The Extraction of Ferulic Acid from the Phenolic Fraction from Rice Bran Oil Using Ultrasonic Methods and Analysis of Antioxidant Effectiveness

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***Abstract*— Rice (*Oryza sativa*) is one of the main cereals in the world, especially in Asian countries. Rice bran, one of the byproducts of rice dehulling, has gained a lot of popularity as a functional foodstuff in recent years. The purpose of this study was to extract Ferulic Acid from the phenolic fraction of rice bran oil using the ultrasonic method, followed by antioxidant effectiveness analyses. Oil was extracted from rice bran using the ultrasonic method in an ethanol solvent. The result was analyzed for phenol yields, and contents and the variables tested include concentrations and volumes of ethanol. Furthermore, Antioxidant effectiveness tests were carried out on the highest yields of FA. The results showed the highest yield, 24%, was achieved at an ethanol concentration of 80%, the volume of 200 ml, and 25 grams of rice bran. The ferulic acid content in the phenolic fraction of rice bran oil was 10-20% with the highest Antioxidant Effectiveness of 10.71%.**

***Keywords*—Extraction, Ferulic Acid, Phenolic, Rice Bran, Ultrasonic.**

I. INTRODUCTION

Rice bran is a byproduct of rice dehulling and contains 16 – 32% (w/w) of oil. However, its contents depend on the variety and dehulling process. The oil is highly nutritious and contains unsaturated fatty acids and antioxidants such as oryzanol, tocopherol, tocotrienol, phytosterol, polyphenol, and squalene. Ferulic Acid (FA), a part of the phenol fraction, might be found in plants in the free or covalently bonded forms [1]. In general, maceration is simple, and the most common method used to extract the phenolic compounds in small or large quantity. Ultrasound-Assisted Solvent Extraction is a modified maceration method using ultrasound at 20 kHz [2]. This method relies on mechanical pressure on cells to induce cellular breakdown, which allows an increased sample solubility and results in higher extraction yields [3]. The purpose of this study was to extract the Ferulic Acid from the phenolic fraction of rice bran oil using ultrasound-assisted solvent extraction method with ethanol. The study also aimed to analyze the antioxidant effectiveness of the Ferulic Acid extract.

II. METHODOLOGY

A. Material and Equipment

The devices used in this study included Spectrophotometer UV-VIS 1240 SHIMADZU, FTIR Q410 (Fourier Transform Infrared) spectroscopy, Ultrasonic WISD WUC-D06H, and Rotary Evaporator B-100 BUCHI. Also, other essential materials include rice bran @25gr, ethanol solvent (volume 100ml, 125ml, 150ml, 175ml, 200ml, and Concentration 40%,50%,60%,70%,80%) Follin ciocalteu solution, and Ferulic Acid standard.

B. Method

Oil extracted from rice bran was dried, roasted, and weighed to obtain 25-gram samples. These samples were dissolved in a binary solvent of ethanol at a concentration of 80% and water [4]. Furthermore, maceration was carried out in ethanol solvent for 12 hours at room temperature. The samples were then immersed in an ultrasonic bath for 15 minutes. Importantly, the extract samples were purified using rotary evaporation and oven drying methods. The ferulic acid yield was analyzed in a series of phenol tests of Spectrophotometer UV-Vis followed by yield and antioxidant assessments using DPPH (2,2-diphenyl-1-picrylhydrazyl) [5].

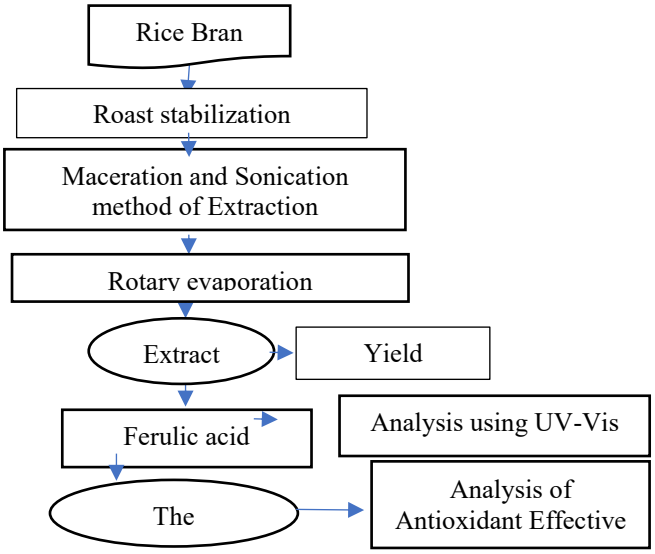

Figure 1. Experimental procedures of preparation and analyses of Ferulic Acid from rice bran oil.

