Original Research Article
Phenol profiling of chilli (*Capsicum annuum* L.) leaves by LC-MS

ABSTRACT

A lab experiment was conducted at the Department of Biotechnology, Anand Agricultural University, Anand, Gujarat, during 2023. The study focused on phenol profiling of 28 genotypes of chilli along with ACCMS 1 (P1) and ACS 18-08 (P2) using LC-MS. Chilli leaf samples were collected three months after transplanting. A total of 20 phenolic acids were used as standards: 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. Among these, six phenolic acids—ferulic acid, caffeic acid, epigallocatechin gallate, sinapic acid, gallic acid, and quercetin—were detected in measurable quantities in the chilli leaves. The result indicated in present study that in parent ACCMS 1 ferulic acid, caffeic acid, epigallocatechin gallate, sinapic acid and gallic acid (0.0075 ppm, 0.0740 ppm, 0.0119 ppm, 0.0083 ppm and 0.1470, respectively) ppm were found lower as compared to parent ACS 18-08. Only one phenolic acid quercetin was only present in ACCMS 1 and not detected in ACS 18-08.

Keywords: chilli, leaves, LC-MS, phenolic acids

1. INTRODUCTION

Chilli (*Capsicum annuum* L.) is not only a significant vegetable and spice crop but also possesses notable biological activities such as antioxidant, anticancer and antidiabetic properties. These activities are mainly attributed to the presence of phenolic compounds, particularly phenolic acids like ferulic acid, caffeic acid, epigallocatechin gallate, sinapic acid, gallic acid and quercetin. Quantifying these phenolic compounds from different chilli genotypes using reference standards is crucial to advancing research for food and industry applications as well as investigating biological properties. Chilli fruits are rich in natural bactericides and capsaicin, which is reputed to have anti-cancerous qualities. They are also abundant in vitamins A, C, and E and are good sources of potassium and folic acid. This nutrient richness makes them beneficial for human health, providing more vitamin A than carrots and more vitamin C than citrus fruits. However, chilli peppers are vulnerable to numerous diseases, including those caused by viruses, which can lead to significant production losses. Begomovirus, transmitted by the whitefly (*Bemisia tabaci*), are particularly harmful, with the leaf curl virus being

the most debilitating, sometimes causing up to 100% yield loss in severe cases (Kenyon *et al*., 2014; Senanayake *et al*., 2007, 2012; Kumar *et al*., 2011, 2015).

Chilli fruits vary widely in shape, size, and color, depending on the cultivar, age, growing conditions, and postharvest treatment. They range in color from vivid purple to dark green, black, yellow and red. Chilli is a rich source of various antioxidant molecules, especially ascorbic acid, quercetin (Hasler, 1998), and carotenoids (Haytowitz *et al*., 1984; Matsufuji *et al.,* 1998), which have garnered significant attention for their antioxidant properties (Ou *et al*., 2002). A substantial body of research supports the health benefits of consuming fruits and vegetables, which can help prevent a range of chronic diseases (FAO, 2004). In recent decades, the potential of vegetable secondary metabolites to prevent cardiovascular disease and cancer has gained considerable attention (Duthie *et al*., 2006; WHO, 2002). This study aims to identify various phenolic compounds in chilli that play vital roles in human health and have the potential to prevent oxidative stress-related illnesses such as cancer, neurological disorders and cardiovascular diseases (Serrano *et al*., 2010).

2. MATERIAL AND METHODS

The investigation was conducted at Department of biotechnology, located in Anand Agricultural University, Anand, Gujarat during 2023. Chilli leaves samples were recorded after three months of transplanting. Fresh chilli leaves were selected for this study.

Experimental materials

Total 120 genotypes were sawn in the field at Main Vegetable Research Station, Anand Agricultural University, Anand, Gujarat during 2023-24**.** For phenol profiling study total 28 contrasting genotypes including P1 (ACCMS 1) and P2 (ACS 18-08).

2.1 Standard Preparation

Ten mL of 100% methanol were used to make the stock solution for each phenol standard. First, stocks of 100 µg/mL of each standard were made. Following this, all standard compounds were mixed to create working standards, with a final concentration of 10 µg/mL. Additionally, using methanol to create the linearity of standard curves, this mixture was generated in the following ranges: 5, 2.5, 1.25, 0.625, 0.3125, 0.1562, 0.0781, 0.0390, 0.01953 and 0.00976.

