Extraction, Purification and Antioxidant Activity of Ferulic Acid by Alkaline Hydrolysis from Sugarcane Bagasse

Ae Mar[1]

Abstract

Ferulic acid is a compound well known for its antioxidant and antimicrobial properties, which was widely used in the food, cosmetic and pharmaceutical industries. In this study, the extraction of ferulic acid from sugarcane bagasse was carried out by alkaline hydrolysis using NaOH and later purified by precipitating hemicelluloses and glucomannans with ethanol. Extracted ferulic acid was examined by Fourier Transform Infrared Spectroscopy (FT-IR) method. FT-IR spectrum of precipitate in purification method performed to prove that the isolated compound was ferulic acid. The antioxidant activity was further determined by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging method for ferulic acid which showed that sugarcane bgasse possesses better antioxidant potential when compare to reference standard ascorbic acid. The main purpose of this research is to develop value-added commodities made from agricultural waste products.

Key words: ferulic acid, sugarcane, hemicellulose, antioxidant, value-added

Introduction

Nowadays, the phenolic compounds in fruit and vegetable-rich diets have attracted researchers' attention because of their health promoting effects such as lowering the risk of cardiovascular disease, cancer, or other conditions associated with aging. The biological mechanisms behind these health-promoting effects include protection against free radicals, free radical mediated cellular signaling, inflammation, allergies, platelet aggregation, ulcers, viruses, tumors and hepatotoxicity. Moreover, the relationship between nutrition and health has become a topic of great interest. There is substantial evidence of the beneficial effects of diets that are rich in fruits and vegetables (Biegelmeyer, *et al*, 2011).

Ferulic acid is a hydroxycinnamic acid with molecular formula $C_{10}H_{10}O_4$ and it is one of the most significant natural phenolic acids generally found in the seeds as well as leaves both in its free form and covalently conjugated with the plant cell wall materials. The extraction of ferulic acid has been found much attention now a day due to the fact that it exhibits wide variety of biological activities including antioxidant, antimicrobial, anti-inflammatory, anti-thrombosis, and anticancer activities (Gogoi, et al, 2016). The syntheis of ferulic acid was established by Dutt in 1935 when ferulic acid was used as a precursor in the manufacturing of vanillin and malonic acid. There are vast numbers of studies documented on the bio-medical properties of ferulic acid such as antioxidant activity, UV-absorbing capacity & its effect of lignin as precursor in plants metabolic pathway. Ferulic acid, being highly abundant, is indeed difficult to synthesize, oryza oil and fat chemical has successfully developed an efficient method to extract ferulic acid from rice bran and suitable for applications in the health and beauty arena (Nomura, *et al*, 2001).

Ferulic acid exhibits biochemical role in the inhibition of seed germination, inhibition of indole-acetic acid and enzyme, inhibition of decarboxylation activity and other protective effect on micro-organisms and pets. It can be absorbed and easily metabolized in the human body. Ferulic acid has been shown to protect against DNA damage and other human disorders. Besides acting as an antioxidant, it reduce inflammation, microbial activity, also prevents allergy and cancer. Liver protective and antithrombotic nature, increase of sperm viability,

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antiviral and vasodilatory action are also some of the important biological activities. Ferulic acid reduces blood glucose level and it has an enormous possibility to become an antidiabetic drug. It can fight coronary disease, lower cholesterol in serum and liver, and increase sperm viability (Raphaela, et al, 2016).

The present investigation was done to extract ferulic acid from sugarcane bagasse and find out the antioxidant activities which give a scientifically proof for its traditional uses.

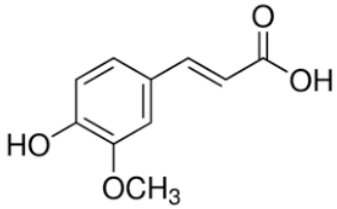

Figure 1. Ferulic Acid

Materials and Methods

Sample Collection

Sugarcane bagasse (SCB) was provided by Nawayat Sugar Factory (Pyi Gyi Tagon Township, Mandalay, Myanmar). The chemicals were used for this study namely sodium hydroxide, hydrochloric acid and 95% ethanol. These were purchased from Able and Golden Lady Chemical Shop, Chan Aye Thar Zan Township, Mandalay Region, Myanmar.

