Original Research Article

Phenol profiling of Brinjal (*Solanum melongena* L.) leaves by LC-MS

.

ABSTRACT

Lab experiment was carried out at department of Agricultural biotechnology of Anand agricultural university, Anand, Gujarat during 2023-24. Study on phenol profiling was carried out on LC-MS of 28 genotypes of brinjal along with parents AB 15-06 (P1) and GRB 5 (P2). Brinjal leaves samples were recorded after three month of transplanting. Total 20 phenolic acids used as standard were; Salicylicacid, gallic acid, hydroquinone, esculin hydrate, pyrocatechol, methylumbelliferone, umbelliferon, quercetin, coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, cinnamic acid, syringaldehyde, fraxetin, 4-hydroxy cinnamaldehyde, aminobenzoic acid, catechin hydrate, sinapic acid and epigallocatechin gallate.Out of Twenty, seven (Ferrulic aicd , Caffeic acid , Epigallocatechin gallate , Quercetin , Salicylicacid , Syringaldehyde , Chlorogenic acid) phenolic compounds were found to be present in detectable quantities in brinjal leaves.

Keywords: Brinjal, leaves, Phenolic acids, LC-MS

1. INTRODUCTION

Solanum melongena, commonly known as brinjal, aubergine, or eggplant, is a warm-season vegetable that is extensively grown for its delicious fruits. Important psychological and morphological traits that can be used to distinguish cultivated varieties of brinjal (eggplant) from their wild relatives include the absence of prickles on the stem, leaves, and calyx, as well as the size and color of the fruits. (Randhawa *et al*., 2016).Brinjal are popular and highly nutritious vegetables that are eaten in many different ways in Asian countries. In terms of overall production, Brinjal is the second most significant vegetable crop, right after tomatoes. (Alam *et al.,* 2021).

Brinjal is found throughout South-East Asia in a variety of forms, colors, and shapes, indicating that this region is a significant center of variation. It is thought that the area between Bangladesh and Myanmar (the former border between India and Burma) is a center of diversity. Zeven and Zhukovsky (1975) claim that it began in India, moved eastward, and reached China by the fifth century B.C. when it became a secondary center of variation. As a result, China has been aware of it for the past 1500 years. Afterwards, Arabic traders were in charge of the migration to Africa and Spain. The Mediterranean region is relatively new to the growing of brinjal. Brazil was colonized by the Portuguese. Nowadays, it is extensively grown for its fruits in warm temperate, tropical, and subtropical climates, particularly in Southern Europe and the Southern United States. Although there are spiky African brinjal plants, Sampson (1936) proposed that this crop originated in Africa. However, there is no proof that *S. melongena* L. is native to that continent.

Brinjal high ranking in terms of oxygen radical absorbance capacity (ORAC) can be attributed to its phenolic constituents, which are known for their antioxidant properties. Phenolic compounds are abundant in eggplant, with more than 4,000 identified phytochemicals, including flavonoids, phenolic acids, and polyphenols. Phenolic acids, another class of dietary phenolics found in eggplant, include hydroxybenzoic and hydroxycinnamic acids. These compounds contribute to the overall antioxidant capacity of eggplant (Whitaker and Stommel 2003). Brinjal is a low-calorie vegetable that offers fiber, iron, calcium, phosphorus, minerals, and vitamins (thiamine and vitamin B-6). This particular food type has inherent medicinal benefits in addition to being delicious and nutritious. (Khan,1979; Konczak and Zhang*,* 2004). White kinds of brinjal fruits, leaves, and roots are beneficial to diabetic people and have significant therapeutic qualities that are employed in many Ayurvedic medications. Additionally, it has been suggested as a great treatment for cholera, asthma, bronchitis, and liver issues. Leafy greens and fruits can help decrease blood cholesterol (Kayamori and Igarashi, 1994). Because of a unique supply of anthocyanin, a key phenolic present in brinjal and one of the most important antioxidants with a range of physiological functions like antimutagenics, anticancer, and vision improvement, it is one of the top ten vegetables in terms of antioxidant capacity (Azevedo *et al.*, 2007; Todaro *et al.*, 2009; Philpott *et al.*, 2009). The main aim of present investigation to identified phenolics compounds which further helps to development of medicines and drugs in pharmaceutical industry.

