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Detection of Ferulic Acid in Maize Plant under Drought Stress Using an RP-HPLC Method

Piyush Kant Rai and Kamlesh Choure*

Dept. of Biotechnology, Amicable Knowledge Solution University, Satna, Madhya Pradesh (485 001), India

Corresponding Author

Kamlesh Choure
e-mail: kamlesh.chaure@gmail.com

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Abstract

The experiment was conducted from April to May 2024 at the Centre for Excellence in Biotechnology Research and Training (CEBRT) lab of Amicable Knowledge Solution University (AKS) University, Satna. In the present study, a simple, routine, and reproducible RP-HPLC method was employed for estimating the levels of Ferulic acid (FA) in different treatments of maize that is essential in the phytochemical analysis of this plant, has been assessed based on ferulic acid (FA). The method used in this study was based on HPLC on a C18 column using a gradient of water, methanol, and acetic acid. The calibration curve showed optimum linearity from 10 to 100 µg ml^{-1}, with R^2=0.981. This method is used to quantify the concentration of FA. This methodology was used to detect FA in maize plants with different treatments: T_1: control plants, T_2: drought-induced plants, T_3: plants inoculated with PKR1, T_4: plants inoculated with PKR5, T_5: plants inoculated with PKR6 An increase in FA was observed in all three Plant Growth-Promoting Rhizobacteria (PGPR)-treated plants (T_3, T_4, and T_5) compared to T_1 (control plants) and T_2 (drought-induced plants). Among these, maximum FA was found in T_4 treatment, and the maximum peak height and area of T_4 were 229259 and 10884, respectively. The validated RP-HPLC method provided a reliable approach for analyzing FA content in maize leaves, supporting phytochemical studies and quality assurance across various plant treatments and derived products.

Keywords: Abiotic, ferulic acid, HPLC, maize, PGPR, phenolic, stress

1. Introduction

Ferulic acid is a hydroxycinnamic acid predominantly located in the plant cell walls, specifically in cereals such as maize (*Zea mays*). Ferulic acid (FA) is one of the major phenolic compounds found in maize cell walls, especially in the pericarp, and the content differs among genotypes (Chateigner-Boutin and Saulnier, 2022); (Razgonova et al., 2022). FA is involved in the resistance of maize to *Gibberella moniliformis* and *F. verticillioides*, which causes ear rot and produces toxic mycotoxins (Xu et al., 2023); (Mesterhazy, 2023). Higher FA content in maize kernels increases with the level of resistance to fungal infection and a reduction in mycotoxin accumulation. Some abiotic stresses mitigated by ferulic acid through regulating antioxidant enzymes are salt, drought, and cold. Additionally, the antioxidant activity of FA contributes to its antifungal properties (Mielniczuk and Skwaryło-Bednarz, 2020); (Chrpová et al., 2021). Genetic studies have shown that the FA concentration in maize grains is highly heritable, with mostly additive genetic variation. Notably, selecting higher FA content does not reduce grain yield and weight. Therefore, the enhancement of FA content in maize varieties could be achieved for both agriculture's nutritional and economic benefits. It is also involved in the physical strength of the structural integrity and the mechanisms of plant defense systems (Lu et al., 2022). A lack of desirable molecular markers has hampered improvements in the physical attributes of the plants that can be resolved by using the identification of quantitative trait loci (QTL) (Katral et al., 2024). Determining ferulic acid in maize leaves is essential for understanding maize physiology, disease resistance quality, and potential adaptation to abiotic stress due to its properties of regulating antioxidant properties (Lephatsi et al., 2022); (González-Rodríguez and García-Lara, 2024). Ferulic acid contributes to the rigidity and robustness of plant cell walls by cross-linking polysaccharides and lignin, which enhances plant resistance to pathogens and environmental stresses i.e. drought, flood, light, salt, and heavy metals alter biological diversity and crop production worldwide (Mnich et al., 2020). Therefore, it is important to know how plants cope with stress conditions. Polyphenols, which are the largest group of plant-specialized metabolites, are generally recognized as molecules involved in stress protection in plants. This diverse group of metabolites

