Journal of Advances in Biology & Biotechnology

Volume 27, Issue 7, Page 929-939, 2024; Article no.JABB.119245
ISSN: 2394-1081

Phenol Profiling of Brinjal (*Solanum melongena* L.) Leaves by LC-MS

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jabb/2024/v27i71054

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/119245

Original Research Article

Received: 16/04/2024
Accepted: 20/06/2024
Published: 24/06/2024

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 by Liquid Chromatography Mass spectrometry (LC-MS) method. Experimental materials for phenol profiling, fresh leaves of 28 genotypes of brinjal along with parents AB 15-06 (P1) and GRB 5 (P2) selected after three months of transplanting. Total 20 phenolic acids used as standard were; salicylic acid, 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,

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Cite as: *Chauhan, B. P., Urja B. Solanki, Y. M. Shukla, and A. A. Sakure. 2024. "Phenol Profiling of Brinjal (Solanum Melongena L.) Leaves by LC-MS". Journal of Advances in Biology & Biotechnology 27 (7):929-39. https://doi.org/10.9734/jabb/2024/v27i71054.*

quercetin, salicylic acid, 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*., [1]. 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.,* [2].

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 [3,4]. 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 [5] 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 [6] proposed that this crop originated in Africa. However, there is no proof that *S. melongena* L. is native to that continent.

The potential of brinjal in high rank in terms of oxygen radical absorbance capacity (ORAC) is because its phenolic content, and it is well known that phenolics are potent antioxidants. Eggplants are rich in phenolic compounds, with over 4000 phytochemicals be discovered falls under the category of flavonoids, phenolic acids and polyphenolics. Phenolic acids in eggplant, a second class of dietary phenolics, include hydroxybenzoic and hydroxycinnamic acids. These compounds all serve to enhance the overall antioxidant capacity of eggplant [7]. A 100 gm serving of brinjal contains water, 27 kcal energy providing by 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, [8] Konczak and Zhang*,* [9]. 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 [10]. 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 [11-13]. The main aim of present investigation to identified phenolics compounds from brinjal leaves which further helps to development of medicines and drugs in pharmaceutical industry.

Phenolic acids are secondary metabolites that distributed various part of plant likes flowers, fruits, leaves, roots and also play significance role in plant physiological process such as color, taste, quality, aroma etc. 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) [14,7]. 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 [15].

Phenolic acids like chlorogenic acid, caffeic acid, ferulic acid were mostly present in brinjal leaves. Chlorogenic acid is usually the most abundant phenolic compound, and may represent >50% of the total leaf phenolic acids at ranging from 1 to 5 mg per gram of dry weight leaves. Caffeic acid, probably comprising 10-20% of the total phenolic content, are also present, generally ranging from 0.5 to 2 mg per gram 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.

Eggplant or brinjal leaves contain a lot of polyphenolic compounds that have an extraordinary premium quality antioxidant profile and wellbeing health advantages. The other phenolic acids detected in brinjal leaves were: p-coumaric acid, vanillic acid, ferulic acid, caffeic acid and chlorogenic acid. Chlorogenic acid, an ester of caffeic acid and quinic acid, is popularly known for its potent anti-inflammatory, antibacterial, and antioxidant features.

It could also be neuroprotective and decrease the risk of cancer and cardiovascular disease. Due to its antioxidant retrospect parameters containing anti-inflammatory, antiepileptic, antimicrobial and antitumor activities hydroxycinnamic acid derivatives(caffeic acerbic and their esters) absorb averted the reversible cellularization-assay which is apropos to beginning diseases associated with oxidative stresses. Another metabolite of hydroxycinnamic acid, ferulic acid is a very popular ingredient in skincare products given its antibacterial and anti-inflammatory action as well as its antioxidant activity which protects cells from oxidative stress. P-coumaric acid, another hydroxycinnamic acid in black rice is anti-inflammatory and has an antioxidant effect that can premised to decrease oxidtative stress related disease. Vanillic acid Methoxybenzoic acid has anti-inflammatory and antioxidant features that diminish the chances of cancer and other chronic diseases [16].