Figure 2. Sugarcane Bagasse (SCB)

Sample preparation

Sugarcane Bagasse (SCB) was soaked in water for 3 hours to extract sugar residues and then it was dried in vacuum oven at 40°C for12 h and ground in a laboratory mill (Panasonic MX-J120-P made in Japan). The powdered sample was passed through a sieve with mesh size 1 mm was taken for further investigations.

Determination of Moisture Content of SCB

About 5g of SCB powder was weighed in a previously weighed porcelain basin. It was heated at 105˚C for 3 hours, than cooled in a desiccator for 15 minutes and weighed again. Heating, cooling and weighing were repeated until the loss in weight become constant. The percentage of moisture content was calculated from the weight loss of rice bran as follows:

$$\% \text{ Moisture Content} = \frac{W_1 - W_2}{W_1} \times 100$$

where,

W_1= weight of SCB before drying

W_2= weight of SCB after drying

Determination of Total Solids Content of SCB

About 5g of SCB powder was added in a previously weighed porcelain basin. It was placed in the oven at 105 ˚C. The SCB was dried to a constant mass for 3hrs. The porcelain basin plus SCB were cooled in a desiccator. The percentage of total solids content was calculated by using the following equation:

$$\% \text{ Total Solid} = \frac{W_1}{W_2} \times 100$$

where,

W_1= weight of SCB

W_2= total mass of SCB

Extraction and Purification of Ferulic Acid

Extraction process was carried out according to the method described by Baranov & Mazza (2009). To extract ferulic acid, 5 g SCB was mixed with 150 ml of NaOH (1 M) in Erlenmeyer flask. The flask was shaken in the water bath shaker at 60 ºC for 4 hours (200 rpm), ensuring total hydrolysis. After cooling down (20 ºC), the hydrolysate was filtered and then neutralized by 6 M hydrochloric acid. The hemicelluloses and glucomannans were precipitated by adding 95% ethanol. The amount of added ethanol corresponded to three times of the original volume. The precipitate was separated by using centrifuge machine. After decanting the supernatant extract, excess ethanol was removed from the extract on rotary vacuum evaporator. This led to the formation of a brown extract which contained ferulic acid (Figure 3). The extract was finally passed through a 0.45-µm filter before further analysis.

Figure 3. Extracted Ferulic Acid

Identification of Ferulic Acid by FT-IR Spectroscopy

Ferulic acid was characterized by using FT-IR -8400, SHIMADZU, Japan, at Universities Research Centre, Yangon. Ferulic acid spectra were recorded on a FT-IR spectrometer in the wavenumber range of 400-4000 cm^{-1} using a potassium bromide (KBr) containing 1 ml of samples.

Determination of Antioxidant Activity of Ferulic Acid

Free radical scavenging activity of ferulic acid of SCB was measured by 2, 2- diphenyl-1-picryl hydrazyl (DPPH). In brief, 0.002 M solution of DPPH in ethanol was prepared. This solution 1 ml was added to 3 ml of extracted ferulic acid in ethanol at different concentration (2.5, 5, 7.5, 10, 12.5 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, the absorbance was measured at 517 nm by using UV-VIS spectrophotometer. Reference standard compound being used was ascorbic acid and

experiment was done in triplicate, (Patel, 2011). The IC_{50} value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity, (Koleva & Beek, 2002). The percent DPPH scavenging effect was calculated by using the following equation:

$$\% \text{ RSA} = [(A_c - A_s) / A_c] \times 100$$

where,

% RSA = % free radical scavenging activity

A_c = absorbance of control

A_s = absorbance of sample

The results were shown in Tables (3 and 4) and Figures (6 and 7) respectively.

