Extraction, isolation, and identification of phytochemical derived from prominent South Asian agricultural waste using analytical tools

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ABSTRACT

Objective: Agricultural waste is foremost source of pollution. South Asian continent farmers are producing large amount of crop residues because of extensive cultivation of rice, wheat. To achieve sustainable and climate deteriorations free agricultural practices, we need to work on the efficient utilisation of residual content. The grass specie *Oryza sativa* (Asian rice) produces rice as its seed. In order to defend themselves against pest attacks as well as plant physiological actions, rice plants create their own chemical defenses. This article focuses on phenolic phytochemicals profiling estimate is done mainly the rice straw morphological portion. A new Reverse Phase Chromatographic method was developed to estimate the phenolic extract from rice straw. Chromatography was performed on a Waters HPLC system, using Shimpak GIST C18 coating on stationary phase, 5 micrometer particle size and 4.6mm diameter x250mm length column as the stationary phase.

Materials and methods: Potassium dihydrogen (KH_2PO_4) solution, pH adjusted to 3.2 ± 0.05 {with dilute 0.1N Hydrochloric acid (HCl) solution}, with 90:10% v/v, acetonitrile (ACN): water as mobile phase. The flow of fluid was kept at a constant 1.0 mL/min throughout the analysis The eluted compounds were detected at wavelength of 280 nm.

Results: The retention time for p-coumaric acid was 37.42 minutes and Ferulic acid was about 38.18 minutes. The concentrations of 3-30µg/mL was found to be acceptable. The method was accurate and precise, reproducible and cheap.

Conclusion: The proposed method is highly accurate and sensitive. The method for achieving success using this approach was found to be effective.

Keywords: Analysis, Phenolic extract, RP-HPLC, Agri-waste, Environment, Applications.

ABBREVIATIONS

RP-HPLC – Reverse phase High Performance Liquid Chromatography
KH_2PO_4 – Potassium dihydrogen orthophosphate
ACN - Acetonitrile
HCl – Hydrochloric acid
RSD – Relative standard deviation

INTRODUCTION

Agricultural biomass is a wide term refers to the residual or organic material making and left over after crop cultivation and harvesting, including crops that is the food and non-food parts of plants like corn, sugarcane, beets, and rice. Southeast Asia is considered as major rice bowl[1], accounting for 40% of international rice exports leading to enormous production of straw[2], bran and other parts[3]. More than 50% world's population, especially in Asia and Africa, eats rice, a cereal grain, as a staple food. The plant produces many defensive phytochemicals in response to environmental adaptation as well some synthetic chemicals, like the herbicide 2,4-D, which increases the plant's resistance to certain pests and survival chances[4]. This study signifies the analysis of phenolic phytochemicals. Skin care products contain ferulic acid & p-coumaric acid (Figure- 3) to prevent skin cancer and photoaging. They can enhance skin elasticity, lessen hyperpigmentation, and brighten skin tone. Many European nations use them as a food additive. In baking, it can also be used as a preservative. Some medications contain phenolics acid. Additionally, has been suggested as a possible treatment for conditions like diabetes, cancer, Alzheimer's disease, and cardiovascular diseases[5].

MATERIALS AND METHODS

The most popular HPLC mode is called reversed-phase HPLC (RP-HPLC), which is simply NP-HPLC in reverse, with the stationary phase being more nonpolar than the eluting solvent. The components in the fractions of interest are separated using the RP-HPLC condition employing the following (Table-1) requirements.

Sr. No.	Name of the material	Manufacturer
1.	Potassium dihydrogen orthophosphate (KH_2PO_4)	Sigma
2.	Hydrochloric acid (0.1 N) 36%	Merck
3.	Potassium Hydroxide	Fisher Scientist
4.	Acetonitrile	Merck
5.	Milli Q Water	In-house

Table 1: List of chemicals and reagents utilized in the study

Instrumentation:

HPLC system (Waters) consisting of a quaternary gradient pump, autosampler, column, oven, and PDA detector was employed for analysis. Chromatographic data were acquired using LabSolutions software.