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III. RESULT AND DISCUSSIONS

A. Yields of Ferulic Acid Extraction in 80% Ethanol solvent

To determine the highest FA extraction from rice bran oil, solvents of volumes 100 ml, 125 ml, 150 ml, 175 ml, and 200 ml were used, as shown in Table 1.

Table I. Ferulic Acid yield as extracted, 80%, from 25 grams of rice bran and purified in a rotary evaporator.

Solvent Volume (ml)	Extract Volume (ml)	Yield (%)
100	3	12
125	3.3	13,2
150	4	16
175	4	16
200	6	24

When the yield of the extract is plotted against the solvent volume, a correlation curve is obtained, as shown in Figure 2.

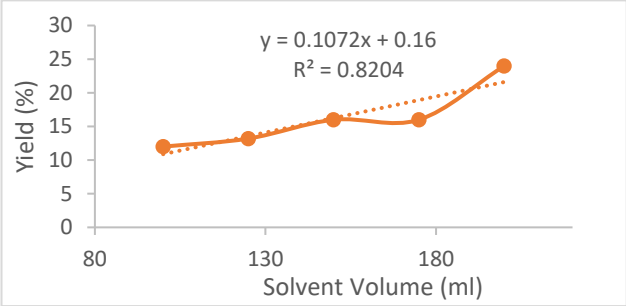

Figure 2. Correlation between solvent volumes and yields of ferulic acid extract purified in a rotary evaporator.

The correlations for FA purifications using rotary evaporator are represented by the equations $y = 0.1072x + 0.16$ and $R^2 = 0.8204$ respectively, in which y represents the yield, and x the solvent volume. These R^2 values indicate strong relationships between the yields and solvent volumes

B. Extraction in Varied Ethanol Solvent Concentrations

The optimal concentration of ethanol in the binary solvent was determined to obtain the highest yield of FA. Also, the effect of extraction conditions was evaluated by the binary solvent and extraction [2]. The tested concentrations were 40%, 50%, 60%, 70%, and 80% for the constant volume of 200 ml. a rotary evaporator was used in the purification of the extract.

Table II. Ferulic Acid yields obtained from 25 grams of rice bran using 200 mL of solvent with varied ethanol concentrations

Solvent Concentration (%etanol water)	Extract Volume (ml)	Yield (%)
40	1.1	4.4
50	1.5	6
60	1.8	7.2
70	2.6	10.4
80	3	12

When FA yield is plotted against ethanol concentrations, a correlation curve is produced and represented in Figure 3.

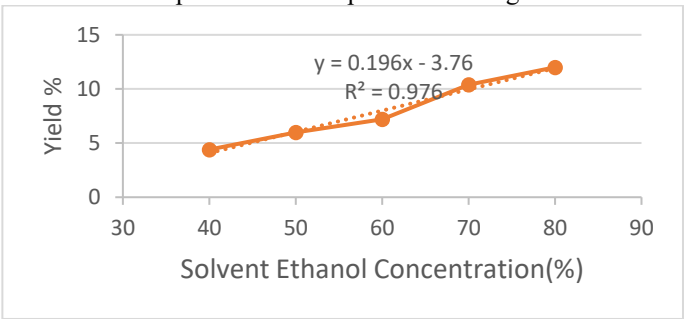

Figure 3. Correlation curve between FA yields and ethanol concentrations.

Figure 3 shows that higher ethanol concentrations correlate positively to higher FA yields. This correlation is formulated with the following equation, $y = 0.049x - 0.94$, and $R^2 = 0.976$, in which y is FA yield and x is ethanol concentration.

C. Determining the Phenolic and Ferulic Acid Contents.

Tests meant to determine the content of phenolic compounds were carried out on the extract using the Follin-Ciocalteu method [6]. Extract samples were titrated using the Follin reagent, and the colour changed from the initial white to light-yellow and finally light-blue. These changes indicate the presence of phenolic compounds.

To determine the nature of compounds present in the phenolic fraction, UV-Vis spectrophotometer tests were carried out. Moreover, the Ferulic Acid amounts in the extracted samples were determined using the FA standards at the wavelength of 765 nm. The results showed rice bran extract purified in rotary evaporator produced an absorbency of 765 nm at the concentration of 0.02 µg/ml.