The *solanaceae* family of plants contains glycoalkaloids, which are responsible for the bitter taste of eggplant. The high concentration of glycoalkaloids (20 mg/100 g fresh weight) typically results in an off flavor and bitter taste. High polyphenol oxidase activity is the cause of the browning in eggplant fruit. Because of its high concentration of 5-O-[E]-caffeoylquinic acid (15) (5-CQA; chlorogenic acid), which normally makes up P70% of all the phenolic compounds in the fruit pulp, eggplant has the strongest ability to scavenge free radicals. These phenolics include derivatives of 5-CQA, other HCA quinate esters, and HCA amides of polyamines (HCAAs) (Winter and Herrmann, 1986; Whitaker and Stommel, 2003). The pulp of eggplant and its close relatives has been shown to contain dozens of other less common phenolic chemicals, principally derivatives of caffeic acid, which, like 5-CQA (15), add to the flavor and health benefits (Ma *et al.,* 2010).

Brinjal (eggplant) leaves are rich in phenolic acids, with chlorogenic acid, caffeic acid, and ferulic acid being the most abundant. Chlorogenic acid is typically the predominant phenolic compound, often constituting over 50% of the total phenolic acids in the leaves, with concentrations ranging from 1 to 5 mg per gram of dry leaf weight. Caffeic acid is also found in significant amounts, usually around 10-20% of the total phenolic content, with typical concentrations between 0.5 to 2 mg per gram of dry leaf weight. Ferulic acid is present in moderate to high concentrations, making up about 5-15% of the total phenolic acids, and is typically found in amounts ranging from 0.2 to 1 mg per gram of dry leaf weight.

Numerous phenolic acids, which are recognized for their antioxidant qualities and possible health advantages, can be found in the leaves of eggplant (also known as brinjal). Brinjal leaves contain significant amounts of phenolic acids, namely p-coumaric acid, vanillic acid, ferulic acid, caffeic acid, and chlorogenic acid. An ester of caffeic acid and quinic acid, chlorogenic acid is well-known for its effective anti-inflammatory, antibacterial, and antioxidant properties. It may also have neuroprotective properties and lower the risk of chronic illnesses including cancer and cardiovascular disease. Derivative of hydroxycinnamic acid, caffeineic acid has anti-inflammatory, anti-tumor, and antioxidant characteristics that help prevent disorders linked to oxidative stress. Another derivative of hydroxycinnamic acid, ferulic acid, is frequently utilized in skin protection products due to its antibacterial, antioxidant, and anti-inflammatory properties that help shield cells from harm. Another hydroxycinnamic acid, p-coumaric acid, has anti-inflammatory and antioxidant properties that may reduce the incidence of oxidative stress-related illnesses. Methoxybenzoic acid vanillic acid has anti-inflammatory and antioxidant qualities that help prevent cancer and other chronic illnesses (Niño-Medina *et al.,* 2017).

Syringic acid and gallic acid are two more phenolic acids found in brinjal leaves. Trihydroxybenzoic acid, or gallic acid, reduces oxidative stress and inflammation and possesses antibacterial, anti-inflammatory, antioxidant, and anticancer activities. Another dimethoxybenzoic acid that has anti-inflammatory, antibacterial, and antioxidant qualities is syringic acid. It also guards against oxidative stress and may have anti-cancer effects. Numerous analytical methods, including Ultraviolet-Visible Spectroscopy (UV-Vis), Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), and Mass Spectrometry (MS), are used to identify and quantify these phenolic acids. The health benefits of brinjal leaves, such as their ability to prevent chronic diseases like heart disease, cancer, and neurodegenerative diseases, as well as their anti-inflammatory and anti-inflammatory effects, as well as their antimicrobial activity that provides resistance against pathogens, are all attributed to the presence of these phenolic acids. This means that because of their pharmacological qualities, brinjal leaves are beneficial for human health in addition to being essential for plant defense (Baek *et al.,* 2016).