Experimental Work

Extraction of Phenolics

Extraction of Free Phenolics: - The previously described technique in the literature was used to extract free phenolic compounds. In short, 20 mL of 80% chilled ethanol was mixed with 5 g of rice straw[5] powder for 10 minutes, and the mixture was centrifuged at 2500g for 10 minutes. After removing the supernatant, the extraction process was carried out once more. After being vacuum-evaporated to 5 mL at 45°C, the supernatant was reconstituted with H_2O to reach a final volume of 10 mL.

Extraction of Bound Phenolics: The previously described technique in the literature was used to extract the bound phenolic compounds from rice straw powder and their byproducts. One gramme of sample was extracted twice using 80% ethanol for every grain of raw maize or maize product, and the supernatant was thrown away. 20 mL of room-temperature 2 M NaOH were used to digest the extraction residue, and the sample was shaken for one hour. Lipids were extracted from the mixture using hexane after it had been neutralized with 4 mL of hydrochloric acid. Ethyl acetate was used five times to extract the final solution. Dry evaporation was performed on the ethyl acetate fraction. 10 mL of distilled water were used to reconstitute phenolic compounds. To develop the method for analysis of four components in given product, a number of trials were taken on HPLC by changing different parameters again and again, e.g.: Mobile Phase, Column, Column Conditions, concentration of the sample etc. Both hydroxybenzoic and hydroxycinnamic acids are part of the significant class of phenolic acids that make up rice's phenolic fraction[6].

After scanning the Sample solution on UV-PDA it was found that maximum wavelength at which all actives can be absorbed is 280nm, so the wavelength was set as 280nm for all the trials.

Chromatographic condition

The Shimpak GIST C18 column (4.6mm × 250 mm, 5 µm) was used as the stationary phase. The Potassium dihydrogen orthophosphate solution, pH adjusted to 3.2 ± 0.05 (with a dilute solution of 0.1 N hydrochloric acid), and 90:10% v/v acetonitrile: water was used as the mobile phase and filtered before use through a 0.5µm filter. A constant flow rate of 1.0 mL/min was maintained throughout the run. Detection was performed using a PDA detector at 280 nm. A degassed mixture of 0.01 N hydrochloric acid: acetonitrile at a ratio of 90: 10% v/v was used as the diluent[7].

Sl. No.	Portion details	Specification
1	Column	Shimpak GIST C-18 (250 mm x 4.6mm, 5.0µm)
2	Flow Rate	Flow gradient
3	Wavelength	280 nm
4	Column oven temperature	25 °C
5	Load	10 µL
6	Test concentration	4 µg/mL
7	Runtime	60 minutes
8	Elution	Gradient

Table 2: List of RP-HPLC experiments specification/parameters.

Sr. No.	Time	Flow	Mobile Phase-B	Mobile Phase-A
1	0	1.00	5.0	95.0
2	5.00	1.00	5.0	95.0
3	40.00	1.00	60.0	40.0
4	45.00	1.00	60.0	40.0
5	46.00	1.00	5.0	95.0
6	60.00	1.00	5.0	95.0

Table 3: Gradient flow RP-LC run time details

Buffer preparation

Dissolved 2 g of KH_2PO_4 in 1000 mL milliQ water and adjust the pH to 3.2 ± 0.05 with dilute hydrochloric acid solution, filtered and de-gased.

Mobile phase A: Use 100% buffer as mobile phase-A.

Mobile phase B: Mix acetonitrile and water in the ratio of 900:100 v/v.

Note:

i. Use 100 mL measuring cylinder for measurement of Acetonitrile in mobile phase preparation.

ii. The quantity of the mobile phase can be changed depending on the analysis requirement.

Preparation of diluent: Mix acetonitrile and water in the ratio of 9:1 v/v

Preparation of system suitability solution: Weighed accurately and transferred 3mg to a 150 mL volumetric flask and added approximately 100 mL of diluent, shaken on a rotary shaker at 200 rpm for 15 min, and shaken gently for 10 min. with intermediate shaking. After sonication and shaking mix 15 mL of acetonitrile and makeup the volume up to 150 mL. Centrifuge a portion of the solution at 500 rpm for the next 10 min[8].