Results and Discussion

Significant efforts have been made for the development of technological processes as the consumption of natural products in food, cosmetics, pharmaceutical and other industries, and are increasing day by day, so the demand and supply of natural products should be maintained. In this research work, the extraction of ferulic acid from SCB was done by using sodium hydroxide and hemicelluloses were removed by using ethanol as a solvent. The residual sugar from the biomass was removed by soaking in the water prior to the NaOH extraction which enhances the quality of the extracted ferulic acid. Table 1 shows moisture content and the total solid content of SCB. The moisture content related to the yield and quality of extracted ferulic acid.

Table 1. Moisture Content and Total Solids Content of Rice Bran

Sr. No.	Parameters	After Degreasing
2.	Moisture Content	4.6%
3.	Total Solid Content	11%

This experiment was carried out at Department of Industrial Chemistry, Yadanabon University, Mandalay.

The alkaline hydrolysis used in this study was for the quantification of ferulic acid in the biomass. The results indicated that the content of ferulic acid in SCB was 55 %. In the purification stage, the ferulic acid obtained from alkaline extract of SCB was analysed by Fourier Transform Infrared Spectroscopy (FT-IR) method. The FT-IR spectrum of the precipitate was compared with the spectrum of the standard ferulic acid to confirm it Table (2) and Figures (4 and 5). The FR-IR spectrum of extracted ferulic acid clearly showed the existence of main functional groups in the ferulic acid structure, and the broad band at 3350 cm^{-1} was corresponding to phenol group which confirm the presence of phenolic compounds in extract. A part from this, C-H stretching of the alkene was at the 2928 cm^{-1} band. The C=O stretching was observed at 1643 cm^{-1}; it is due to the presence of carbonyl group. The peak at 1452 cm^{-1} indicates CH_2 bending vibration of alkane. Thus, FT-IR spectrum confirmed the presence of phenols, carboxylic acid, alkane, ester, polysaccharide, cellulose and lignin in ferulic acid of SCB.

Table 2. Functional Groups of Extracted Ferulic Acid

Sr. No.	Extracted Ferulic Acid		Standard Ferulic Acid		Functional Groups
	Peak Values (cm^{-1})	Bond	Peak Values (cm^{-1})	Bond	
1.	3350	O-H	3334	O-H	Alcohols, Phenols
2.	2928	C-H	2922	C-H	Alkenes
3.	1643	C=O	1641	C=O	Esters, Ethers and Carboxylic Acids
4.	1452	CH_2	1460	CH_2	Celluloses

This experiment was carried out at Universities Research Centre, Kamayut Township, Yangon.