contains various structures, from simple forms consisting of one aromatic ring to more complex ones consisting of large number of polymerized molecules. Consequently, depending on their structure, all these molecules may show different roles in plant growth, development, and stress protection. Numerous previous studies have shown how different polyphenol structures affect their biological activity and their roles in responding to abiotic stress. Therefore, understanding how plants deal with such stress conditions is crucial. Polyphenols, the largest group of plant-specialized metabolites, are generally recognized as molecules involved in stress protection in plants (Samec et al., 2020). This diverse group of metabolites contains various structures, from simple forms consisting of one aromatic ring to more complex ones consisting of a large number of polymerized molecules (Li et al., 2021); (Stompor-Gorący and Machaczka, 2021). Oxidative stress can affect plant physiology, such as metabolism and nutrition, leading to reduced seed germination and altering the correct function of organs and systems. ROS accumulates in the plant body through endogenous and exogenous mechanisms. Increasingly, studies point to ROS's involvement in the physiopathology of various chronic diseases requiring prolonged pharmacological treatment periods. Long-term treatments may contribute to changes in systemic ROS (García-Sánchez et al., 2020).

The involvement of reactive oxygen species (ROS) in plant pathological mechanisms, their defense responses' antioxidant effects, and potential protective strategies. Plant diseases such as bacterial blight, fungal infections, and viral infections contribute to increased oxidative stress and crop damage. Plant defense mechanisms, including systemic acquired resistance (SAR) and induced systemic resistance (ISR), help reduce disease progression through enhanced antioxidant production. The plant immune response to pathogens has a dual role - initially stimulating ROS production as a defense signal and antimicrobial agent, while simultaneously activating antioxidant systems to protect plant cells from oxidative damage. Secondary metabolites, such as flavonoids and phenolic compounds, serve as natural antioxidants that help decrease systemic ROS induced by pathogen infection (Samec et al., 2020). Ferulic acid determination in maize leaves can be achieved by various electrochemical methods using modified electrodes (Bounegru and Apetrei, 2022). Previous Studies demonstrated the successful detection of ferulic acid in food samples, including sweet corn, using techniques such as square wave voltammetry and cyclic voltammetry with modified electrodes, such as carbon mesoporous fabricated glassy carbon electrodes (Forzato et al., 2020); (Buffon and Stradiotto, 2021). A novel electrochemical strategy using a carbon paste electrode modified with a NiO-embedded single-wall carbon nanotube nanocomposite has been proposed for determining ferulic acid in maize samples (Tolun and Altintas, 2023); (Jadon et al., 2024). Furthermore, the GMC of maize hybrids contains variable amounts of phenolic compounds, such as ferulic acid, highlighting the importance of these compounds in the adaptability of maize to different environmental conditions (Feregrino-Perez et al., 2024).

There are various applications, and the quantification of FA in maize leaves has wide applications in biotechnology (Wang et al., 2023). The ferulic acid content in maize leaves can be helpful in agricultural applications to increase maize resistance causing disturbances in physiological, biochemical, and metabolic processes (Linić et al., 2021). The exogenous application of natural metabolites is a useful strategy to reduce the adverse effects of stress on crops. Techniques such as genetic engineering can be used to modify organisms' ferulic acid biosynthesis to enhance crop traits (Alharby and Fahad, 2020). Determination of the ferulic acid content in maize contributes to the development of dietary, functional foods, and nutraceuticals aimed at improving human health (Santos-Buelga et al., 2019); (Deepak and Jayadeep, 2022). This study focused on the presence of ferulic acid in different maize plant treatments to assess its role in mitigating drought stress.

