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Research Article

Estimation of ferulic acid from selected plant materials by Spectrophotometry and High performance liquid chromatography

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Abstract
Ferulic acid (FA) is an abundant phytophenolic compound present in plant cell wall. Ferulic acid possesses anticancer, antioxidant, and anti-aging properties. A simple, sensitive and reproducible spectrophotometric method has been exploited for quantitative estimation of ferulic acid from selected plant materials such as rice bran (*Oryza sativa*), wheat bran (*Triticum aestivum*) and bamboo shoot (*Bambusa vulgaris*). The blue coloured chromogen obtained after the Folin–Ciocalteu assay was measured at a wavelength of 718 nm for ferulic acid against the blank reagent. The chromogen obeyed linearity over the range of 1µg/ml - 8µg/ml. High-Performance Liquid Chromatography (HPLC) method was also developed for the estimation of ferulic acid from selected plant materials. In HPLC analysis, ferulic acid got eluted and the amount of FA was found to be higher in rice bran (14.03mg/kg) compared to bamboo shoot (1.92mg/kg) and wheat bran (11.03mg/kg). The method can be used for the routine analysis of ferulic acid from various plant species and can be applied for nutritional and clinical investigations in a variety of samples.

Keywords: Bamboo shoot, Ferulic acid, Rice bran, Spectrophotometry, Wheat bran

INTRODUCTION

Antioxidants are compounds needed by most organisms, where they prevent oxidative damage caused by free radicals. The formation of free radicals causes the development of various diseases like cancer, cardiovascular diseases and cataracts in humans (Zhang *et al*., 2007). The antioxidant activities of plants have been mainly due to their phenolic content, which is one class of natural antioxidants (Castaneda *et al*., 2009). Thus, plants containing a high-level of phenolic acids considered a source of potent natural antioxidants (Ranusova *et al*., 2021). Ferulic acid (FA), together with dihydroferulic acid, could be a component of lignocelluloses, confers plasma membrane rigidity by cross linking lignin and polysaccharides. It is commonly found in seeds of plants like rice, wheat and oats (Buranov and Mazza, 2009). Ferulic acid will be easily absorbed by the body and stays within the blood longer than the other antioxidant, even longer than vitamin C. Thus FA can be considered as an important antioxidant and commonly used in nutrition purposes and food supplements (Silva and Batista, 2017).

Ferulic acid is found in many vegetable sources and occurs in particularly high concentration in popcorn and bamboo shoots. As an antioxidant, FA plays a major role in the body's defence against carcinogenesis by inhibiting the formation of N-nitroso compounds (Kuenzig *et al*., 1984; Lee *et al*., 2009; Aarabi *et al*., 2016). Ferulic acid possesses anticancer, antioxidant, and anti-aging potentials and can decrease blood glucose levels. Like other phenolic compounds, FA showed radioprotective abilities and reduced ionizing radiation-induced damages to DNA and membranes in biological systems (Roginsky & Lissi, 2005; Divakaran *et al*., 2013; Kumar and Goel, 2019).

Therefore, we exploited a simple, repeatable, sensitive and cost-effective VIS Spectrophotometric method for the quantification and determination of FA from various plant materials. Folin–Ciocalteu assay is used for the quantification of phenolic acid in the presence of alkali (15 % sodium carbonate). High-Performance Liquid

Chromatography (HPLC) method is considered to be as an appropriate method for estimation of chemical constituents from plant materials. Therefore, HPLC analysis has also been used for the quantitative determination of ferulic acid.

MATERIALS AND METHODS

Instruments

Soxhlet apparatus, VIS spectrophotometer (Systronics), HPLC (Agilent Technologies 1200 Infinity Series) were used.

Chemicals

AR grade chemicals such as ethyl acetate, Folin – Ciocalteu reagent and Sodium carbonate were obtained from Nice chemicals. Double distilled water was obtained after purification. Ferulic acid (FA) (Fig. 1) of 98% purity was purchased from NICE.

Plant materials

Plant materials selected such as rice bran (*Oryza sativa*), wheat bran (*Triticum aestivum*) and Bamboo shoot (*Bambusa vulgaris*) were collected from homestead region. Rice bran and wheat bran as whole and young bamboo shoot tip were dried, powdered with the help of blender and kept in sealed containers for future use.

Folin–Ciocalteu assay

Folin–Ciocalteu assay is based on of oxidation-reduction reaction, containing molybdates, tungstates as the main components of the reagent. This assay is a commonly used method for the quantification of phenolic acids in samples. The phenolic compounds reduces the heteropolyphosphotungstates–molybdates into a blue coloured chromogen. The reaction is carried out only under basic conditions in the presence of washing soda solution. The Phenolate anion formed from phenolic compound reduces Folin–Ciocalteu reagent to form the blue coloured substance. Spectrophotometer can be used to measure the colour intensity of blue chromogen (Jadhav *et al*., 2012).