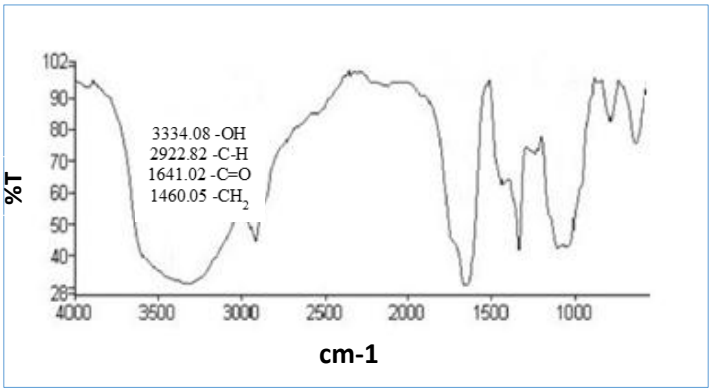

Figure 4. FT-IR Spectra of Standard Ferulic Acid

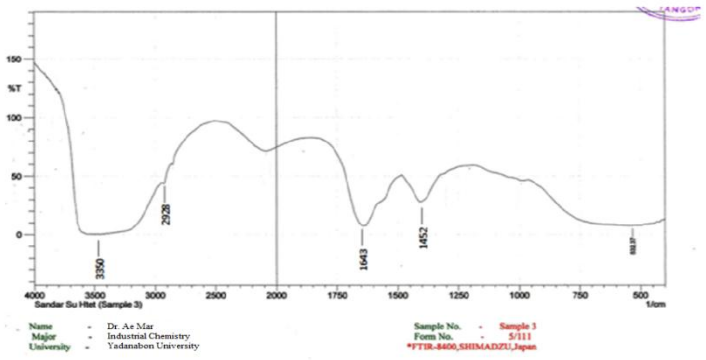

Figure 5. FT-IR Spectra of Extracted Ferulic Acid

The DPPH radical has been extensively applied method to assess the antioxidant potential of food items, such as vegetables, olive oils, fruits, juices and wines etc. Stable organic radical DPPH has utilized in determination of the antioxidant activity of ferulic acid as well as purification compounds. The ability of antioxidants for DPPH radical scavenging is supposed to be free radicals due to their hydrogen donating property. After acceptance of an electron or a hydrogen atom, a stable diamagnetic molecule will emerge which will result in vanishing the absorption band at 517 nm. The radical scavenging activity of the sample corresponds to the remaining DPPH in an inverse manner. The antioxidant potential of SCB extracts to scavenge free radical varied from 30.12 % to 70.74%. Highest antioxidant potential

of ferulic acid in present study (70.74 %) was found in agreement with standard ferulic acid (78 %) (Alanon, *et al*., 2011) but was lower than that reported for ascorbic acid (91.42%). From the point of IC_{50} value, the value of standard ascorbic acid and extracted ferulic acid were 5.4 µg/ml and 9.5 µg/ml, respectively as shown in Tables (3 and 4) and Figures (6 and 7). The DPPH radical scavenging activities of tested ascorbic acid and extracted ferulic acid from SCB are shown in Table (5). The total phenolic content of ferulic acid of SCB was found to be high. For this reason, there was correlation between the antioxidant activity of the ferulic acid and its total phenol content. Earlier studies indicated strong in vitro antioxidant activity of ferulic acid of SCB in many models, including a few studied in the present work (Kumaran & Karunakaran, 2006). Our studies also confirmed this finding. As the IC_{50} value and the antioxidant capacity have inversely proportional values, ferulic acid of SCB had the strong antioxidant properties. Thus, ferulic acid is considered to be a superior antioxidant.

Table 3. Antioxidant Activity of Standard Ascorbic Acid

Sr. No.	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
1.	2.5	26.08	5.4
2.	5	46.05	
3.	7.5	69.51	
4.	10	90.77	
5.	12.5	91.42	

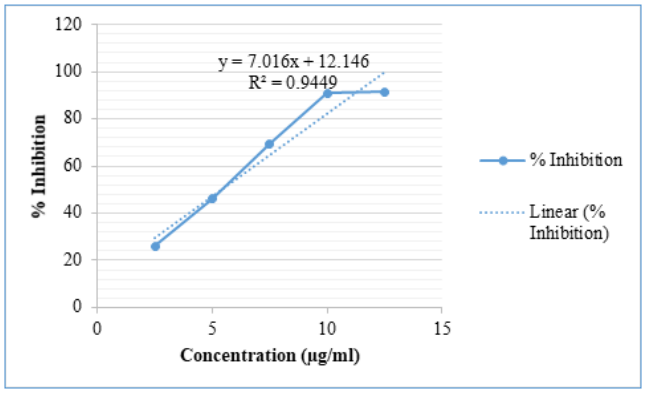

Figure 6. Plot of Concentration vs % Inhibition of Standard Ascorbic Acid

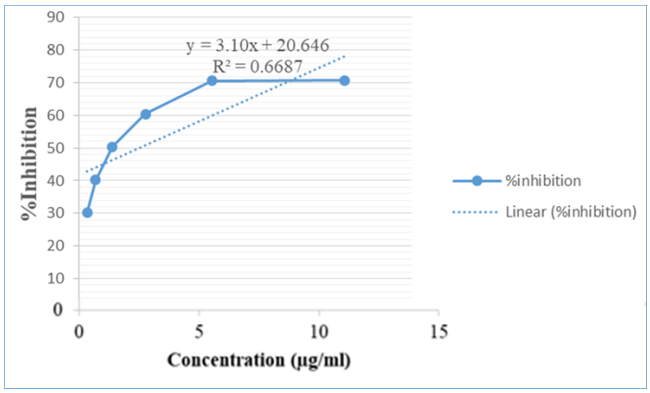

Figure 7. Plot of Concentration vs % Inhibition of Extracted Ferulic Acid

Table 4. Comparative % Inhibition Effect of Different Concentration of Extracted Ferulic Acid and Standard Ascorbic Acid