2. Materials and Methods

2.1. Extraction and analysis of FA

FA content in maize was measured in leaves, which required multiple stages, including extraction, isolation, and quantification (Erenstein et al., 2022). Centre of excellence in biotechnology research and training (CEBRT) lab of AKS University in April–May 2024 (L24.5683° N, N 80.7931° E). Fresh leaves were collected, washed, and dried to eliminate any moisture. Subsequently, the dried leaves were pulverized into a fine powder to aid in the extraction process (Bento-Silva et al., 2018).

Ferulic acid was commonly obtained through extraction using solvents, such as methanol, ethanol, or a combination of water and alcohol. Before extraction, the samples were dried at 40°C for 12 h, pulverized, and collected for the study. A 2 g sample was mixed with 60 ml of 2 M NaOH, saponified for 24 h, and treated with sodium hydrogen sulfite to prevent deterioration. The resulting hydrolysate was centrifuged, and the supernatant was acidified and extracted using 60 ml of ethyl acetate, which was repeated thrice. Subsequently, the organic fractions were condensed to 2–3 ml in a rotating vacuum evaporator. Quantitative and qualitative analyses were conducted after mixing the concentrate extract with 2 ml of acetonitrile and water in a 1:1 ratio (Mahato et al., 2019). Incorporated three distinct PGPR strains (PKR1, PKR5, PKR6) enabled the assessment of their individual effects on maize plants, potentially under both normal and drought stress conditions. The PGPR-inoculated plants (T_3, T_4, T_5) exhibited higher ferulic acid content than the control (T_1), which indicated that these PGPRs can individually enhance the plant's secondary metabolite production (Table 1). These findings were supported by the previous findings where research reported that there was an increase in the phenolic

acid in the plant under stress condition (Sharma et al., 2019). However, our study uniquely explored how different PGPR strains vary this response.

Table 1: Table representing different treatments in maize and PGPR

Sl. No.	Treatment provided	Treatment symbol
I.	control plants	T_1
II.	drought-induced plants	T_2
III.	plants inoculated with PKR1	T_3
IV.	plants inoculated with PKR5	T_4
V.	plants inoculated with PKR6	T_5

2.2. Quantification by HPLC

The quantification was done by isolating phenolic acids and aldehydes at room temperature on a C18 HPLC column. The samples were separated on a gradient composed of solvent A, water/methanol/acetic acid 80:19:1, and solvent B, methanol/water/acetic acid 80:19:1, for 35 min with some modifications, as indicated in Table 2.

Table 2: Time Program for FA estimation using the HPLC program

Sl. No.	Time	Module	Command	Value
I.	0.01	Controller	start	
II.	2.00	Pumps	Pump B conc	40
III.	5.00	Pumps	Pump B conc	60
IV.	13.00	Pumps	Pump B conc	100
V.	15.00	Pumps	Pump B conc	20
VI.	16.00	Pumps	Pump B conc	40
VII.	20.00	Controller	Stop	

The flow rate used for separation was 0.8 ml min^{-1}. Using an ultraviolet monitor, quantitative data were obtained from chromatograms captured at 320 nm. Calibration curves were generated using suitable combinations of phenolic acids and aldehydes. The method was validated for linearity, specificity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision according to the criteria established by the International Council for Harmonization (ICH) (Tungmunnithum et al., 2022).

2.3. Evaluation of linearity, specificity, LOD, and LOQ

The linearity of FA in the standard solutions (n=3) was examined using optimum chromatographic conditions. The concentration range of the standard solutions was 10–100 µg ml^{-1}. The linearity range of standard solutions of FA was evaluated (n=3) using optimal chromatographic conditions, covering a concentration range of 10–100 µg ml^{-1}. The plots were made based on the primary peak area on the y-axis against the concentration on the x-axis to generate the calibration curve. Further linear regression analysis was employed for the linearity (Bueno-Herrera and Perez-Magarino, 2020).

The retention times (rt) of various standards were compared and assessed sample specificity, authenticated substance identification and purity, and reduced errors in the results. The LOD and LOQ were calculated according to ICH recommended use for the standard deviation of the response and slope of the linear equation.