Standard solution of FA preparation (Stock)

The stock solution of FA (1 mg/ml) was prepared by dissolving 10 mg FA in ethyl acetate, final volume was made up to 10 ml with ethyl acetate in volumetric flask. From this stock, 1 ml was taken out and added to 10 ml volumetric flask, and the volume was adjusted to 10 ml by adding double distilled water to get 100 µg/ml concentration. This solution was used for further analysis for making calibration curve.

Calibration curve of FA

From the stock solution (100 *µ*g/ml) of FA, 0.1 ml to 0.8 ml aliquots were added to a volumetric flask (10ml). To this flask, 2ml of sodium carbonate solution (15 %) and Folin–Ciocalteu reagent (0.5 ml) diluted with double distilled water (1:2 ratio) were added. The final volume was added with double distilled water to get a solution ranging in concentration from 1*µ*g/ml - 8*µ*g/ml of FA. The mixture showed maximum absorption at 718 nm when calculated against the blank solution. The absorbance of all solutions can be measured, and a calibration curve was plotted.

Preparation of ethyl acetate extract of plant materials

Accurately weighed 10 g of maize, wheat bran, rice bran, and bamboo shoot was extracted separately with 100ml of ethyl acetate with the help of the Soxhlet apparatus. The resulting crude extract was used for further analysis.

Preparation of sample solution

The extract solution (1ml) was added to the volumetric flask (10ml). To the flask, Folin – Ciocalteu reagent (0.5 ml) diluted with double distilled water (1:2 ratio) and 2 ml sodium carbonate solution (15 %) was added, the final volume was made up to 10ml with double distilled water. Absorbance was measured at 718 nm with a Spectrophotometer.

Validation of the proposed method:

Linearity

The linearity was determined by constructing the calibration curve and evaluating it by linear least square regression analysis.

Chromatography

Column : C18 4.6×250mm×5µm
Flow rate : 1.0mL/Minute
Inj.Volume : 20µL
Wave length : 319nm
Run time : 10 minute
Column temperature: 30^0C
Mobile phase: 5% Glacial Acetic acid in HPLC water : Acetonitrile (80:20)

Standard preparation

Ferulic acid Stock solution of 1000ppm was prepared in Methanol. From this, working standard of 2.5, 5, 10, 15, 20 ppm were prepared by serial dilution of the stock solution with methanol.

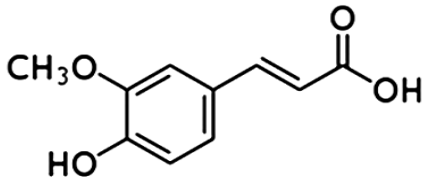

Fig. 1. *Structure of ferulic acid.*

Sample preparation

The ethyl acetate extract of plant samples were concentrated, filtered with 0.45µm disposable filter, 20µL samples were injected to the HPLC system for the analysis.

RESULTS AND DISCUSSION

Ferulic acid is a strong scavenger of free radicals and it has been accepted as a food additive to prevent lipid peroxidation. The selected plant materials such as rice bran, wheat bran, bamboo shoot are recognized in different systems of traditional medicine for the treatment of various diseases (Kumar and Pruthi, 2014). The mixture of ferulic acid along with Folin Ciocalteu reagent in an alkaline medium yielded a maximum absorbance at 718 nm. A linear relationship was obtained when a graph was plotted for concentration v/s absorbance within the concentration range of 1µg/ml - 8 µg/ml with a correlation coefficient value r2=0.988 and therefore, the rectilinear regression equation was y= 0.094x – 0.001 (Table.1).

The various samples used for the studies yielded different concentrations of FA, calculated by using the standard graph (Fig. 2). The bamboo shoot sample used for the study contained 1.7 µg/ml of FA, the wheat bran sample contains 7.3 µg/ml, and the rice bran sample yielded a maximum amount as 8 µg/ml (Table 2).

Chromatographic studies

In the HPLC analysis, Ferulic acid got eluted at 6.82 min (Fig. 4) and the peak for the same was found to be at 6.794 in the bamboo shoot sample (Fig. 5), 6.804 for the wheat bran sample (Fig. 6) and 6.791 for rice bran sample (Fig. 7) under the conditions of detection at 319 nm and temperature 30°C. The amount of ferulic acid in bamboo shoot, wheat bran and rice bran was found to be 1.92mg/kg, 11.03mg/kg and 14.03mg/kg, respectively, as calculated from the calibration curve of FA (Fig.3). The present study showed that rice bran yielded a higher amount of FA than bamboo shoot and wheat bran. Recent studies also unveiled the fact that rice bran is a rich source of antioxidant molecules such as γ-oryzanol and ferulic acid (Arumsari *et al*., 2019; Tam *et al*., 2021).