2. MATERIAL AND METHODS

The present investigation was conducted at department of Agricultural biotechnology, Anand agricultural university, Anand, Gujarat during 2023-24.

Experimental material:

Total 28 genotypes were selected for phenol profiling study along with their parent AB 15-06 and parent GRB 5. The fresh leaves of brinjal after three moth of transplanting were selected for phenolic acid profilingas the method described by Kowalski and Kowalska (2005) with some modifications.

2.1 Standard preparation:

The stock solution of each Phenols was prepared in 10 ml of 100 % methanol. Initially, 100 µg/ml of stock of each standard were prepared. From this, working standards were prepared by mixing all standard compounds so that final concentration was 10 µg/ml. Further this mixture was prepared in the range of 5, 2.5, 1.25, 0.625, 0.3125, 0.1562, 0.0781, 0.0390, 0.01953 and 0.00976 using methanol for generating the linearity of standard curves.

2.2 Sample preparation:

A homogenized 500 mg of each sample was taken in a 15 ml capacity centrifuge tube and 10 mL of methanol was added. The samples were sonnicated at 40-45˚C for 5 mins. Followed by centrifugation at 5000 rpm for 5 mins. Supernatant was taken in another tube and 10 m volume make up with methanol and the process was repeated once. The sample was concentrated to 10 ml and filtered through a 0.22 µ nylon membrane filter. The sample was further diluted 40 times and was loaded in LC/MS-MS system.

2.3 Instrument detail

Separations was carried out using an EKsigout Expert Ultra LC 100 connected to AB Sciex QTRAP 4500 using electron spray ionization (ESI) positive mode. To separate the bioactive compound, a reverse-phase C18 column (1.7 µm, length 2.1×100 mm) was utilized. Column elution was comes out using the A: Water (0.1 % formic acid) and B: ACN (0.1 % formic acid).The flow rate of the mobile phase was maintained at 0.3 ml/min, and a fixed injection volume of 5µl was used. The identification of each compound’s peaks was accomplished by comparing their retention times to standard references peak and the peak area was automatically calculated by integrated analyst 6.0% software determined by applying an equation based on the ratio of peak areas between the respective standard and sample.

