, rest of the three namely *B. carinata* (n=17), *B. napus* (n=19) and *B. juncea* (n=18) are amphidiploids (Nagaharu, 1935)^[14]. It originated in Asia and spread to Afghanistan and other countries (Vaughan, 1977)^[19]. *Brassica spp.*, commonly known as rapeseed-mustard, plays an important role in the Indian economy by providing edible oils, vegetables, condiments and animal feed (Jat *et al.*, 2019)^[9].

Global vegetable oil consumption is projected to increase by more than 3 percent in 2023-24 to 218.0 million metric tons, which is led by growth in oils consumption in Brazil, China, India, Indonesia, and the United States. India is the 4th largest oilseeds producer in the world. India is ranked fourth after European Union, Canada and China sharing about 14.0 percent of the global rapeseed-mustard production 88.39 million metric tonnes (mmt) during 2023-24 (USDA website)^[18].

In India, groundnut, soybean, and rapeseed-mustard are the major oilseeds, contributing approximately 80% of oilseed production. The major oilseed *Brassicacae* grown in India are *B. juncea*, *B. Rapa*, *B. napus*, and *B. carinata*. These crops are grown in diverse agro-climatic conditions and are consumed as vegetables, fodder, oil sources, and condiments. In India, Rajasthan is the largest production (46.63%), followed by Madhya Pradesh (14.36%), Haryana (11.63%), Uttar Pradesh (8.81%), and Gujarat (4.27%) (Anonymous, 2022)^[3]. In Gujarat, it is mostly grown in districts of Banaskantha, Mehsana, Sabarkantha, Kheda, Ahmedabad, Kutch and Gandhinagar.

Brassica play an important role in the world agriculture as oilseeds, vegetables, forage and fodder, green manure and condiments. They are the main source of edible oil in Indian diet after groundnut. The important members of this group viz., *B. napus*, *B. rapa*, and *B. juncea* are sources of canola and industrial oil. Oil cake is used as an animal feed. The *Brassica* seed meal (oil cake) contains protein (36-38 percent), carbohydrate (14-16 percent), fiber (10-15 percent), moisture (6-8 percent), ash (4-6 percent), mineral (3-4 percent), vitamins (0.7-0.9 percent) glucosinolate (2-3 percent), phytic acid (3-6 percent), sinapine (1-1.5 percent) and 1.6-3.1 percent of tannin (Agnihotri and Kumar, 2004) [1].

Brassica also plays a significant role in world agriculture as oilseeds, vegetables, forage and fodder, green manure, and condiments. Oil cake, a by-product of *Brassica*, contains protein, carbohydrates, fiber, moisture, ash, minerals, vitamins, glucosinolates, phytic acid, sinapine, and tannin. Glucosinolates are plant secondary metabolites which are hydrolyzed by the plant myrosinases activated by chewing or grinding, giving rise to biologically active by-products (isothiocyanates, thiocyanates, and nitriles). When present in high amounts, these active by-products can also induce multiple disorders in animals such as thyroid dysfunction and organ abnormality, reproduction, teratogenicity, growth impairment and a decreased intestinal protein intake (summarized in Scientific Panel on Contaminants in the Food Chain, 2008). The rape seed meal had high protein content, is not considered good as livestock feed due to the presence of glucosinolates, which produce toxic sulphur compounds upon degradation in the intestinal tract of the animals. Canadian Oil Association introduces canola, a double low *Brassica* oilseed cultivar, ideal for cattle feed due to its low erucic acid and glucosinolate content (Kimber, 1995) [11]. It contains less than 30µMg-1 glucosinolate of the deoiled cake (Downey, 1990) [7] and is considered ideal for cattle feed purposes.

To improve quality of *Brassica oil seed* spp. the presence of sufficient genetic diversity is very important. Wide genetic diversity for improved crop productivity is largely unexplored in the wild relatives of *Brassica oil seed* crops and there is not a clear association between genetic distance and taxonomic classification within the *Brassicaceae* (Warwick *et al.*, 2000) [21]. The concentration of glucosinolate has been found to vary between *Brassica* species as well as between cultivars of the same species (Bradshaw *et al.*, 1984) and (Rangkadilok *et al.*, 2002) [4, 16]. To maximize the potential benefits it is essential to evaluate glucosinolate levels in *Brassica* species. The aim of the present study was to investigate the glucosinolate (sinigrin) concentrations in different *Brassica* species.