Transfer 2mL of the above solution to a 10 mL volumetric flask, dilute to volume with the diluent, and mix. The solution was filtered through a 0.5 µm syringe filter and discarded for the first 5 mL, specifications are given in Table- 4.

Preparation of sample solution: Weighed accurately and transferred 5mg to a 250 mL graduated flask and added approximately 150 mL of diluent, shaken on a rotary shaker at 200 rpm for 15 min, and shaken gently for 10 min with intermediate shaking. After sonication and shaking mix 25 mL of acetonitrile and make up the volume up to 250 mL. Centrifuge a portion of the solution at 500 rpm for the next 10 min.

Transferred 4mL of the above solution to a 25 mL volumetric flask, dilute to volume with the diluent, and mixed. The solution was filtered through a 0.5 µm syringe filter and discarded for the first 5 mL.

Procedure: The column has been equilibrated for 30min with the initial composition of the mobile phase at a flow rate of 1.0mL/min.

Separately 1microliter of diluent was injected as blank (2 injections), Test solution (one injection) into the chromatographic system. Recorded chromatograms and measured the peak responses and retention time.

Sl. No	Name of the solution	No. of injections
1	Blank	2
2	System suitability solution	1
3	Test solution	1
4	Blank	Minimum 1(Bracketing Blank)
5	System suitability solution	1(Bracketing standard)

Table 4: Summary of System Suitability and sample run sequence

Note:

1. Use 0.1 N HCl in water and acetonitrile in the ratio 85:15 as injection with needle wash.
2. Disregard impurities below 0.05 % from test sample after calculation.
3. Identify the specified impurities in the test solution based on the RRT's of the respective impurities in System suitability solution[10].

Calculation: % purity (%w/w) = Test area x Standard wt. x Test dilution x Purity of standard/Standard area x Standard dilution x Test wt.

Blank Chromatogram

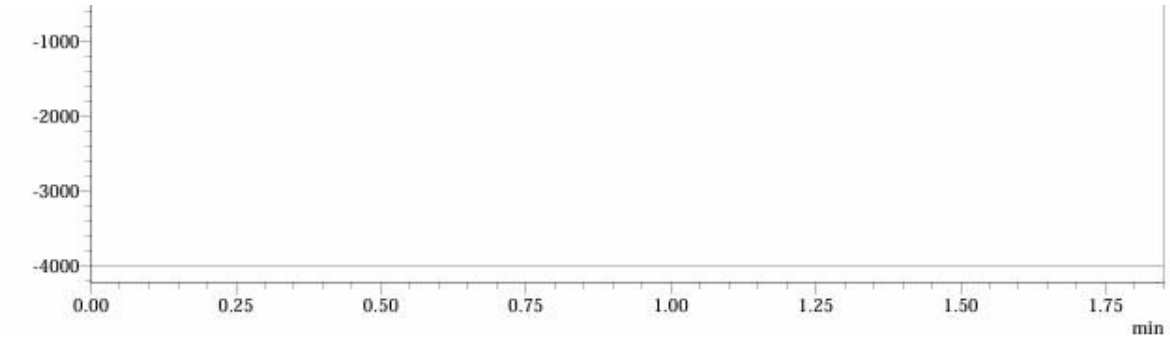
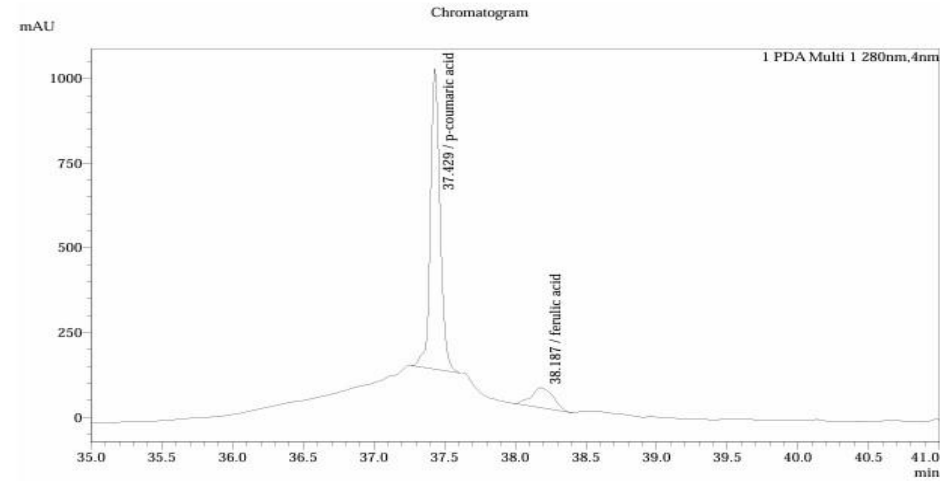