Other phenolic acids such as syringic acid and gallic acid present in brinjal leaves. Gallic acid also known as trihydroxybenzoic acid that exhibits antibacterial, anti-inflammatory, antioxidant, and anticancer properties while combating oxidative stress and inflammation. Syringic acid also identified as dimethoxybenzoic acid that demonstrates anti-inflammatory, antibacterial qualities with antioxidant effects; it also fights oxidative stress potentially through anti-cancer mechanisms. Several analytical methods including Ultraviolet-Visible Spectroscopy (UV-Vis), Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS) are employed to detect these phenolic acids present in brinjal leaves which contribute towards various health benefits like preventing chronic diseases and boosting immunity by protecting against pathogens that cause disease. This means that because of their pharmacological qualities, brinjal leaves are beneficial for human health in addition to being essential for plant defense [17].

2. MATERIALS AND METHODS

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

2.1 Experimental Material

Total 28 genotypes were selected for phenol profiling study along with their parent AB 15-06 (P_1) and parent GRB 5 (P_2). The fresh leaves of brinjal after three months of transplanting were selected for phenolic acid profiling as the method described by Kowalski and Kowalska [18] with some modifications.

2.2 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. Standard of analytical grade were procured from Sigma Aldrich. From this, working standards were prepared by mixing all standard compounds (salicylic acid, 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), 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.

2.3 Sample Preparation

A homogenized 500 mg of brinjal leaves sample was taken in a 15 ml capacity centrifuge tube containing 10 mL. LC-MS grade of methanol was used for sample extraction. The samples were sonicated and dry at at 45˚C for 5 mins. Followed by centrifugation at 5000 rpm for 5 mins. Supernatant was taken in another tube and 10 mL volume make up with methanol and the process was repeated once. The sample was concentrated to 10 ml and filtered through a 0.22 µm PVDF membrane filter. The sample was further diluted 40 times and was loaded in LC/MS-MS system or stored at -80 ˚C.

2.4 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 UPLC BEH-C-18 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. Mobile phase was selected based on compound resolution. 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 (P_1) and GRB 5 (P_2). 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, salicylic acid**,** syringaldehyde, chlorogenic acid) phenolic compounds were found to be present in leaves of brinjal in detectable quantities of 28 genotypes of brinjal and both of the parents AB 15-06 and GRB 5 (Table 1) and (Fig. 2).