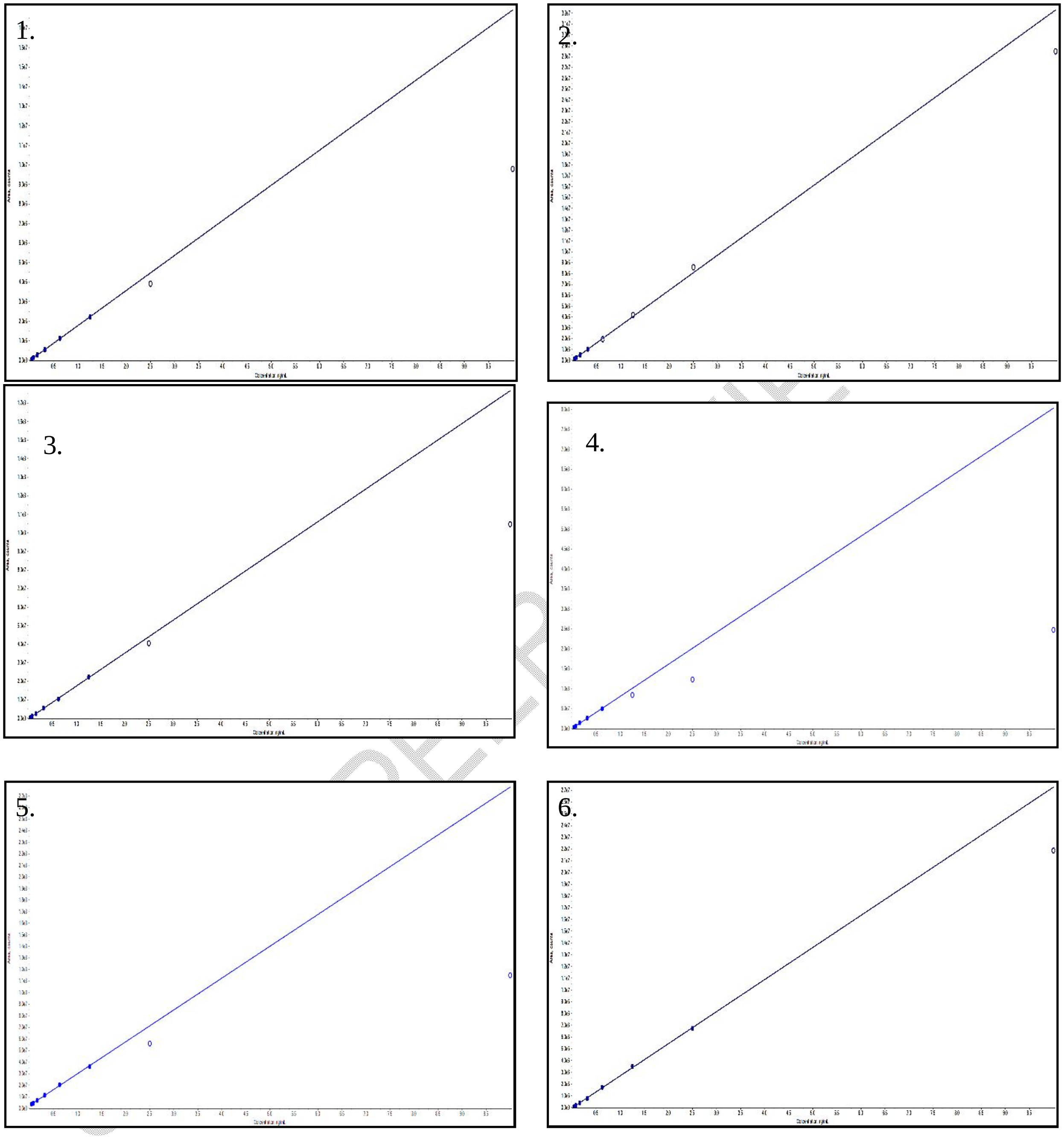
3. RESULTS AND DISCUSSION

3.1 Phenol profiling by LC-MS from brinjal leaves

Different phenolic compounds were used to studied from 28 genotypes along with their parents AB 15-05 (P1) and GRB 5 (P2). Total 20 phenolic compound like Salicyclic acid, Gallic acid, Hydroquinone, Esculin hydrate, Pyrocattecole, Methylumbelliferone, Umbelliferon, Quercetin, Caffeic acid, Coumeric acid, Chlorogenic acid, Ferrulic aicd, Cinamic acid, Syringaldehyde, Frexetin, 4- hydroxi-cinnamaldihyde , Aminobenzoic acid, catechin hydrate, Epigallocatechin gallate, Sinapic acid were used to indentified phenols which presented in the brinjal leaves. Out of 20, 7 (Ferrulic aicd **,** Caffeic acid **,** Epigallocatechin gallate **,** Quercetin , Salicylicacid **,** Syringaldehyde , Chlorogenic acid) phenolic compounds were found to be present in detectable quantities in brinjal leaves and presents AB 15-06 and GRB5 (Table 1) and (Fig. 2).

All phenolic acid like Ferrulic aicd **,** Caffeic acid **,** Epigallocatechin gallate **,** Quercetin , Salicylicacid **,** Syringaldehyde , Chlorogenic acid were found lower in genotype 6 where as higher amount of phenolic acid was observed in genotype 22. In parent GRB 5 chlorogenic acid (4.4 ppm) detected in higher amount where as caffeic acid remarkably higher in parent AB 15-06. Ferulic acid was remarkably higher in genotype 22 (0.0934 ppm). Caffeic acid and Chlorogenic acid were distinctly higher in genotype number 16 (5.33 ppm) and genotype 22 (5.23 ppm). Maximum amount of Epigallocatechin gallate and Syringaldehyde found in genotype 1 (0.047 ppm) and genotype 22 (0.023 ppm). Quercetin amount found higher in genotype 47 (0.137 ppm). Salicylic acid detected in genotype 1, 10, 16, 17, 23, 27 and 28 and parent GRB 5 while absent in parent AB 15-05. Out of them maximum amount of Sylicilic acid was found in genotype 1 (0.037 ppm).