Sr. No.	Test Drugs Concentration (µg/ml)	2.5	5	7.5	10	12.5	IC_{50} Value (µg/ml)
1.	% Inhibition Std. Ascorbic Acid	26.08	46.05	69.51	90.77	91.42	5.4
2.	% Inhibition Ferulic Acid	30.12	40.25	50.34	60.46	70.74	9.5

This experiment was carried out at Ministry of Industry, Myanma Pharmaceutical Industrial Enterprise, Research Department, Yangon-Insein Road, Insein Township, Yangon Region.

Conclusion

From the above results, it is concluded that ferulic acid a wide range of therapeutic properties like anti-inflammatory, anti-diabetic, antiageing, neuroprotective, radioprotective and hepatoprotective effects. Many of these properties can be attributed to its potent antioxidant capacity because of its phenolic nucleus and extended side chain conjugation. Ferulic acid works well in all herbal antioxidant formula, vitamin and herbal health supplements. Thus our body's immune system can be benefited from ferulic acid.

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References

Alanon, M. E., Castro, I., Diaz, M.C., Cordon, M. H., & Perez, M. S., (2011). A study of the antioxidant capacity of oak wood used in wine aging and the correlation with polyphenol composition. *Food Chemistry* 128, pp. 997-1002.

Baranov, A. U., & Mazza, G., (2009). Extraction and purification of ferulic acid from flax shives, wheat and corn bran by alkaline hydrolysis and pressurized solvents. *Food Chemistry*, 115(4), pp. 1542-1548.

Biegelmeyer R, Andrade JM, Aboy AL, Apel MA, Dresch RR, Marin R, Raseira Mdo C, Henriques AT. Comparative analysis of the chemical composition and antioxidant activity of red (Psidium cattleianum) and yellow (Psidium

cattleianum var. lucidum) strawberry guava fruit. J Food Sci 2011;76: C991e6.

Gogoi, P., Gogoi, A., Rajbangshi, C., Bhuyan, P., & Bhuyan, B.K. (2016). Extraction, Purification and Spectroscopic Characterization of Ferulic Acid by Alkaline Hydrolysis from Brans of Assam, India.

Koleva, I. I, Beek, T.A.V, (2002). "Screening of Plant Extracts for Antioxidant Activity: *Comparative Study on Three Testing Methods" Phytochemical analysis, 2002*, vol-13, pp. 8-17.

Kumaran A, Karunakaran RJ (2006): Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromatics. *Food Chem* 97, pp. 109-114.

Nomura H., *et al*. Acceleration of ferulic acid and related compounds on insulin secession . Research report of Wakayama industrial technology center, 17-9 (2001).

Patel, R. M, Patel, N. J, (2011). "In vitro antioxidant activity of coumarin compounds by DPPH, superoxide and nitric oxide free radical scavenging methods" *Journal of advanced pharmacy education & research*, vol-1, 2011, pp. 52-68.

Raphaela, G., Bitencourt, Fernando A., Cabral, and Antonio J. A., "Ferulic acid solubility in supercritical carbon dioxide, ethanol and water mixtures", The Journal

of Chemical Thermodynamics, 103, December 2016, pp. 285-291.

Yoshinori K. & Seichiro F; a comparative study of the radical scavenging activity of the phenol carboxylic acids caffeic acid, p-coumaric acid, ferulic acid, without or with 2-mercapto ethanol and thiol using indication period method; Molecules, 2008; 13: 2488-2499.