2.2 Sample Preparation

Using the method described by Herrera-Pool *et al*. (2021), phenol profiling from chilli leaves was ascertained. A 15 mL centrifuge tube was filled with 500 mg of each homogenized sample, and 10 mL of methanol was then added. The samples were sonicated for five minutes at 40-45 ºC. centrifugation for five minutes at 5000 rpm came next. The procedure was performed once using the supernatant in a new tube filled with 10 mL of methanol. A 0.22 µ nylon membrane filter was used to filter the sample after it had been condensed to 10 mL. After being further diluted forty times, the material was fed into an LC/MS-MS apparatus.

3. Instrument Method

The Electron Spray Ionization (ESI) positive mode of an EKsigout Expert Ultra LC 100 coupled to an AB Sciex QTRAP 4500 was used to perform the separations. A reverse-phase C_{18} column (1.7 µm, length 2.1×100 mm) was used to isolate the bioactive component. A: Water (0.1%) and B: ACN (0.1%) formic acid were being used for the column elution. 5 µl of fixed injection volume was utilized, and the mobile phase flow rate was kept at 0.3 mL/min. Each

compounds peaks were identified by comparing its retention times to the peak of a standard reference. The peak area was then automatically calculated by the integrated analyst 6.0 software using an equation based on the ratio of peak areas between the corresponding standard and sample.

3. RESULTS AND DISCUSSION

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 these 20 phenolic acids 6 (ferulic acid, caffeic acid, epigallocatechin gallate, sinapic acid, gallic acid and quercetin) were found to be present in detectible quantities in leaves of chilli. (Fig.1). Ferulic acid, a common hydroxycinnamic acid derivative, found in fresh leaves of chilli ranging from 0.0068 to 0.0152 ppm (Table 1) it is significant to note that the phenolic acids are known to offer several health benefits such as antidiabetic, antioxidant, hyper cholesterol, antimicrobial effects and protection from diet related chronic disease to its regular consumers.

Naturally occurring in plants, ferulic acid (FA) is a phytochemical that can be found in fruits and vegetables like rice bran, sweet corn, and tomatoes. Antioxidant, anti-inflammatory, antiviral, and antibacterial qualities are only a few of its various biological actions. FA has antioxidant qualities because of its phenolic hydroxyl group, which gives electrons to free radicals to squelch them. Naturally occurring in plants, ferulic acid (FA) is a phytochemical that can be found in fruits and vegetables like rice bran, sweet corn, and tomatoes. Antioxidant, anti-inflammatory, antiviral, and antibacterial qualities are only a few of its various biological actions. FA has antioxidant qualities because of its phenolic hydroxyl group, which gives electrons to free radicals to squelch them.

Caffeic acid was also present ranging from 0.1010 to 0.5050 ppm. Epigallocatechin gallate, sinapic acid, gallic acid, and quercetin were detected in ppm with range 0.0102 to 0.0163, 0.022 to 0.097, 0.1058 to 0.1820 and 0.0306 to 0.512 respectively. In addition to micro- and macro-nutrients, it is also a good source of phytochemicals mainly phenolic compounds which assist in reducing chronic diseases like diabetes, cancer and cardiovascular diseases. The main function of caffeine is to aid in the formation of lignin, which shields leaves from ultraviolet B radiation (UV-B). Additionally, by preventing the growth of bacteria, fungi, and insects, it protects against pests, predators, and diseases. The following are some possible benefits of caffeine acid for human health: Cancer prevention: Certain heterocyclic amines, which are carcinogens and mutagens, can have their synthesis inhibited by caffeine. Diabetes: Caffeic acid helps normalize blood insulin levels and lower blood glucose levels. Atherosclerosis: The effects of caffeine are anti-atherosclerotic. Potential effects of caffeine on the body include anti-inflammatory and antioxidant properties. Blanco-Rios et *al*, (2013).