The used equation is: LOD=3.3 s/S and LOQ=10 s/S (Bueno-Herrera and Perez-Magarino, 2020).

2.4. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 8.0. The data were shown with standard error means to differentiate between the control and treated plants for analytical purposes.

3. Results and Discussion

In this study, the plants were inoculated with PGPR, namely PKR1, PKR5, and PKR6, and reported the presence of FA using HPLC with an Rt of 6.2 at 320nm, as shown in Figures 1 to 7. Figure 1 confirmed the presence of FA in the control sample (non-inoculated), from which it was observed that the concentration of ferulic acid increased upon inoculation with PGPR, as shown in Tables 1 and 2.

This method was tested to quantify the concentration, of FA with different treatments, viz. T_1: control plants, T_2: drought induced plants, T_3: plants inoculated with PKR1, T_4: plants inoculated with PKR5, T_5: plants inoculated with PKR6. Overall maximum increase in FA was observed in all three PGPR-treated plants (T_3, T_4, and T_5) as compared to T_1 (control plant) and T_2 (drought-induced plants). Among these, maximum FA was found in the T_4 treatment, which showed maximum peak height and area of T_4 with 229259 and 10884, respectively, as presented in Table 3. It was also supported by the previous findings that developed the concept of the strain specificity of the PGPR effects on the physiology of plants (Ansari and Ahmad, 2018) plant growth promoting rhizobacteria (PGPR. However, it brought new insights into their impacts on accumulated phenolic compounds. The most notable aspect of our study was that, in addition to mitigating drought-induced FA reduction, PGPR inoculation could be more in

Table 3: Table representing different treatments in maize and PGPR

Sr. No.	Treatment symbol	Peak area	Peak height
I.	T_1	109212	5179
II.	T_2	2994	408
III.	T_3	229259	10884
IV.	T_4	235107	13479
V.	T_5	89511	6735

comparison with control values. Our finding thus suggested that PGPR could promote plant stress tolerance due to actively enhancing and not simply ameliorating stress symptoms through beneficial metabolic changes. This observation added a new dimension to the previous findings of PGPR-mediated stress tolerance mechanisms, focusing on improved water uptake or hormone modulation (Vurukonda et al., 2016).

Calibration curves were created systematically by taking a range of standard FA sample concentrations, from 10 to 100 µg ml^{-1} (Chen and Chen, 2022) higher-order polynomial, exponential rise to maximum and power equations. A constant variance test was performed to assess the suitability of calibration equations for this dataset. The data sets are checked for suspected outliers to verify their presence. The standard error of the estimate errors, s, was used as criteria to determine the fitting performance. The Prediction Sum of Squares (PRESS. The dilutions were prepared precisely and then added to the system to ensure a high signal-to-noise ratio (between 3 and 10) while running the method on an HPLC machine. The developed method provided more accuracy for detecting FA, with relative standard deviation (RSD) values within the range of 0.24–1.43. LOQs were within the 0.05–0.75 µg ml^{-1} range for the studied analytes. The linear calibration curves for the mentioned analytes with a specified range were expressed by R^2=0.981. The possible matrix effects on analyte quantification were also examined. While quantification was performed, no matrix effect was observed in the quantification of the FA molecule. The RP-HPLC method was validated to analyze the amount of ferulic acid in maize leaves to ensure that FA could be measured. The results demonstrated the reliability and applicability of the technique to compositional studies on maize plants, which were relevant in research or product development studies (Raposo and Barceló, 2021). Specifically, FA concentration upon PGPR inoculation was significantly higher than in control and drought-induced plants at inoculation. The result followed previous studies that demonstrated a role for PGPR in modulating plant secondary metabolism (Goswami et al., 2016); (Etesami and Beattie, 2018); (Wang et al., 2022). Nevertheless, under these conditions, our report was among very few linking PGPR inoculation to the increase in the FA content of maize leaves.