Ferulic acid (0.0103 ppm) found lower in genotype 15. Epigallocatechin gallate(0.0118 ppm) found lower in genotype 25. Quercetin (0.000129 ppm) found lower in genotype 13. Salicylic acid (0.00146 ppm) found lower in genotype 10. Caffeic acid(0.32 ppm), Syringaldehyde (0.0006 ppm) and Chlorogenic acid (0.87 ppm) found lower in genotype 6.


1.
2.
3.
4.
5.
6.

7.

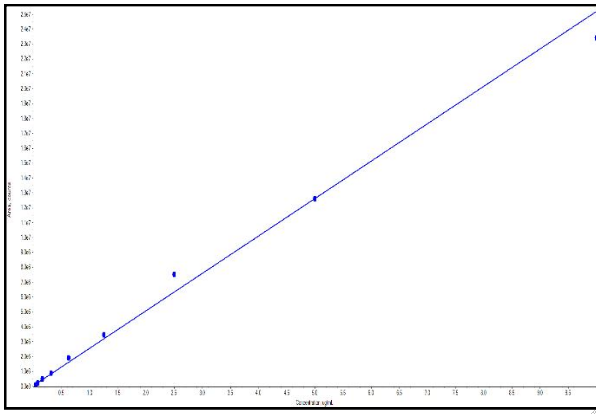


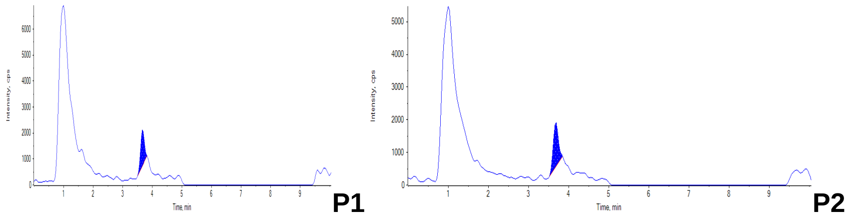
Fig. 1.Calibration curve of Standard for phenol profiling from brinjal leaves
Note: 1. Ferrulic aicd , 2. Caffeic acid , 3. Epigallocatechin gallate , 4. Quercetin , 5. Salicylicacid , 6. Syringaldehyde , 7. Chlorogenic acid

Ferulic acid (FA) is a widely distributed natural phenolic phytochemical found in seeds and leaves. It can be found both unbound and covalently attached to polysaccharides, glycoproteins, polyamines, lignin, and hydroxy fatty acids found in plant cell walls. Ferulic acid has a wide range of biological activities antioxidant, anti-inflammatory, antimicrobial, antiallergic and anticarcinogenic effects. Ranged of ferulic acid in brinjal found between 0.73-3.5 ppm. (Kumar & Pruthvi 2014).

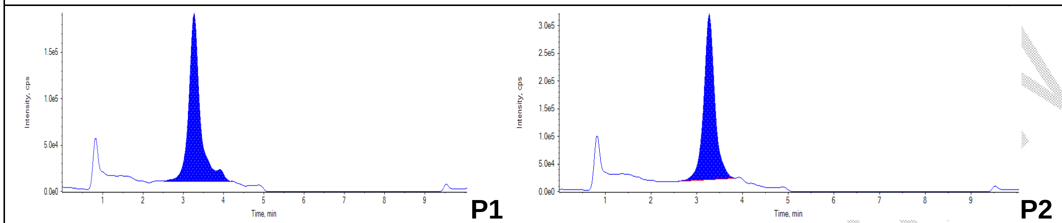
Rodrigues *et al.* (2022) identified different phenolic compound from chilli. quercentic ranges from 0.07-1.3 ppm. epigallocatechin gallate and syringaldehyde also reported from 0.02-0.45 ppm. ferulic acid (0.40-5.2 ppm), chlorogenic acid (0.13-0.81 ppm), caffeic acid (0.10-1.2 ppm) also detected varies in ranges.However, information pertaining to phenolic profile study in brinjal leaves is trace and hence it is compared with other solanaceae plants.