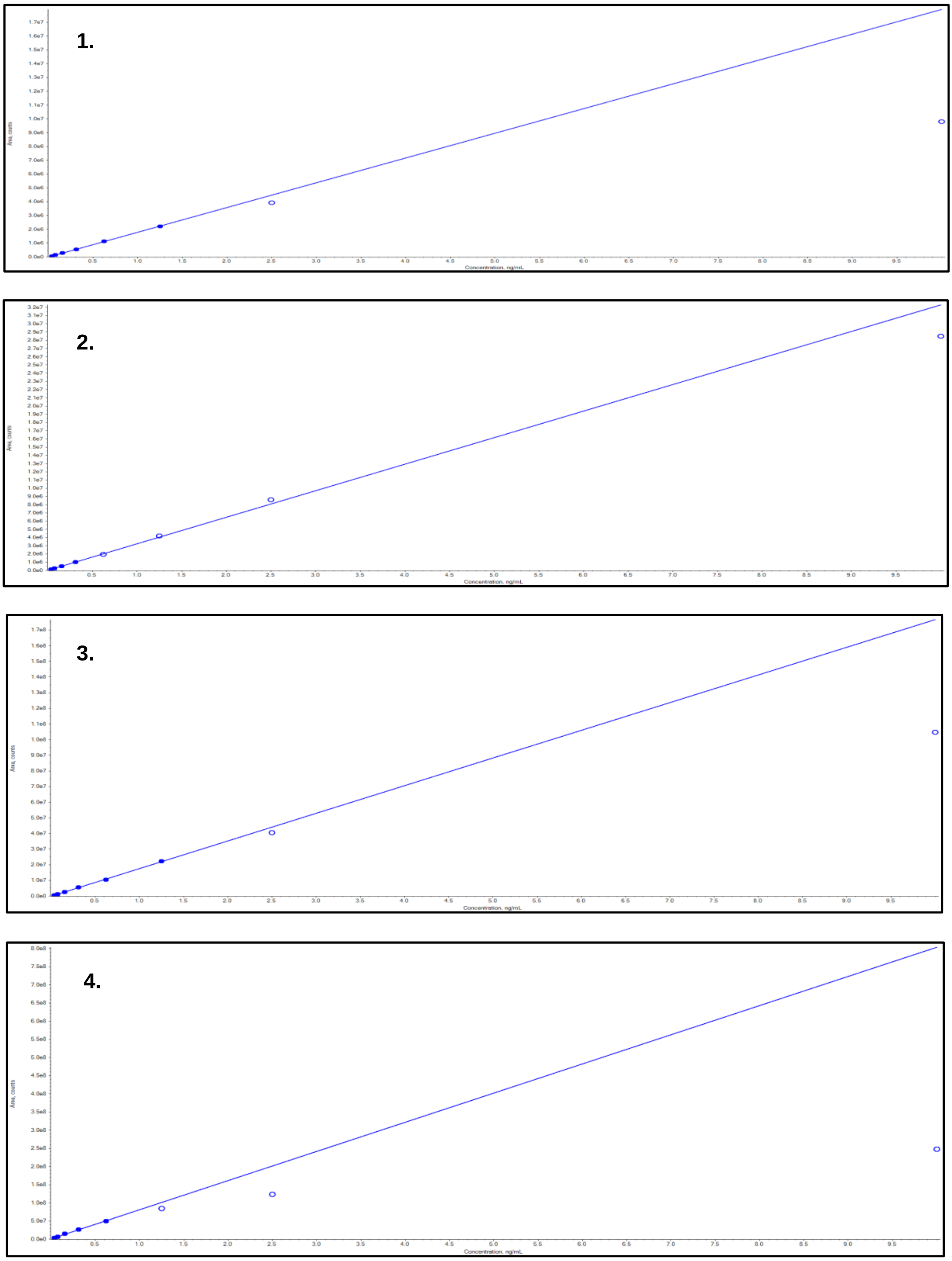
All phenolic acid like ferrulic aicd**,** caffeic acid**,** epigallocatechin gallate**,** quercetin, salicylic acid**,** syringaldehyde, chlorogenic acid were found lower in genotype 6 where as higher amount of phenolic acid was observed in genotype 22. In parent GRB 5 chlorogenic acid (4.4 ppm) detected 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 salicylic 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.

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 [19].

Rodrigues *et al.* [20] 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.