3.1. Development of the method

In this study, we utilized formic acid (FA) to enhance this method. Separation was performed on a reverse-phase chromatography C-18, 250, 4.6 mm column with acetonitrile and water. Acetonitrile was set at 80%, while in water, 0.1% formic acid was used to maintain a pH above 4 and achieve optimal stability, while pH 7 was set for reducing tailing. This analytical method was developed to detect the presence of FA in a solution for an extended period at an optimal pH, with a detection wavelength of 320 nm. This was based on the absorption maximum of FA and had been consistent with previous studies on the analysis of phenolic acid at 320 nm, which ensured better sensitivity and specificity (Mattila anf Kumpulainen, 2002). This novel strategy eliminated the reliance on ion-pairing reagents (Oiram Filho et al., 2018). This new approach avoided hazards, and tailing peaks were less desirable for separating substances in chromatography without ion-pairing agents. Changing the pH and strength of the buffer made the HPLC method easy to develop without using an ion-pairing reagent. The method was validated and exhibited a favorable regression value of 0.975, with a serum calibration curve range of 10–100 µg ml, similar to Bhattacharyya and Sogali, 2018. The method showed adequate accuracy, with RSD values of approximately 0. 4 to 1.43, which were at par with the acceptable limits for analytical methods. The LOQ of FA was identified to be 0 µg ml^{-1}, while in the case of LOD, it was observed at 10 µg ml^{-1} for FA, proving that the method was sensitive. The recovery rates fluctuated but remained above 98%. 88% to 101.24% enabled accurate quantification of FA in Maize leaf samples. This achieved high sensitivity and was very useful for detecting subtle changes in FA content that could occur under various stress conditions or by PGPR treatments.

3.2. Linear relationship

A calibration curve demonstrated linearity across the 10–100 µg ml^{-1} concentration range was observed. The peak area data were subjected to linear regression analysis concerning concentration. The linear equations for SA, GA, FA, and FA were Y=10911x-16054, respectively. The coefficient of determination (R^2) for FA was determined to be 0.97 (Chen and Chen, 2022) higher-order polynomial, exponential rise to maximum and power equations. A constant variance test was performed to assess the suitability of calibration equations for this dataset. Suspected outliers in the data sets are verified. The standard error of the estimate errors, s, was used as criteria to determine the fitting performance. The Prediction Sum of Squares (PRESS. The specificity test indicated that the well-defined curve supported the calibration of ferulic acid at a wavelength of 320 nm, as presented in Figure 1 and 2. Another important innovation of our methodology was optimizing the mobile phase composition by pH. Keeping the pH greater than 4 and optimal stability at pH 7 avoided the problem that peak trailing in FA analysis usually brought about. The current approach used in this study was similar to that of Oiram Filho et al., 2018, in particular, highly improved the shape and resolution of the peaks.

Maize was an essential crop in food agriculture and had a significant influence on numerous global commercial items (Ranum et al., 2014). An accurate assessment of phytochemicals in crops was crucial to ensure the reliability and replicability of studies. Ferulic acid (FA), an essential indicator, was recognized as pivotal in assessing the excellence of plants in their defense system in response to abiotic stress in its plant defense system (Srinivasan et al., 2007); (Linic et al., 2021)causing disturbances in physiological, biochemical, and metabolic processes. The exogenous application of natural metabolites is a useful strategy to reduce the adverse

effects of stress on crops. We investigated the effect of foliar application of salicylic acid (SA. In the present study, the inoculated plants with three different PGPRs, PKR1, PKR5, and PKR6, reported the presence of FA using HPLC, as presented