Table 1. Phenol profiling from leveas of brinjal genotype (ppm)

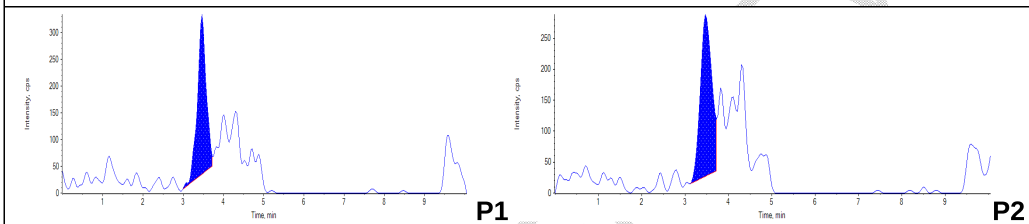
Genotypes	Ferrulic aicd	Caffeic acid	Epigallocatechin gallate	Quercetin	Salicylic acid	Syringaldehyde	Chlorogenic acid
1	0.0234	1.38	0.047	0.137	0.0307	0.0347	1.38
2	0.0117	1.28	0.0122	0.0119	N/A	0.0118	1.11
3	0.0113	1.24	0.0121	0.0225	N/A	0.0118	1.26
4	0.0112	1.2	0.0137	0.0135	N/A	0.0305	1.2
5	0.0129	2.18	0.0136	0.0193	N/A	0.0349	1.14
6	N/A	0.32	N/A	0.0003	N/A	0.0006	0.87
7	0.0114	1.58	0.013	0.0606	N/A	0.0116	1.11
8	0.0113	1.65	0.0129	0.0597	N/A	0.0315	1.36
9	0.0112	1.2	0.0119	0.0112	N/A	0.0305	1.35
10	0.0133	1.85	0.0125	0.0811	0.00146	0.0327	2.09
11	0.0108	1.67	0.0127	0.0269	N/A	0.0345	1.73
12	0.0202	1.5	0.0123	0.014	N/A	0.0256	1.63
13	0.0107	1.59	0.0122	0.0003	N/A	0.0273	1.85
14	0.011	1.77	0.0122	0.0138	N/A	0.0285	1.64
15	0.0103	2.74	0.0124	0.00937	N/A	0.0237	3.29
16	0.0238	5.33	0.0122	0.0832	0.00204	0.0251	2.28
17	0.0141	2.38	0.0122	0.0095	0.012	0.0251	3.38
18	0.0112	2.59	0.0123	0.00781	N/A	0.0282	3.25
19	0.0131	2.29	0.012	0.107	N/A	0.0473	2.24
20	0.0149	2.56	0.0122	0.0266	N/A	0.0324	3.27
21	0.0114	3.12	0.012	0.0383	N/A	0.0279	3.3
22	0.0934	2.99	0.0118	0.0159	N/A	0.023	5.23
23	0.0163	3.11	0.0121	0.0353	0.0154	0.0273	4.75
24	0.0323	2.42	0.0119	0.0349	N/A	0.0285	2.46
25	0.0296	1.81	0.0118	0.021	N/A	0.0217	3.61
26	0.0119	2.64	0.012	0.00398	N/A	0.0266	3.09
27	0.0212	2.37	0.0119	0.0207	0.0151	0.0307	2.97
28	0.0132	2.75	0.0119	0.00341	0.00838	0.0234	3.86
AB 15-06 (P1)	0.0129	1.13	0.0119	0.00626	N/A	0.0276	1.11
GRB 5 (P2)	0.0932	3.55	0.0219	0.00921	0.0907	0.0796	4.4