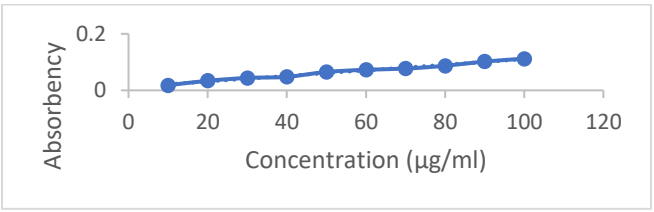

Figure 4. Wavelength calibration curve using Ferulic Acid standards at 765 nm.

Based on the calibration results in Figure 5, the concentration of phenol (Ferulic Acid) was 10-20%.

D. Result of FTIR Test

FTIR tests were conducted to determine the phenol groups in the Ferulic Acid isolate.

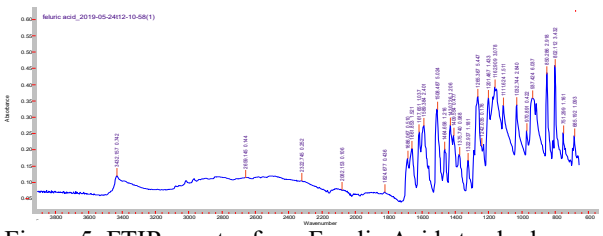

Figure 5. FTIR spectra from Ferulic Acid standard

Table III. The Ferulic Acid Standard

Number	Peak Number	Wavenumber(cm^{-1})
1	25	2322,745
2	26	2659,145
3	27	3432,157

Table IV. The phenolic groups in Ferulic Acid isolate determined with FTIR analysis

Number	Peak Number	Wavenumber(cm^{-1})
1	10	2852,341
2	11	2921,494
3	12	3008,586

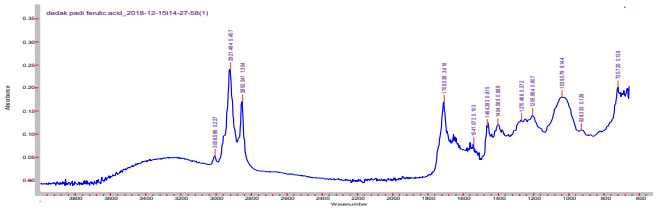

Figure 6. FTIR spectra revealing phenolic groups in Ferulic Acid isolate from rice bran oil.

The results from FTIR analysis compared with Ferulic Acid standard show the presence of absorbency bands for C-OH, C-C, C-H, C=O, at wavelengths of 3008.586 cm^{-1}. These bands indicate the extract contains phenolic groups found in Ferulic Acid, while the IR spectra show the presence of carbonyl, hydroxyl, and C=C groups.

E. The result from the Antioxidant Content Test

The results from rice bran oil extraction were tested for antioxidant contents using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay [7]. This process involves colour changes with the addition of DPPH. In the end, a positive result was purple. The tests showed the presence of antioxidants in the samples, confirmed using the UV-Vis spectrophotometry. The DPPH wavelength and absorbency were verified at the maximum absorbency of 508 -517 nm. Moreover, the control absorbency at a wavelength of 508 nm was 3.436, and 3.08 for the DPPH. Antioxidant effectiveness of the extract was tested using inhibition activity procedures [8], and the results were 10.71%. The samples were reanalyzed with UV-Vis spectrophotometry to determine the wavelengths and absorbency. The results showed control and maximum absorbency of 517 nm at 3.214 and 3.0682, respectively. Antioxidant effectiveness of the extract at a wavelength of 517 nm was 4.536%.

IV. CONCLUSION

The objective of this study was to extract Ferulic Acid from the phenolic fraction of rice bran oil using the ultrasonic method along with the analysis of the antioxidant effectiveness. Oil was extracted from rice bran using the ultrasonic method in an ethanol solvent. The result was analyzed for phenol yields and contents. The variables tested were concentrations and volumes of ethanol. Additionally, antioxidant effectiveness tests were performed to determine the highest yields of FA. The results showed 24% maximum yield from 25 grams of rice bran was achieved at an ethanol concentration of 80% in 200 ml. The ferulic acid content in the phenolic fraction was 10-20%, with the highest Antioxidant Effectiveness of 10.71%.

- In this study, ferulic acid was successfully extracted from the phenolid fraction of rice bran oil using ultrasonic method and antioxidant effectiveness analysis.
- The highest oil yield from 25 grams of rice bran was 24%, obtained therough the ultrasonic method in 200 mL of 80% ethanol solvent.
- The concentrations of phenolic compounds were 10 – 20%, determined from the Ferulic Acid qualitative tests through the Follin-Ciocalteu method.
- FTIR tests confirmed the presence of phenolic groups in the samples at absorption bands of 3008.586 µ.
- Antioxidant effectiveness tests on the extract at Authors and Affiliations were successful.

ACKNOWLEDGMENT

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