Materials and Methods

Plant Material

Plant material thirty genotypes of *Brassica* species genotypes were collected from Oilseeds Research Centre of S. D. Agricultural University, Sardarkrushinagar (Gujarat), India. The detail of the genotypes given in table 1.

Glucocinolate (Sinigrin) through analyzed by High performance liquid chromatography

Reagent /chemicals: Standard of sinigrin, HPLC grade water, Ammonium sulfate sigma grade (99.5 % pure).

Standard preparation

Take 10 mg standard sinigrin was dissolved in 1 mL HPLC grade Water to prepare a stock standard mixture. The stock mixture was further diluted to prepare calibration standard solution the 100 ppm, 50 ppm, 25 ppm, 10 ppm, 5 ppm, 1 ppm all standard solution were stored at 4 °C until use.

Extraction of sinigrin method

Take 75 mg Defatted mustard meal in capped vial. Add the 1.5 mL boiling water and incubated for 15 min. in water bath at 70 °C. The extracts were then centrifuged at 12000 rpm for 10 min the supernatant was collected in other new capped vial and discard pellet. The supernatant were stored at 4 °C until HPLC analysis. The sample was filtered using a 0.22 µm PTFE membrane syringe filter and placed into a vial and the put in Sample Manager (Cools K, and Terry LA) [16].

Analysis of sinigrin

A Waters Acquity UHPLC Class System equipped with a Quaternary Solvent Manager, a Sample Manager FTN was coupled to a UV Photodiode array detector (PDA). Analyses were performed on a bridged ethylene hybrid (BEH) C18 analytical column (2.6 µm, 3 mm x 100 mm). The mobile phase consisted of isocratic HPLC grade water with 0.2 M ammonium sulphate at a flow rate of 0.2 mL min⁻¹, a column temperature of 25 °C and sample temperature of 4 °C. The samples (1 µl) were injected into an UPLC system. An UV Photodiode array detector (PDA) eλ detected eluted sinigrin at a wavelength of 229 nm and detection set at 229 nm in steps of 4.8 nm, with a data acquisition rate of 10 points/sec. The column was returned to starting conditions over 0.2 min, and allowed to re equilibrate for 2.0 minutes. The Thermo Scientific instrument Chromeleon 7.2.10 software was used for chromatographic data analysis and in order to correct shifts in retention times, identification and confirmation of antinutritional compounds were based on comparing retention times with standards and ultraviolet absorption spectrum data. Detection limit (LOD) and quantification limit (LOQ) were determined at 10 ppm. The content of Sinigrin was calculated using its standard curve and expressed in µmol/g (dry weight). (Based on the mean of the three extractions for that genotype).

Results

Glucosinolate (Sinigrin content µmole/g) The *Brassica* species genotypes contained great variability for glucosinolate (GSL) content. Glucosinolate, the pungent principle in oil, has anti-bacterial, anti-fungal and anti-carcinogenic properties and hence account for many medicinal utilities. In seeds, the dominant glucosinolate was gluconapin (GNA), with sinigrin (SIN) as the next most abundant component.

The individual sinigrin concentration (µmole/g) in extracts from the defatted seed of thirty genotypes of *Brassica* oil seed species samples studied is listed in the (Table 1). Peak of standard (sinigrin) was obtained at retention time 2.293 minute shown in figure 2. Stoin *et al.* and Michael [13, 17] also observed the sinigrin retention time at 2.26 minutes in *Brassica nigra* and *Brassica juncea* respectively. Sinigrin concentration in *Brassica juncea* genotypes ranged from 4.93 µmol/g DM (GDM5) to 90.29 µmol/g DM (SKM1744). PM 25 had higher amount of sinigrin 48.28 µmol/g DM. Concentration of sinigrin in *Brassica*