Green tea contains a polyphenol called epigallocatechin gallate (EGCG), which is thought to be the cause of many of its health advantages. Research has indicated that EGCG may offer several advantages for human health, such as: Effects of EGCG as an antioxidant: It can prevent lipid peroxidation and scavenge free radicals. Effects against inflammation: EGCG has the ability to lessen inflammation and the generation of inflammatory mediators. Cancer prevention: By controlling angiogenesis, metastasis, cell proliferation, and survival, EGCG may aid in the prevention of cancer. Cardiovascular health: EGCG might make things better in this area. Weight reduction: By encouraging fat oxidation, EGCG may aid in weight loss. Brain health: EGCG may aid in defending the brain against neurodegenerative illnesses and aging. Skin health: EGCG may lessen the formation of melanin and shield the skin from UV damage. Effects against bacteria: EGCG might be antibacterial.

All phenolic compound like ferulic acid, caffeic acid, epigallocatechin gallate, sinapic acid, gallic acid and catechin were detected lower (0.0469 ppm) in ACCMS 1parent and higher (0.1421 ppm) in ACS 18-08 parent. Maximum amount of ferulic acid (0.0152 ppm) catechin (0.0512 ppm) was found in genotype 18. Caffeic acid (0.5050 ppm) and sinapic acid (0.0097 ppm) was remarkably higher in ACS 18-08 parent. In genotype 26 epigallocatechin gallate (0.0163 ppm) was noted higher amount.

Sinapic acid is a potent antioxidant that helps protect plant cells from oxidative stress by scavenging free radicals. It plays a crucial role in plant defense mechanisms against pathogens and pests. Sinapic acid can enhance the plant's ability to resist diseases and herbivore attacks. Sinapic acid is involved in the biosynthesis of lignin, a key component of plant cell walls. This contributes to the structural integrity and rigidity of the plant, providing mechanical support and protection. It is implicated in the regulation of plant growth and development. Sinapic acid influences various physiological processes such as seed germination, root growth, and flowering. The compound aids in the plant's response to abiotic stresses such as drought, salinity, and extreme temperatures, helping the plant to survive and adapt to challenging environmental conditions.

Table 1. Phenol profiling from leaves of chilli (ppm)

Genotype No.	Ferulic acid	Caffeic acid	Epigallocatechin gallate	Sinapic acid	Gallic acid	Quercetin
1	0.0096	0.1520	0.0112	0.0072	0.1660	N/A
2	N/A	0.1420	0.0107	0.0076	0.1440	0.0329
3	0.0092	0.0974	0.0128	N/A	0.1500	N/A
4	0.0089	0.2270	0.0131	0.0081	0.1120	N/A
5	0.0088	0.1220	0.0103	0.0078	0.1820	0.0326
6	0.0068	0.1010	0.0104	0.0064	0.1640	0.0321
7	0.0072	0.1710	0.0132	N/A	0.1670	0.0334
8	0.0118	0.2040	0.0116	0.0074	0.1680	0.0314
9	0.0090	0.1540	0.0121	0.0088	0.1540	0.0334
10	0.0130	0.2220	0.0116	0.0063	0.1120	N/A
11	0.0077	0.1400	0.0102	0.0022	0.1058	0.0306
12	0.0081	0.1970	0.0131	0.0077	0.1750	0.0361
13	0.0097	0.0771	0.0127	0.0064	0.1780	0.0342
14	0.0134	0.1820	0.0113	0.0088	0.1650	N/A
15	0.0077	0.2460	0.0117	0.0056	0.1480	N/A
16	0.0118	0.3090	0.0108	0.0067	0.1710	0.0308
17	0.0114	0.2830	0.0134	0.0067	0.1800	0.0357
18	0.0152	0.1690	0.0116	N/A	0.1660	0.0512
19	0.0078	0.2020	0.0122	N/A	0.1540	N/A
20	0.0121	0.2850	0.012	0.0047	0.1960	0.0325
21	0.0101	0.3150	0.0118	N/A	0.1680	N/A
22	0.0082	0.2850	0.0115	0.0057	0.1500	0.0346
23	0.0082	0.4480	0.0123	0.0031	0.1870	0.0340
24	0.0101	0.3650	0.0125	N/A	0.1570	0.0324
25	0.0084	0.2770	0.0113	0.0068	0.1710	0.0421
26	0.0092	0.3500	0.0163	N/A	0.1650	N/A
27	0.0101	0.2010	0.0111	N/A	0.1570	N/A
28	0.0093	0.3190	0.0119	0.0072	0.1540	N/A
ACCMS 1	0.0075	0.0740	0.0119	0.0083	0.1470	0.0324
ACS 18-08	0.0087	0.5050	0.0125	0.0097	0.1750	N/A