Figure 1: Chromatogram of Blank/Diluent



Peak Table

PDA Ch1 280nm

Peak#	Name	Ret. Time	Area	Height	Area%
1	p-coumaric acid	37.429	4204581	887603	86.590
2	ferulic acid	38.187	651171	59037	13.410
Total			4855752	946640	100.000

Figure 2: Chromatogram of test sample solution

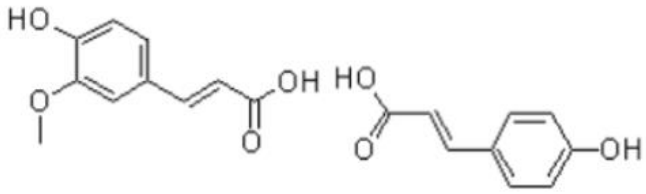


Figure 3: Chemical structures of the isolated and estimated Phenolic

RESULT: The R_t values of standard and sample solutions were given in Table 2 and Table 3 respectively. The estimation of Phenolic extract was done by RP-HPLC and in the optimized method, the mobile phase consists of potassium dihydrogen orthophosphate, pH adjusted to 3.2±0.05 (with 0.1N of hydrochloric acid solution) and Acetonitrile and water in the ratio of 90:10%v/v and was filtered before use, through 0.45 µ membrane filter. A constant flow of 1.0 mL/min was maintained throughout the analysis[11]. Detection was carried out using a PDA detector at 280 nm. The retention time, and chromatograms of the blank, system suitability and sample of phenolic extract were given figures 1 & 2.

DISCUSSION:

The retention time for phenolic extracts was found to be 37 minutes and 38 minutes for p-coumaric acid and ferulic acid respectively at 3-30µg/mL. By the proposed method of analysis, obtained retention time, peak area and peak height of chromatogram. In another Lab research work, various samples of rice from Japan were examined for ferulic and p-coumaric acids[9]. They found the concentrations of Ferulic and p-coumaric acid in brown rice varied between 309 to 607 mg/kg and 49 to 100 mg/kg, respectively[10].

CONCLUSIONS:

Based on our interest in agricultural waste study and phytochemical analysis[11], we summarized pathways and strategies for renewable practices to agriculture waste management and its contribution to the enhancement of our understanding in latest pharmaceutical developments. The proposed technique turned into determined to be simple, precise, accurate, and fast for the estimation of phenolic extract from rice straw. The cell segment utilized in this technique is easy to prepare, and the runtime was found to be 60min, so much less time-consuming technique. The studies showed that there may be no interference of components used for the preparation of samples. Hence, the method developed for the estimation of phenolic extract can be applied to quality control testing for phytochemical profiling of rice straw/rice plant. Other significant phytochemicals like Monilactones[12] and Cafenstrole[13] metabolites have also been reported in rice straw samples under separate lab. findings[14]. From this significant agri-waste rice straw many useful materials[15] have been processed[16, 17].

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CONFLICT OF INTEREST

The authors confirms that there is no conflict of interest related to the manuscript.

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