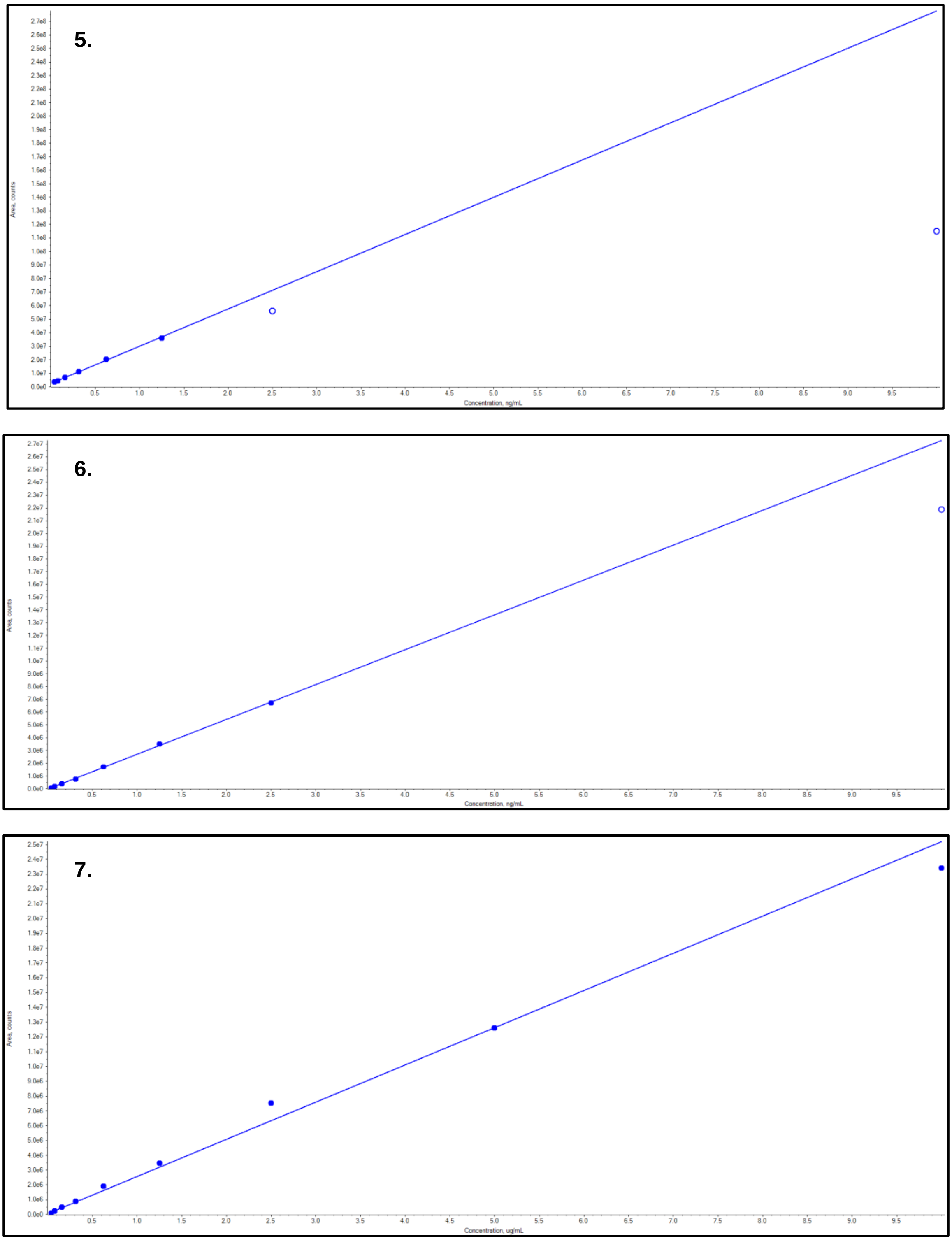


Fig. 1. Calibration curve of Standard for phenol profiling from brinjal leaves

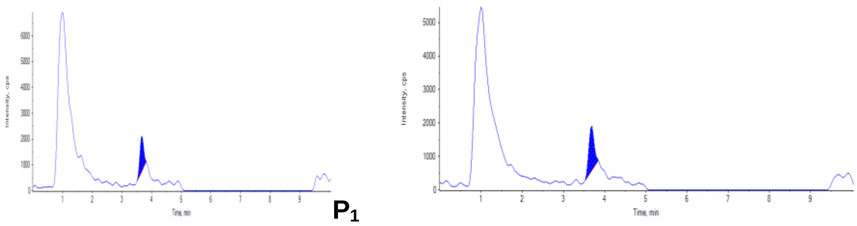
Note: 1. Ferrulic aicd, 2. Caffeic acid, 3. Epigallocatechin gallate, 4. Quercetin ,5. Salicylicacid, 6. Syringaldehyde, 7. Chlorogenic acid

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

Genotypes	Ferrulic acid	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 (P_1)	0.0129	1.13	0.0119	0.00626	N/A	0.0276	1.11
GRB 5 (P_2)	0.0932	3.55	0.0219	0.00921	0.0907	0.0796	4.4