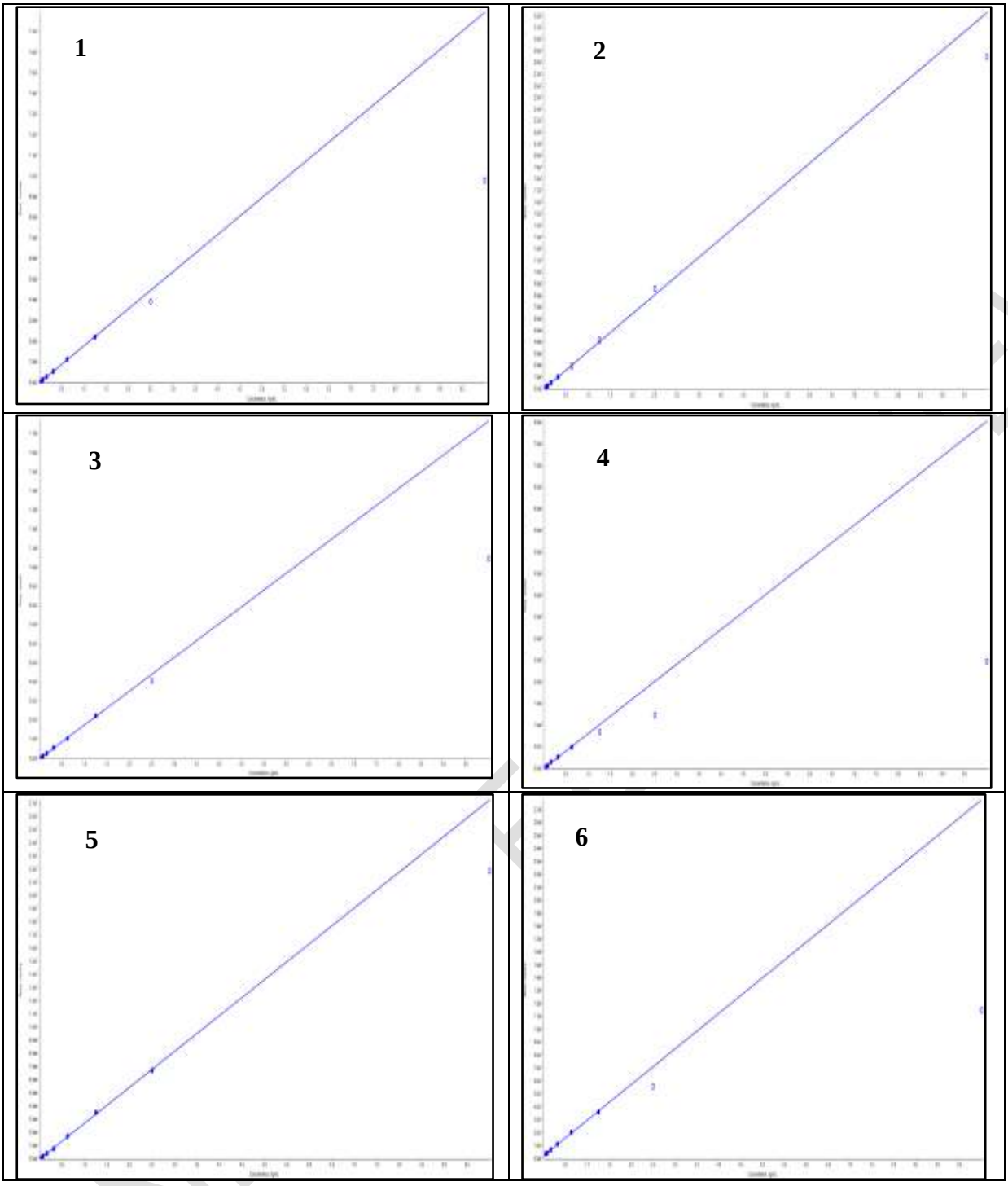


Fig. 1. Calibration curve of standard reported in phenol profiling

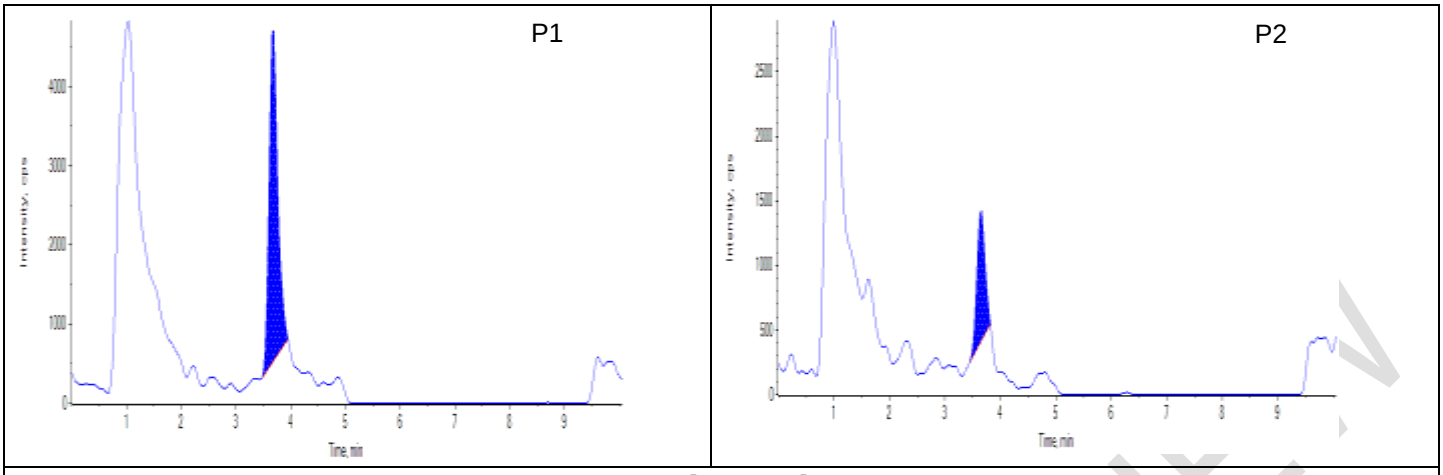
(Note: 1-Feluric acid, 2- Caffeic acid, 3- Epigallocatechin gallate, 4- Sinapic acid, 5- Gallic acid and 6- Quercetin)

Gallic acid is a powerful antioxidant that helps protect plant cells from oxidative damage caused by reactive oxygen species (ROS). This protection is vital for maintaining cellular integrity and function. It plays a crucial role in plant defense mechanisms by exhibiting antimicrobial properties. Gallic acid can inhibit the growth of various bacterial and fungal pathogens, thereby enhancing the plant's ability to resist infections. Gallic acid can act as an allelopathic compound, which means it can influence the growth and development of neighboring plants by releasing chemicals into

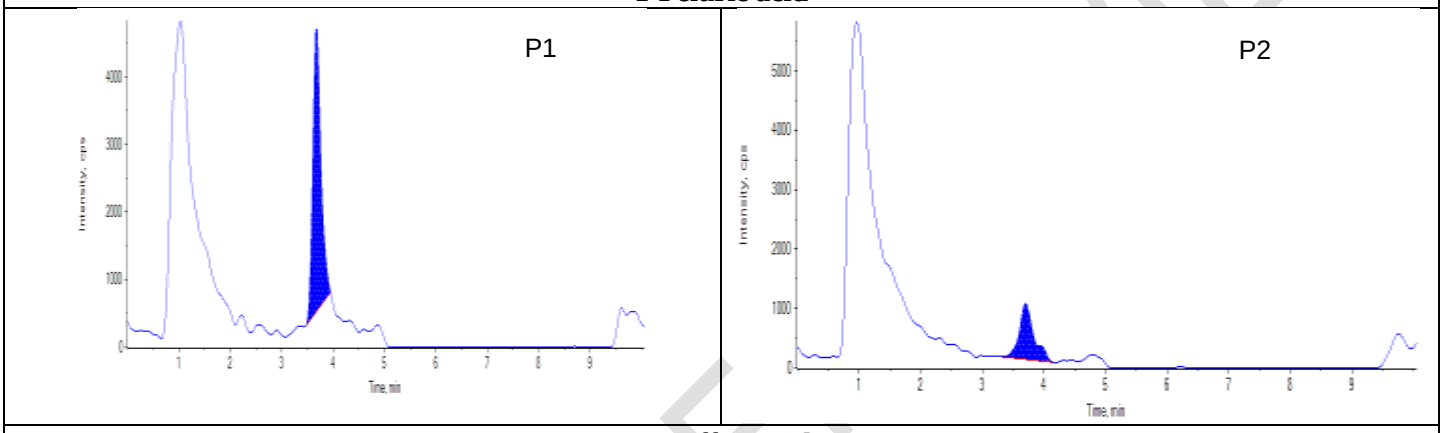
the soil. This can help reduce competition for resources such as nutrients, water, and light. It contributes to the plant's ability to tolerate abiotic stresses such as drought, salinity, and extreme temperatures. Gallic acid can modulate stress-responsive pathways, helping plants to adapt and survive under adverse environmental conditions. Gallic acid is involved in the regulation of plant growth and development. It can influence various physiological processes, including seed germination, root elongation, and leaf expansion, by modulating hormone levels and signaling pathways. As a phenolic compound, gallic acid is a key intermediate in the biosynthesis of other important phenolics, such as tannins and flavonoids. These compounds play diverse roles in plant physiology, including UV protection, pigmentation, and interaction with pollinators. Gallic acid can deter herbivores by making the plant tissue less palatable or more toxic. This chemical defense strategy helps reduce damage caused by herbivorous insects and animals. In general, almost all the traditional varieties analyzed exhibited a content of gallic acid undoubtedly superior if compared to that present in some commercial varieties. Hallmann *et al.* 2012.