campestris 8.18 $\mu\text{mol/g}$ DM (NDTC 9502) and 16.09 $\mu\text{mol/g}$ DM (BR 54). In *Brassica rapa*, concentration of sinigrin was ranged from 10.61 $\mu\text{mol/g}$ DM (NRCY93) to 128.06 $\mu\text{mol/g}$ DM (GS1). Sinigrin concentration in *Brassica carinata* genotypes ranged from 21.30 $\mu\text{mol/g}$ DM (PC 5) to 165.42 $\mu\text{mol/g}$ DM (BCRC3). Concentration of sinigrin in *Erucas sativa* 2.92 $\mu\text{mol/g}$ DM (RTM314) and 5.05 $\mu\text{mol/g}$ DM (T27). Sinigrin concentration in *Brassica*

napus genotypes ranged from 7.51 $\mu\text{mol/g}$ DM (PBN2001) to 173.14 $\mu\text{mol/g}$ DM (NRCG35). In *Brassica nigra*, concentration of sinigrin was ranged from 6.84 $\mu\text{mol/g}$ DM (BHC 46) to 102.28 $\mu\text{mol/g}$ DM (BSH1). Genotypes of *Brassica juncea* were all genotypes sowed <30 $\mu\text{mol/g}$ while *Brassica napus* NRCG35 genotype sowed highest sinigrin content 173.14 $\mu\text{mol/g}$.

Table 1: List of selected genotypes of seven oil seed *Brassica* species

Sr. No.	Genotypes	Sr. No.	Genotypes
<i>Brassica juncea</i>		<i>Brassica carinata</i>	
1	GM-1	17	BCRS-3
2	GM-2	18	DLSC 1
3	GM-3	19	PC 5
4	GDM-4	<i>Erucas sativa</i>	
5	GDM-5	20	RTN-314
6	Kranti	21	T-27
7	PM-25	<i>Brassica napus</i>	
8	SKM 1620	22	HNS-0004
9	SKM 1731	23	NRCG-35
10	SKM1744	24	PBCM-2002
<i>Brassica campestris</i>		25	PBN- 2001
11	BR-54	<i>Brassica nigra- black sarson</i>	
12	NDTC-9502	26	BHC-46
<i>Brassica rapa</i>		27	BSH-1
13	GS-1	28	NDYS-9504
14	NRCY 93	29	SSK-16
15	NRCYS-219	30	SSK-9205
16	PS-66		

In all the thirty genotypes sinigrin content of individual species varies considerably. The sinigrin content in all thirty *Brassica* species genotypes found in the ranged from 2.92 to 173.14 $\mu\text{mole/g}$. Genotype NRCG35 had the highest concentration of sinigrin (173.14 $\mu\text{mole/g}$) and RTM314 had the lowest concentration (2.92 $\mu\text{mole/g}$) as sown in figure 4 and 5 respectively. These findings are similar to those found in other glucosinolate containing species studied by Kumar *et al.*, (2023) [12]. The *Brassica juncea* species and other species genotypes which had low content of sinigrin where all genotypes good for animal feed and used for the double zero varieties by breeding program. The glucosinolates have also been applied in the prevention of bacterial and fungal spoilage of food products in advanced atmospheric packaging technology which improves the shelf-life of these products. These issues are important priorities for rapeseed mustard breeders. The range of total glucosinolates was in agreement with the reports showing glucosinolate content to be 21.89–187.29 (Font *et al.* 2004), 62.8–125.2 (Chauhan *et al.* 2010), 94.4–169.2 (Velasco and Becker 2000) [20] and 57.2–104.6 $\mu\text{mole/g}$ defatted seed meal (Ali *et al.* 2015). Gupta *et al.* (2012) and Rangkadilok

et al. (2002) [2, 7, 16] reported sinigrin concentration ranged from the 0.194 to 51.90 $\mu\text{mole/g}$ in *Brassica juncea*. Kaushik and Agnihotri (1999) [10] studied the levels of sinigrin varied from 6.94 to 32.47 $\mu\text{mole/g}$ in mustard seed. Our results provide evidence for large genotypic variability within the studied *Brassica* species collection.