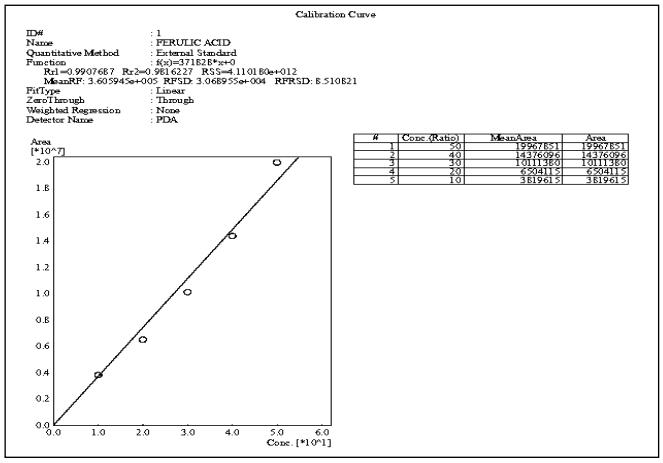

Figure 1: The standard FA was used to compare the FA present in maize plants

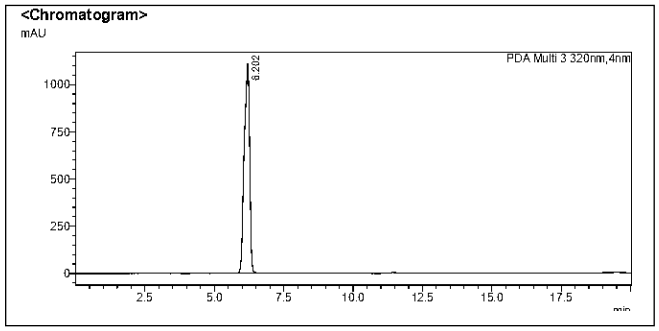

Figure 2: The peak with a retention time (Rt) of 6.2 for ferulic acid at 320 nm was presented in the control sample of maize plants

in Figures 3–7. The following were some of the reasons likely to be responsible for this increase in FA concentration, as evidenced by the PGPR-treated plants. PGPR was known to increase nutrient assimilation and plant development (Rafique et al., 2024) biofilm-based root colonization by these microorganisms has emerged as a promising strategy for agricultural enhancement. The current work aims to characterize biofilm-forming rhizobacteria for wheat growth and yield enhancement. For this, native rhizobacteria were isolated from the wheat rhizosphere and ten isolates were characterized for plant growth promoting traits and biofilm production under axenic conditions. Among these ten isolates, five were identified as potential biofilm-producing PGPR based on in vitro assays for plant growth-promoting traits. These were further evaluated under controlled and field conditions for their impact on wheat growth and yield attributes. Surface-enhanced Raman spectroscopy analysis further indicated that the biochemical composition of the biofilm produced by the selected bacterial strains includes proteins, carbohydrates, lipids, amino acids, and nucleic acids (DNA/RNA, which could increase the synthesis of secondary products, such as FA. Second, PGPR was reported to elicit systemic resistance in plants (Zhu et al., 2022) which might have activated the phenylpropanoid route, synthesizing FA in plant defense reactions.

The calibration curve was generated by systematically injecting a range of sample concentrations from 10 to 100 µg ml^{-1}. Different dilution standards were added to the system during the experimental method to ensure a high signal-to-noise ratio (between 3 and 10). The method showed an accurate estimation for FA, in which the RSD ranged from 0.24 to 1.43. The LOQs ranged from 0.05 to 0.75 µg ml^{-1}, and the compounds demonstrated high linearity (R^2=0.981) across a wide concentration range. An assessment found no significant impact of the matrix on the quantification of the FA molecules. The study aimed to validate an RP-HPLC method for analyzing

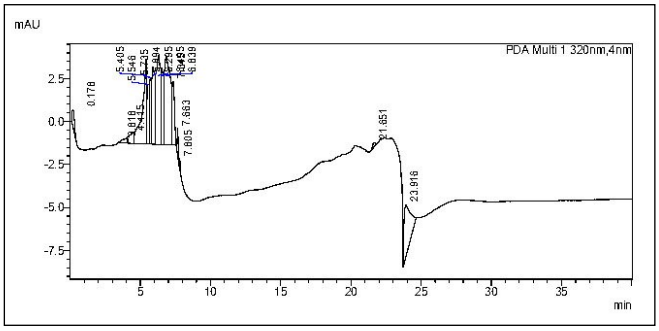

Figure 3: The peak with a retention time (Rt) of 6.2 for ferulic acid at 320 nm was presented in the control sample of maize plants