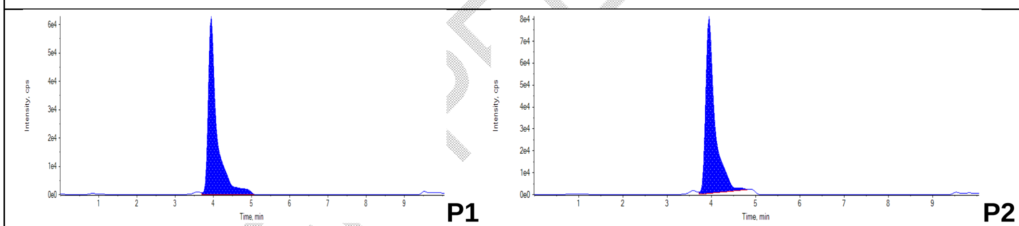
1. Ferrulic acid



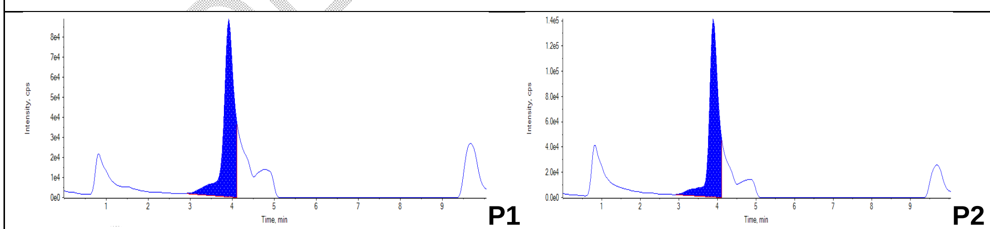
2. Caffeic acid



3. Epigallocatechin gallate



4. Quercetin



5. Salicylicacid

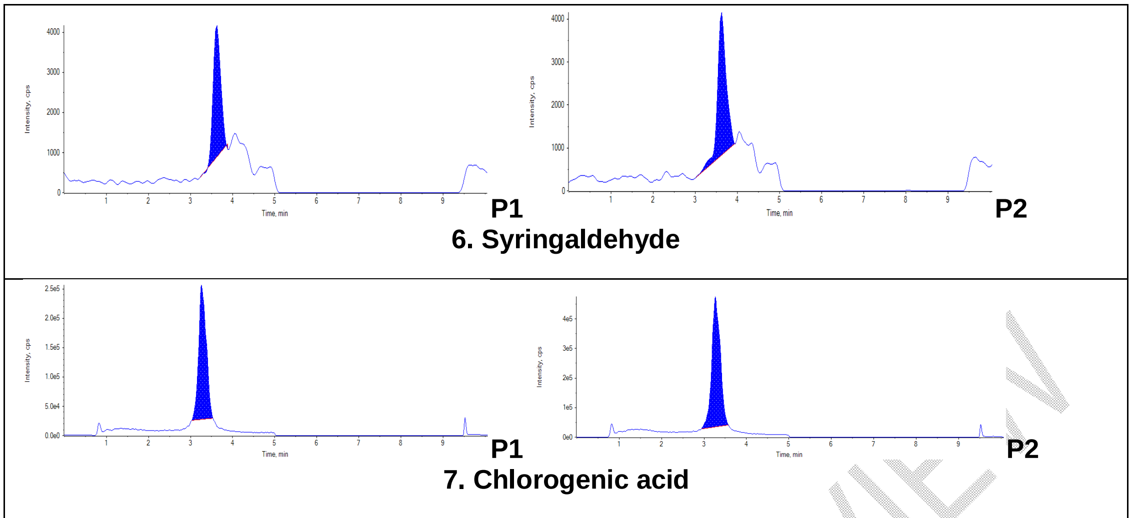


Fig. 2. LC-MS chromatograms of phenolic acid fraction released from (P1) AB 15-06 and (P2) GRB 5 parents of brinjal

4. CONCLUSION

The result revealed that caffeic acid was observed higher amount in AB 15-06 (1.13ppm) and GRB 5 (3.55 ppm) leaves compare to other phenolics acid. Salicylicacid only detected in genotype 1, 10, 16, 17, 23, 27 and 28 and parent GRB 5 while absent in parent AB 15-05. Out of them maximum amount of Salicylic acid was found in genotype 1 (0.037 ppm). Other phenolic compound also present in detectable amount.