The lower sinigrin content (>30 $\mu\text{mole/g}$) were found in the RTM314, GDM5, T27, GM3, GDM4, BHC46, SKM1731, PBN2001, HNS004, NDTC9502, NRCY93, GM1, PS66, NRCY219, KRANTI, BR54, SKM1620, GM2 and PC5 genotypes. The seed glucosinolate content has been drastically reduced to contents of 10 $\mu\text{mole/g}$ seed and lower (Raney *et al.*, 1999) [15] by conventional plant breeding, having advantage of spontaneously arisen mutants. Considerable efforts have been made to lower glucosinolates from the seeds of commercial varieties of canola (*Brassica campestris*) and oilseed rape (*Brassica napus*) as these compounds reduce the nutritional value of rapeseed meal. Some studied genotypes showed high levels of sinigrin and could be introduced in breeding programs for reduce of sinigrin content in particular.

Table 2: Sinigrin content from meal of different genotypes of *Brassica* species

Sr. No.	Genotypes	Sinigrin (μmole/g)	Sr. No.	Genotypes	Sinigrin (μmole/g)
1	GM1	11.30	18	DLSC1	158.17
2	GM2	17.94	19	PC5	21.30
3	GM3	5.35	20	RTM314	2.92
4	GDM4	5.75	21	T27	5.03
5	GDM5	4.93	22	HNS0004	7.88
6	KRANTI	13.45	23	NRCG35	173.14
7	PM25	48.28	24	PBCM2002	34.64
8	SKM1620	17.12	25	PBN2001	7.51
9	SKM1731	7.12	26	BHC46	6.84
10	SKM1744	90.29	27	BSH1	102.28
11	BR54	16.09	28	NDYS9504	65.87
12	NDTC9502	8.18	29	SSK16	55.32
13	GS1	128.06	30	SSK9205	48.09
14	NRCY93	10.61		S.Em.	0.87
15	NRCY219	12.81		C.D. at 5 %	2.46
16	PS66	12.50		C.V. %	3.58
17	BCRS3	165.42		Average	42.14