Ferulic acid (0.068 ppm) and caffeic acid (0.1010 ppm) were noted lower amount in genotype 6. In genotype 11 remining four phenolic acids like epigallocatechin gallate (0.0102 ppm), sinapic acid (0.022 ppm), gallic acid (0.1058 ppm) and quercetin (0.0306 ppm) was remarkably lower amount. Ferulic acid was not detected in genotype 2. In genotypes 3, 7, 18,19, 21, 24, 26 and 27 sinapic acid is not present. Quercetin was absent in genotypes1, 3, 4, 10, 14, 15, 21, 26, 27 and 28. ACCMS 1 ferulic acid, caffeic acid, epigallocatechin gallate, sinapic acid and gallic acid (0.0075 ppm, 0.0740 ppm, 0.0119 ppm, 0.0083 ppm and 0.1470, respectively) ppm were found lower as compared to parent ACS 18-08. (Fig. 2).

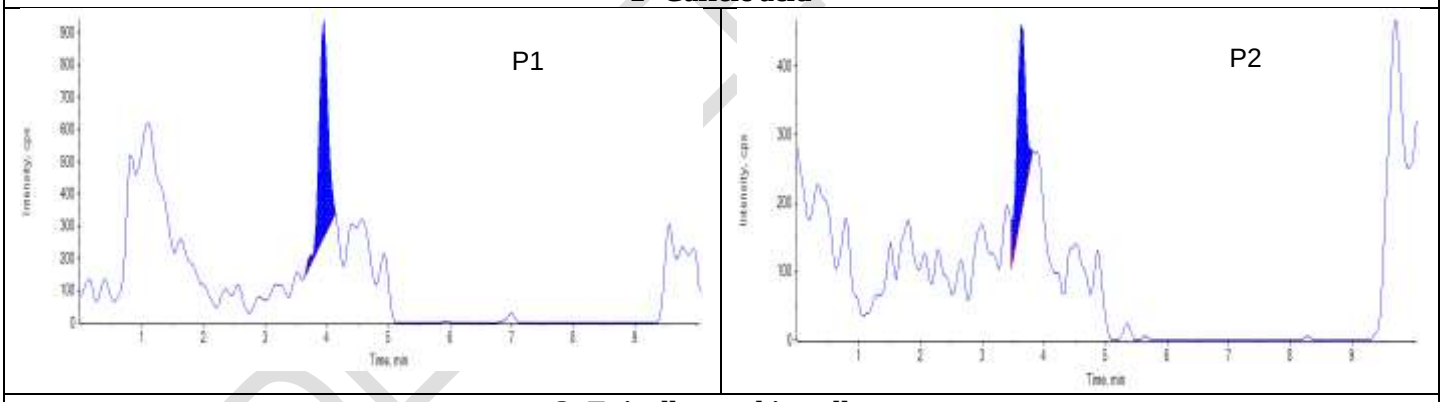
Quercetin is a potent antioxidant that protects plant cells from oxidative damage by scavenging reactive oxygen species (ROS). This helps in maintaining cellular integrity and overall plant health. Quercetin can absorb UV radiation, protecting plant tissues from harmful UV-B rays. This is especially important for plants exposed to high levels of sunlight, as it prevents DNA damage and other detrimental effects caused by UV exposure. Quercetin exhibits antimicrobial properties that help plants defend against various pathogens, including bacteria, fungi, and viruses. By inhibiting the growth and spread of these pathogens, quercetin enhances the plant's immune response. Quercetin contributes to plant resilience against abiotic stresses such as drought, salinity, and extreme temperatures. It helps modulate stress-responsive pathways, enabling plants to adapt to and survive under challenging environmental conditions. Similar to other phenolic compounds, quercetin can act as an allelopathic agent. It influences the growth and development of neighbouring plants by releasing chemicals into the soil, thereby reducing competition for resources like nutrients, water, and light. Blanco-Rios et *al*, (2013). Quercetin plays a role in regulating various plant growth processes, including seed germination, root development, and leaf expansion. It can influence hormone levels and signaling pathways that are critical for plant growth and development. Quercetin contributes to the coloration of flowers and fruits, which is crucial for attracting pollinators and aiding in seed dispersal. The pigmentation can also serve as a visual cue to deter herbivores and attract beneficial insects.