REFERENCES

1. Whitaker BD, & Stommel JR. Distribution of hydroxycinnamic acid conjugates in fruit of commercial eggplant (*Solanum melongena* L.) cultivars. Journal of Agriculture Food Chemestry, 2003;51:3448-3454.
2. Khan R. *Solanum melongena* L. and its ancestral forms. The biology and taxonomy of the Solanaceae. In: Linnean Society symposium series. Academic Press. 1979; 629-635.
3. Konczak I, & Zhang W. Anthcyanins-more than nature's colours. Journal of Biomedicine and Biotechnology. 2004;5:239-240.
4. Kayamori F, & Igarashi K. Effects of dietary nasunin on the serum cholesterol level in rats. Bioscience, Biotechnology and Biochemistry. 1997;58(3):570-571.
5. Azevedo L, Alves de Lima, P, Gomes JC, Stringheta PC, Ribeiro DA, Salvadori D. Differential response related to genotoxicity between eggplant (*Solanum*

melanogena) skin aqueous extract and its main purified anthocyanin (delphinidin) *in vivo*. Food and Chemical Toxicology. 2007;45(5):852- 858.

6. Todaro A, Cimino F, Rapisarda P, Catalano AE. Recovery of anthocyanins from eggplant peel. Food Chemistry. 2009;114(2):434-439.
7. Philpott M, Lim CC, Ferguson LR. Dietary protection against free radicals, a case fortiple testing to establish structure-activity relationships for antioxidant potential of anthocyanin plant species. International Journal of Molecular Sciences. 2009;10(3): 1081-1103.
8. Kowalski R, Kowalska G. Phenolic acid contents in fruits of aubergine (*Solanum melongena* L.). Polish Journal of Food *and* Nutrition Sciences. 2005;55(1):37-42.
9. Rodrigues AC, Zomer APL, Rotta EM, Visentainer JV, Maldaner L. A µ-QuEChERS method combined with UHPLC-MS/MS for the analysis of phenolic compounds in red pepper varieties. Journal of Food Composition and Analysis. 2022;112:104647.
10. Winter M, Herrmann K. Esters and glucosides of hydroxycinnamic acids in vegetables. Journal of Agriculture Food Chemistry. 1986;34:616-620.
11. Ma C, Whitaker, BD, Kennelly EJ. New 5-O-caffeoylquinic acid derivatives in fruit of the wild eggplant relative *Solanum viarum*. Journal of Agriculture and Food Chemistry. 2010;58:11036-11042.
12. Zeven & Zhukovsky. Dictionary of cultivated plants and their centres of diversity Excluding ornamentals, forest trees and lower plants, center for agricultural publishing and documentation, wageningen. 1975:10-218
13. Sampson HC. 1936. Bulletin ofmiscellaneous information. Roy. Bot. Gdn. Kew, Add. Ser. 12:159. Cited in: Som, M. G. and Malty, T. K. 1986. Brinjal In: Vegetable Crops in India. Eds. Bose, T.K. and Somm, M.G. pp. 293-342. Noya Prokash Calcutta. India.
14. Randhawa MA, Abid, QUZ, Anjum FM, Chaudhary AS, Sajid MW, &Khalil AA. Organo-chlorine pesticide residues in okra and brinjal collected from peri-urban areas of big cities of punjab pakistan.Pakistan Journal of Agricultural Sciences. 2016;53(2).
15. Alam MA, Kamarzaman AB, Jalloh MB, & Lassim MBM. Evaluation of varietal performance for yield and yield contributing attributes of local brinjal (*Solanum melongena* L.) germplasm collections. Journal of Agrobiotechnology. 2021;12(1):1-9.
16. Niño-Medina, GUILLERMO, Urías-Orona VANIA, Muy-Rangel, MD, & Heredia JB. Structure and content of phenolics in eggplant (Solanum melongena)-a review. South African Journal of Botany. 2017;111:161-169.

17. Baek, DR, Lee MJ, Baek NI, Seo, KH, & Lee Y H. Phenolic compounds from the leaves of eggplant (Solanum melongena L.). Journal of Applied Biological Chemistry. 2016;59(2):103-106.