The extraction of Ferulic acid has been found much attention nowadays because it exhibits a wide variety of biological activities, including antimicrobial, anti-inflammatory, anti-thrombosis, anticancer, and antioxidant activities. However, the extraction procedure of phenolic acids from biomass is very complicated and proper methodology is yet to be developed (Zavala-Lopez and Garcia-Lara, 2017; Zhong *et al*., 2019).

The extraction and purification of phenolic acid viz. ferulic acid from rice bran and orange peels by solvent extraction method was studied by Gogoi *et al*. (2017). One of the major drawbacks of this procedure is its requirement for large quantities of different solvents and chemicals, which generates a significant quantity of toxic solvent waste (Acosta-Estrada *et al*., 2014). Ideia *et al*. (2020) reported the use of autoclave to perform alkaline hydrolysis and partial purification by adsorption on a synthetic resin to obtain ferulic acid from brewer's spent grain. The procedure is additionally very time consuming, making the handling of several samples without delay a challenging task.

In the present study, the estimation of phenolic acid viz. ferulic acid from rice bran, wheat bran and bamboo shoot by spectrophotometric method and the HPLC technique seems to be simple, sensitive, reproducible

Table 1. Regression analysis.

Regression equation	y= 0.094x – 0.001
Range	1µg/ml - 8 µg/ml
Co-relation coefficient r2	0.988
Slope m	0.094
y-intercept	0.001

Table 2. Concentration of FA from various plant samples.