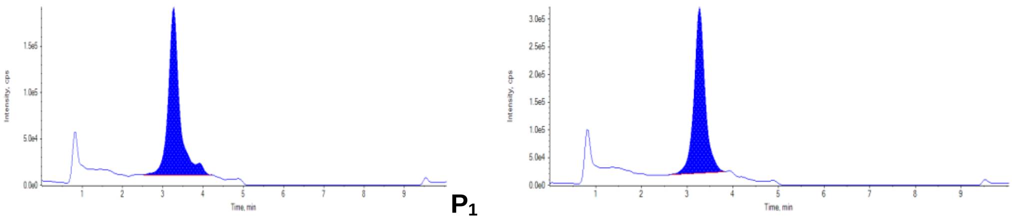
Plate 1. (AB 15-06)

Plate 2. (GRB 5)



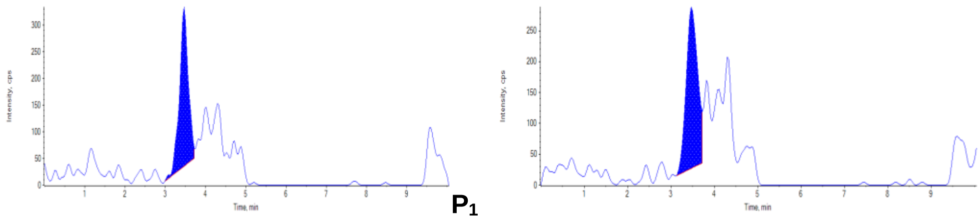
P_1 **P_2**

1. Ferrulic acid



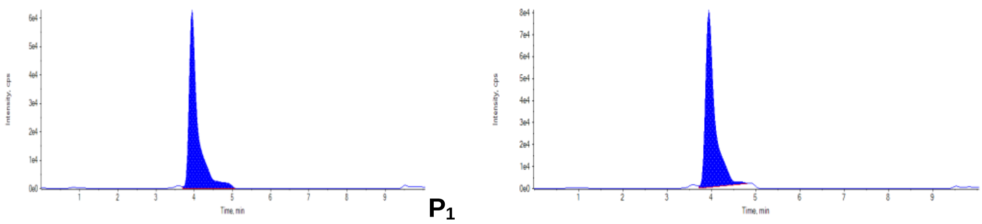
P_1 **P_2**

2. Caffeic acid



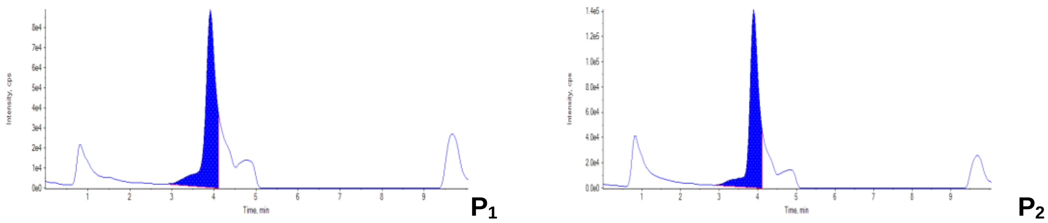
P_1 **P_2**

3. Epigallocatechin gallate

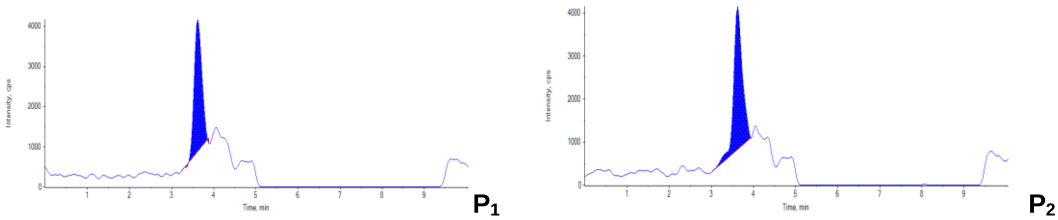


P_1 **P_2**

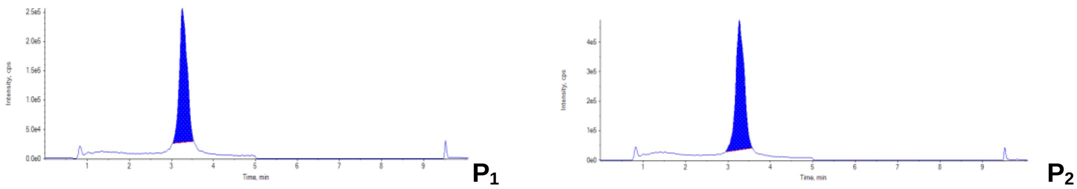
4. Quercetin



5. Salicylicacid



6. Syringaldehyde



7. Chlorogenic acid

Fig. 2. LC-MS chromatograms of phenolic acid fraction released from (P_1) AB 15-06 and (P_2) GRB 5 parents of brinjal

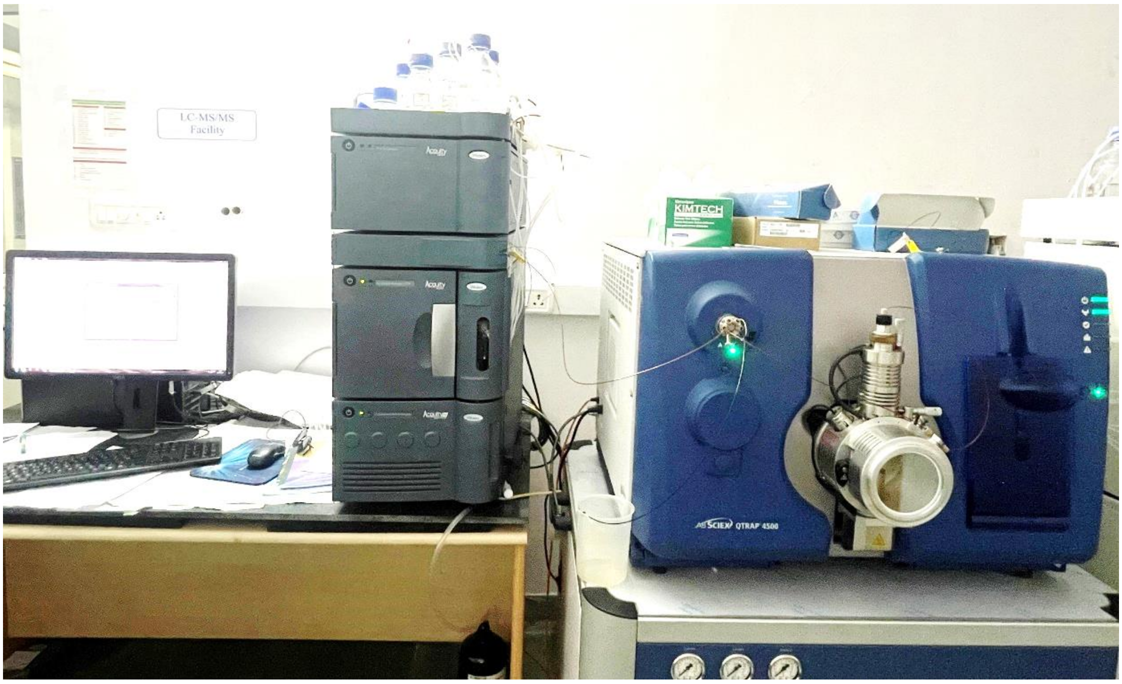


Fig. 3. Instrumental system of LC-MS

4. CONCLUSION

The result revealed that GRB 5 has highest amount of all seven (ferrulic aicd**,** caffeic acid**,** epigallocatechin gallate**,** quercetin, salicylic acid**,** syringaldehyde, chlorogenic acid) phenolic acids compare to AB 15-06. Chlorogenic acid recorded highest amount in GRB 5 (4.4 ppm) whereas caffeic acid was observed higher amount in GRB 5 (3.55 ppm) compare to other phenolics acid. Salicylic acid 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). Ferrulic acid and Epigallocatechin gallate were absent in genotype 6. Other phenolic compound also present in detectable amount in brinjal leaves.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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