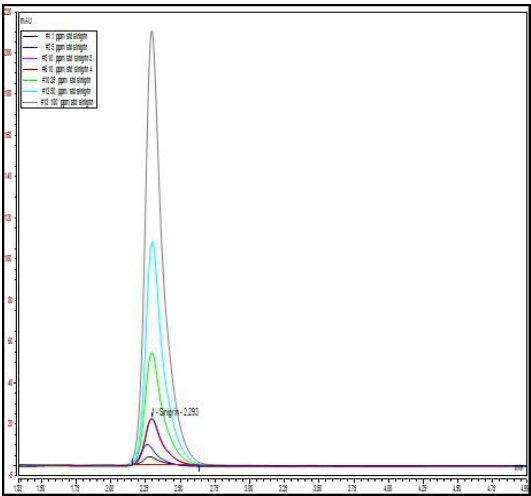


Fig 1: Chromatograph of standard sinigrin overlay

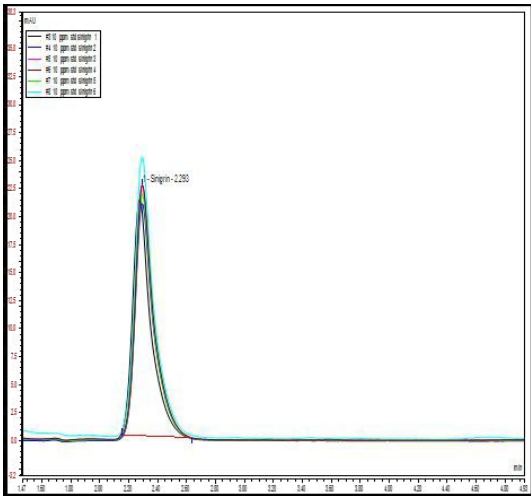


Fig 2: Chromatograph of 10 ppm standard sinigrin overlay

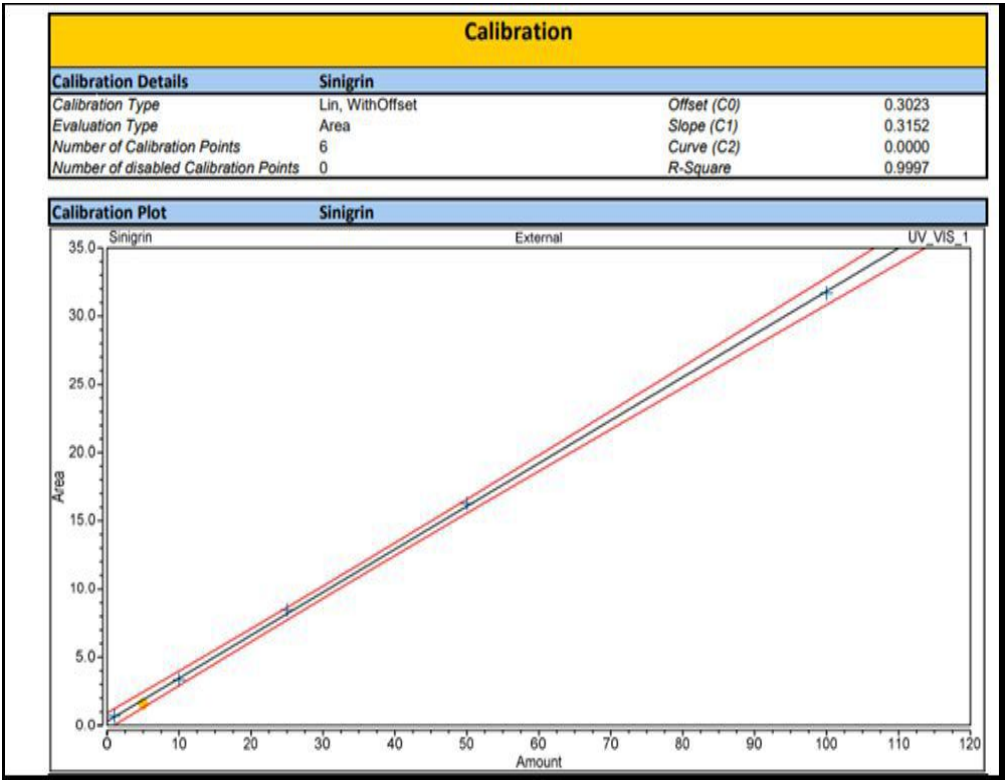


Fig 3: Chromatograph of standard sinigrin calibration curve