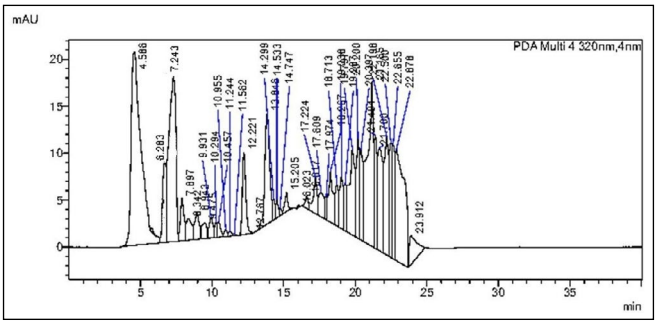

Figure 4: The peak with a retention time (Rt) of 6.2 for ferulic acid at 320 nm was presented in drought-induced plants

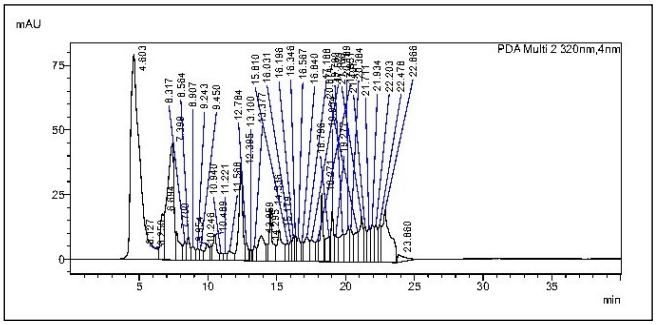

Figure 5: The peak with a retention time (Rt) of 6.2 for ferulic acid at 320 nm was presented in PKR1-inoculated plants

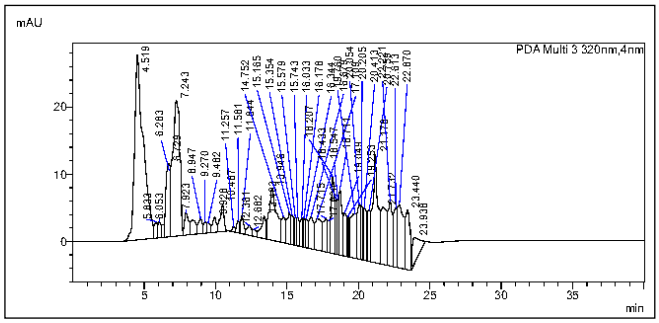

Figure 6: The peak with a retention time (Rt) of 6.2 for ferulic acid at 320 nm was presented in PKR5-inoculated plants

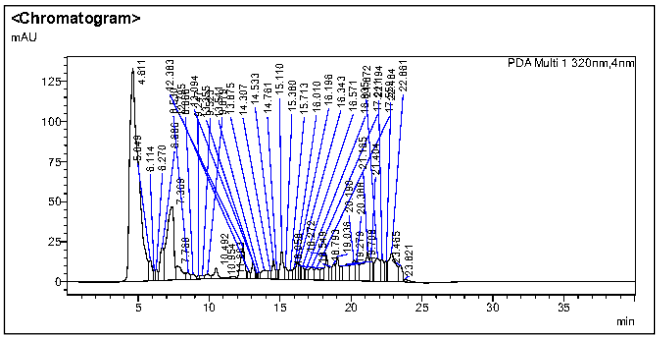

Figure 7: The peak with a retention time (Rt) of 6.2 for ferulic acid at 320 nm was presented in PKR6-inoculated plants

the amount of FA in the leaves of maize plants. The results confirmed this technique's reliability and proved its usefulness for estimating phytochemical constituents in maize plants.