Sample	Unknown yield (µg/ml)
Bamboo shoot	1.7
Wheat bran	7.3
Rice bran	8.0

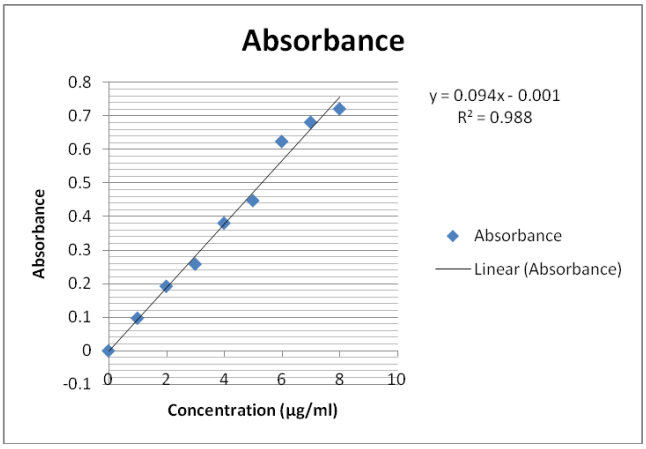

Fig. 2. *Calibration curve of ferulic acid.*

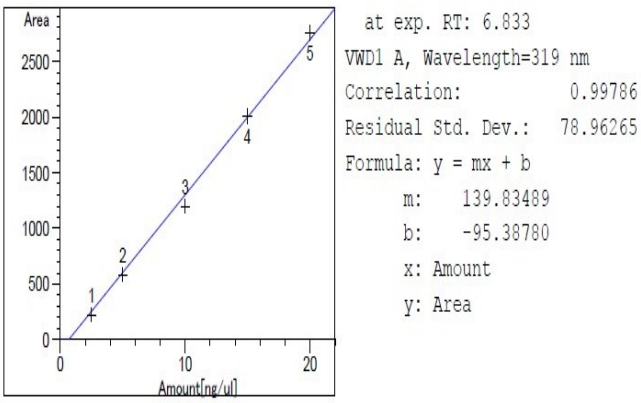

Fig. 3. *Calibration curve of ferulic acid.*

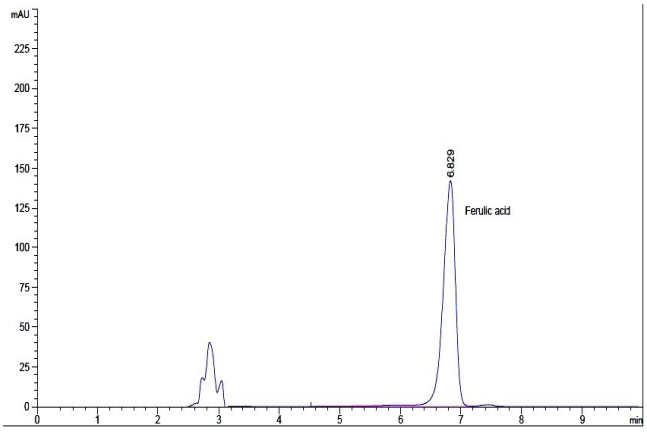

Fig. 4. *HPLC chromatogram of standard ferulic acid.*

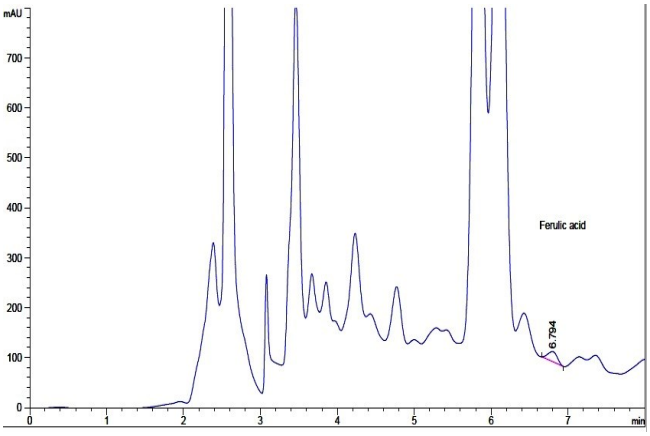

Fig. 5. *HPLC chromatogram of ethyl acetate extract of bamboo shoot.*

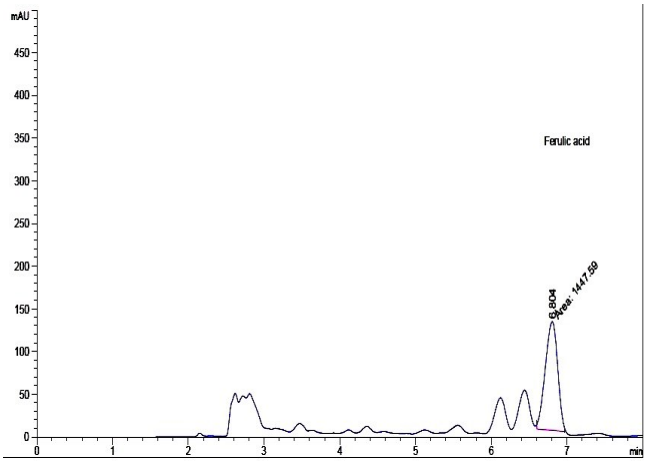

Fig. 6. *HPLC chromatogram of ethyl acetate extract of wheat bran.*

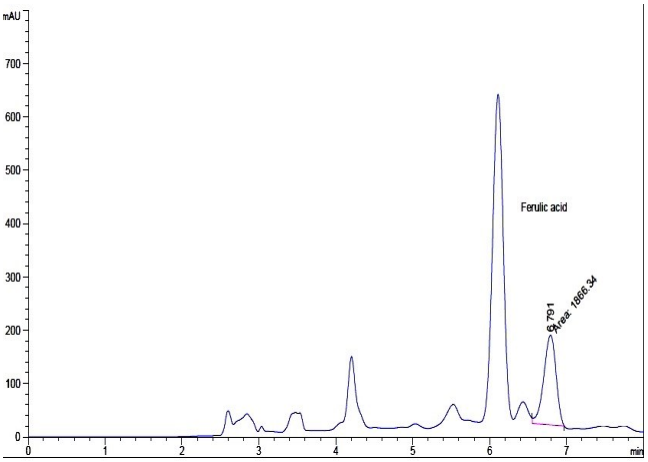

Fig.7. *HPLC chromatogram of ethyl acetate extract of rice bran.*

with minimum sample quantity, solvent and extraction time compared to methods proposed by Gogoi *et al*. (2017) and Ideia *et al*. (2020). The result obtained from the quantitative estimation of FA by Spectrophotometry and HPLC shows a parallel relationship. The chromatogram developed from HPLC also explains the isolation of ferulic acid with minimum impurities; hence can be recommended as a precise technique for the estimation of ferulic acid from plant samples.

Conclusion

A simple, sensitive and reproducible VIS-Spectrophotometric method has been exploited to estimate and quantify ferulic acid in various plant materials like bamboo shoots, rice bran and wheat bran etc., using Folin Ciocalteu reagent in the presence of an alkaline medium. The method can be employed for the routine analysis of ferulic acid from the various plant species. Moreover, they can prove to be helpful for nutritional and clinical investigations of ferulic acid levels in a variety of samples. The method for estimating ferulic acid in bamboo shoots, rice bran and wheat bran by HPLC is accurate, precise and reproducible.

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Conflict of interest

The authors declare that they have no conflict of interest.

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