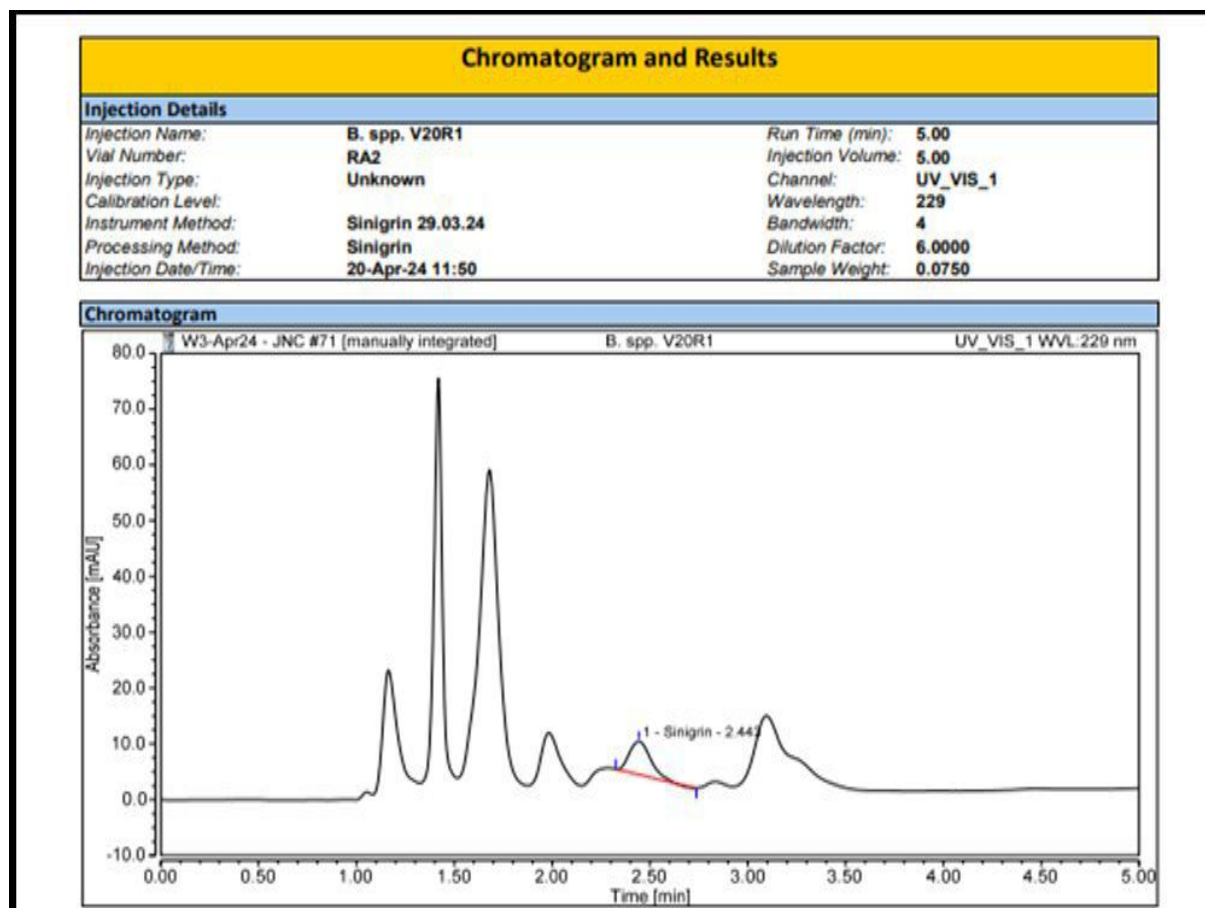


Fig 4: Chromatograph of sinigrin from RTM314

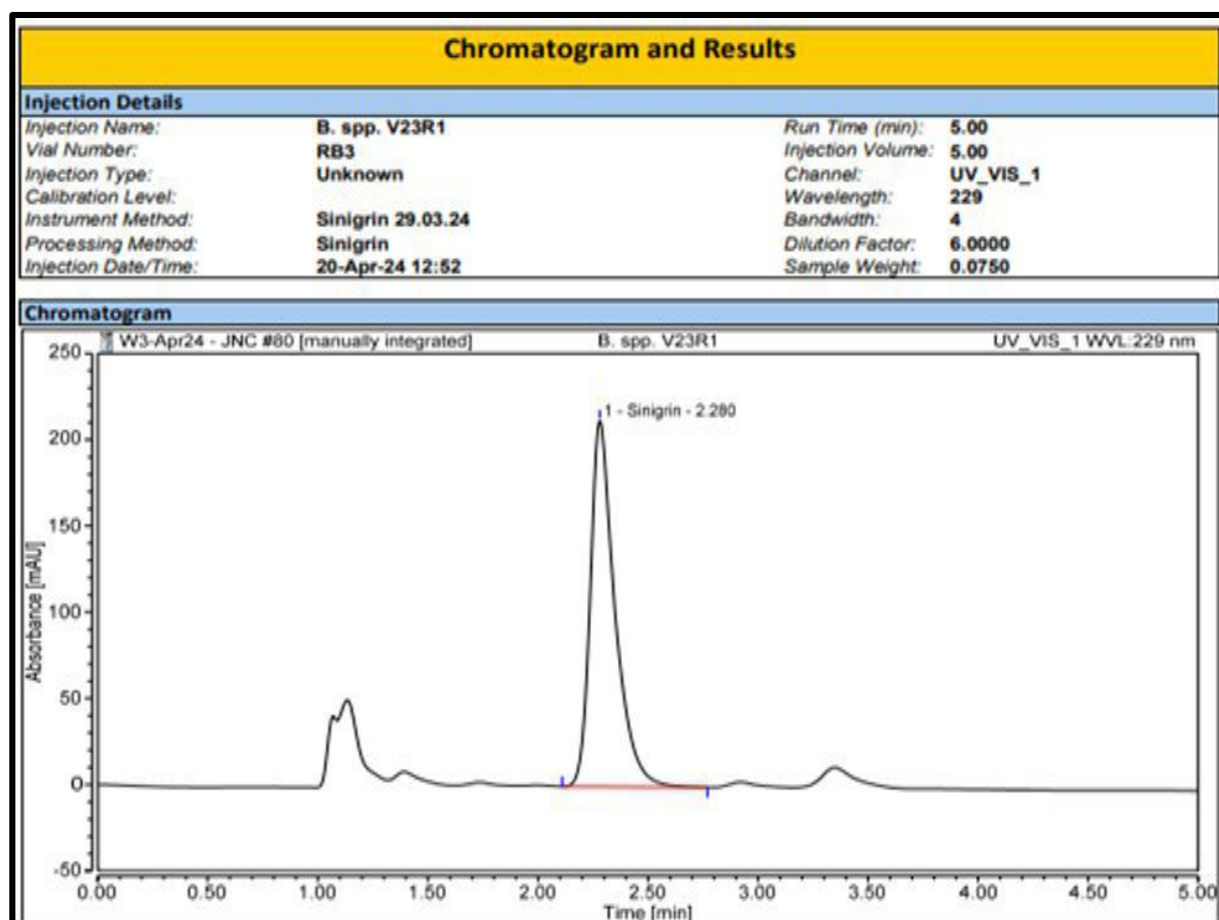


Fig 5: Chromatograph of sinigrin from NRCG35

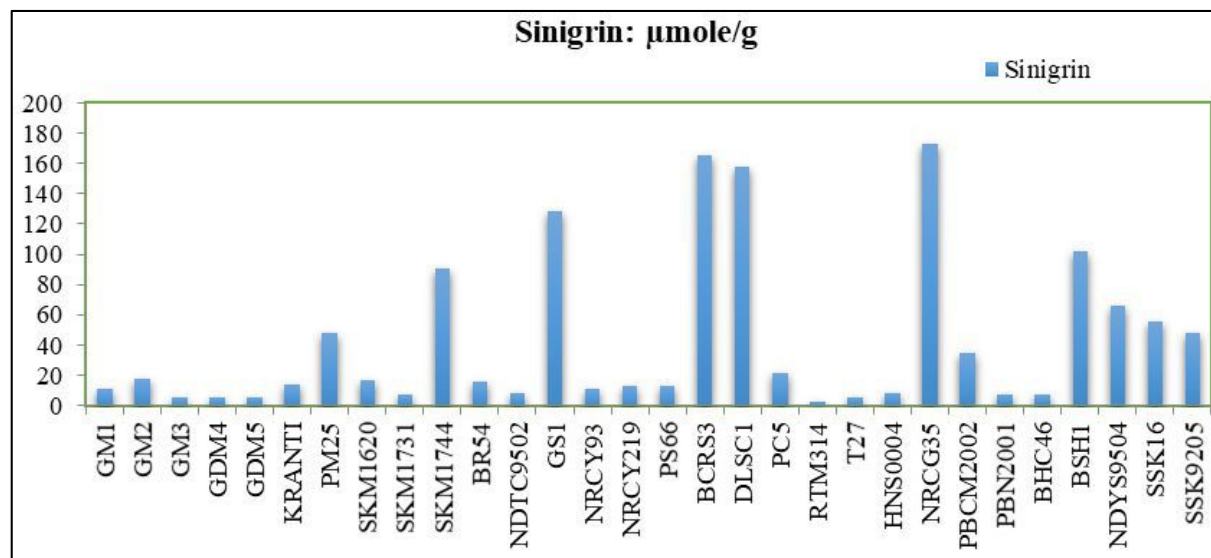


Fig 6: Sinigrin content from different oil seed genotypes of *Brassica* species

Conclusion

Thus, it can be concluded that this study on sinigrin anti-nutritional factor analysis will provides useful information in crop improvement strategies for rapeseed mustard quality improvement through selection of germplasms with low sinigrin content.

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