3.3. Accuracy

The approach exhibited significant consistency, with a relative standard deviation (RSD) of less than 2%. For instance, Song et al., 2023 They reported RSDs ranging from 1.5% to 4.2% in their analysis of ferulic acid in plant samples, while our method consistently was achieved with lower variability.

3.4. Precision and resilience

Sample chromatograms at concentrations of 20%, 40%, 60%, 80%, and 100% were presented in Figures 3–7. The accuracy findings for the FA were presented in Table 2. The approach achieved nearly 100% recoveries for FA, ranging from 98.88 to 101.24%. The resilience of the method was validated by purposely changing the composition of the mobile phase, flow rate, and pH. Slight modifications made to the method parameters did not affect its performance. All data obtained were consistent with the original conditions. The percentage of relative standard deviation (% RSD) obtained for the assay under the altered conditions was below 2%, indicating the robustness of the proposed method. This indicated that the sample remained unchanged under the specified mobile phase conditions but only for the duration of this specific period, as presented in Figure 4.

3.5. Limit of detection (LOD) and limit of quantification (LOQ)

The study lower FA LOD and LOQ were 10 µg ml^{-1} and 0.2 µg ml^{-1}. The RP-HPLC method was very sensitive; the spiked concentrations found were 2 µg ml^{-1} for both compounds. These values were within the range or even higher than those in previous literature. For instance, a study that employed a paper-based platform found an LOD of 7 ppm and LOQ of 20 ppm for FA, which was higher than the detected LOD and LOQ in this study (Tee-ngam et al., 2013). A similar study utilizing HPLC-DAD also found the limit of detection (LOD) and limit of quantification (LOQ) for FA to be 56 ng $spot^{-1}$ and 164 ng $spot^{-1}$, indicating satisfactory sensitivity despite the differences in the measurements (Kareparamban, 2013). Also, the assessment of the method was used to determine free and total available FA in various types of samples described the LOD and LOQ of the method as 4. 5 µg g^{-1} and 13 µg g^{-1}. Unrestricted FA was 8 µg g^{-1}, and total FA was 5 µg g^{-1}, 8 µg g^{-1}, and 18 µg g^{-1}. Also, compliance with the maximum permissible limits for the total available FA and VFA was achieved at 5 µg g^{-1} for the former and 5 µg g^{-1} for the latter. These comparisons illustrated that the current method's LOD and LOQ were situated in the range of previously published values. However, it had certain advantages in specific situations. The limit of detection of FA was 10 µg ml^{-1}. At the same time, the sample was quantified at 0.2 µg ml^{-1}, which was sufficient stability and efficiency of the method in various analytical practices, thereby increasing confidence in detecting the concentration of FA and its related compounds. Such high sensitivity was highly beneficial because it allowed for accurate detection of FA, which often occurred in relatively low concentrations in the samples, and accurate analyte quantification, which might have been necessary for some experimental work. Due to the low LOD and LOQ, this method could be applied in any practice that required the determination of minute quantities of phytochemicals, quality control in pharmaceuticals and food products, and environmental analysis.

3.6. Implications and future directions

Thus, the RP-HPLC method described in this work could be used as a reliable tool for determining FA content in the maize leaves with perspectives to investigate plant responses to stress factors and the influence of beneficial microorganisms on plant metabolism. In this regard, the developed method was accurate, precise, and sensitive. Thus, it can be applied in further research or the commercial aspect to determine the quality of the crop. The following studies could have investigated the modified method for more phenolic compounds or its application to UHPLC for shorter analysis times and lower LODs.

4. Conclusion

This newly developed, inexpensive HPLC method could separate with only a simple mobile phase and no ion-pairing chemicals within 30 minutes. It further validated its potential for detecting material changes and met the recommendations under ICH. Implementing this method on maize plants detected ferulic acid (FA) levels, which regulated cellular

metabolism. The different plant treatments (T_1, T_2, T_3, T_4, T_5) showed varying levels of FA, indicating that *Pseudomonas* sp. (PKR1) could be used to combat drought stress in plants.

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