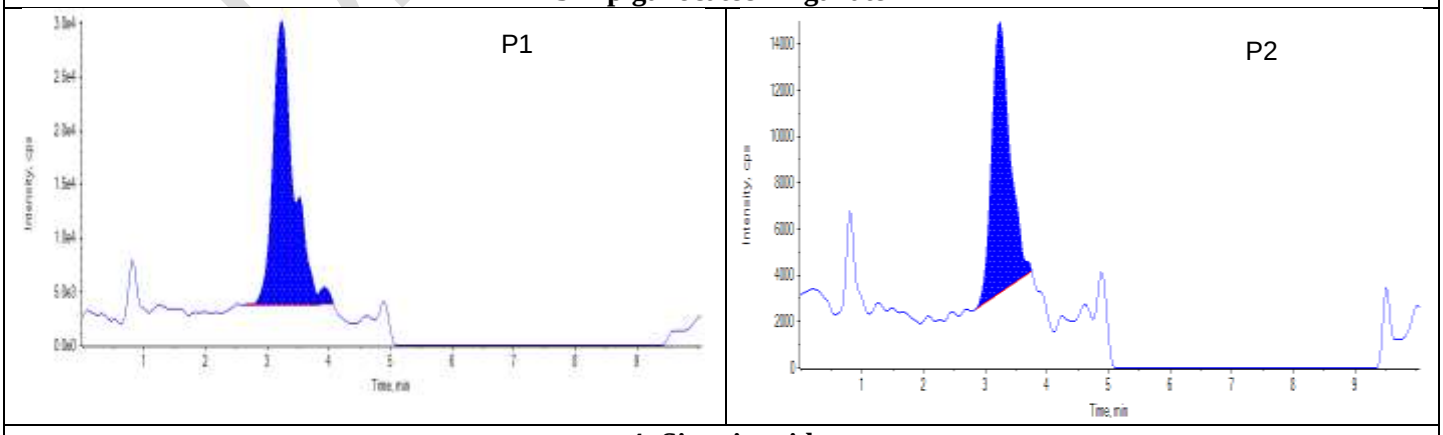
1-Feluric acid



2- Caffeic acid



3- Epigallocatechin gallate



4- Sinapic acid

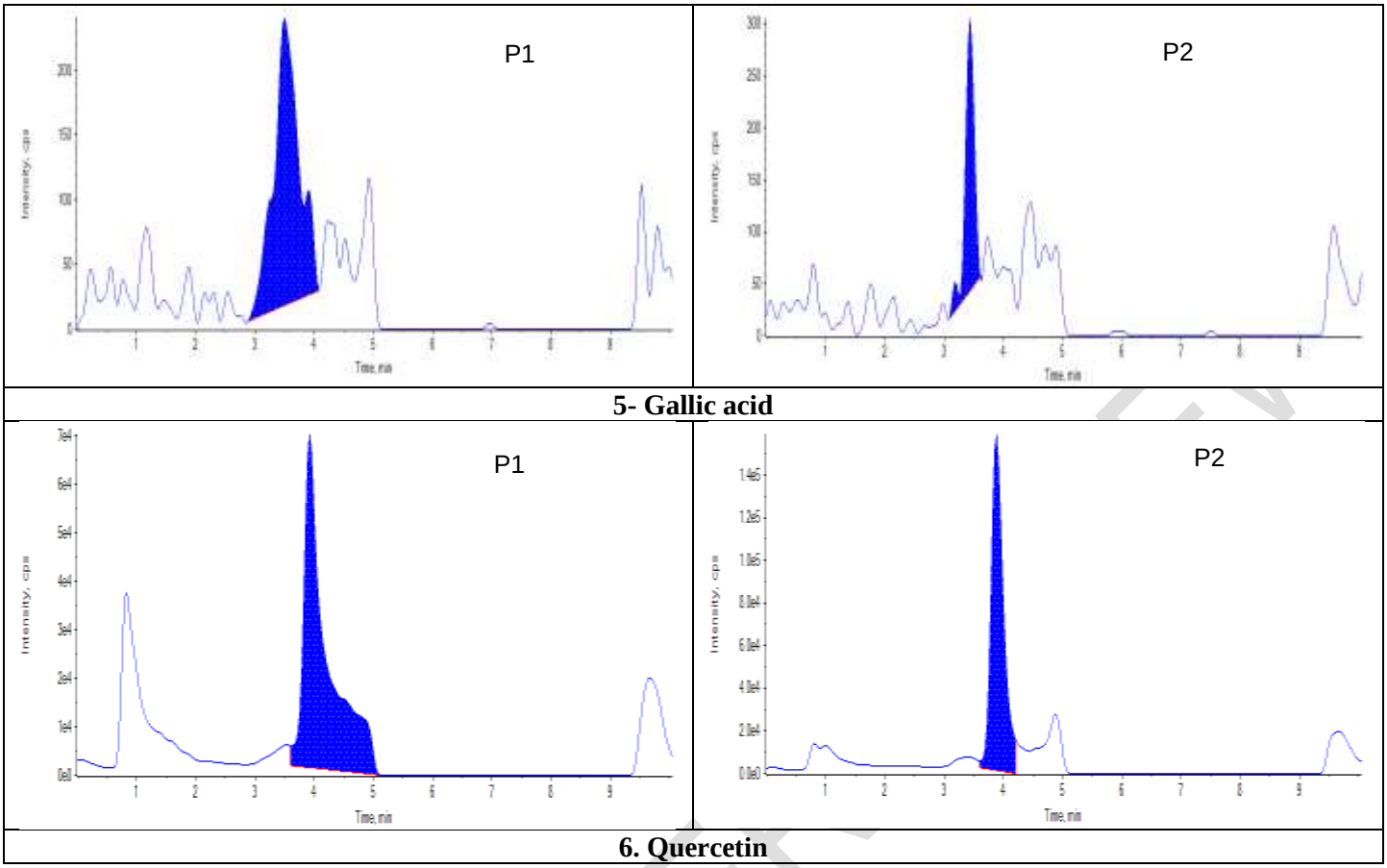


Fig. 2. LC-MS chromatogram of phenolic acid in P1(ACCMS 1) and P2(ACS 18-08) parents of chilli

Phenolic acids like ferulic acid (0.40-5.20 ppm), caffeic acid (0.10-1.20 ppm), epigallocatechin gallate (0.02-0.45 ppm), sinapic acid (0.09-1.90 ppm), gallic acid (0.18-1.10 ppm) and quercetin (0.07-1.30 ppm) reported by Rodrigues *et al*. (2022) in chilli.

4. CONCLUSION

The result indicated that in parent ACCMS 1 ferulic acid, caffeic acid, epigallocatechin gallate, sinapic acid and gallic acid (0.0075 ppm, 0.0740 ppm, 0.0119 ppm, 0.0083 ppm and 0.1470, respectively) ppm were found lower as compared to parent ACS 18-08. Only one phenolic acid quercetin was only present in ACCMS 1 and not detected in ACS 18-08.

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