

Production and Characterization of Ferulic Acid from Pineapple Waste and Its Anticancer Activity Using HeLa cell Line

J. Jayashree¹, D. Priya¹, S. Karthikeyan² and N.G. Ramesh Babu³

U.G Student, Department of Biotechnology, Adhiyamaan College of Engineering, Hosur, Tamilnadu, India¹

Assistant Professor, Department of Biotechnology, Adhiyamaan College of Engineering, Hosur, Tamilnadu, India²

Professor and Head, Department of Biotechnology, Adhiyamaan College of Engineering, Hosur, Tamilnadu, India³

ABSTRACT: Ferulic acid is a hydroxycinnamic acid, a type of organic compound. It is an abundant phenolic photochemical found in plant cell wall components such as commelinid plants as covalent side chains. It is related to trans-cinnamic acid and is found in invasive plant species and contributes to the antibacterial, anticancer activity and antioxidant properties of the plant. The present study showed that ferulic acid extracted from pineapple peel was identified and quantified by using TLC and HPLC. The antioxidant potential for extracted and standard ferulic acid was calculated by DPPH assay of 87.3% and 81.92% respectively and the total antioxidant capacity was calculated by FRAP assay which is expressed as 2315 μM and 3985 μM . The anticancer activity of extracted ferulic acid was evaluated based on viability of cells using MTT assay. The optimum cell viability was 48.04% at a concentration 125 $\mu\text{g/ml}$ and the maximum cell viability was 91.63% obtained at the concentration of 7.8 $\mu\text{g/ml}$ using MTT assay. The current study showed that pineapple is a potent source of ferulic acid that can be extracted and used as anticancer and antioxidant.

KEYWORDS: Ferulic acid, pineapple, antioxidant, anticancer, HPLC, HeLa cells

I. INTRODUCTION

Ferulic acid is a phenolic compound of plant origin with chemical formula $\text{C}_{10}\text{H}_{10}\text{O}_4$. Ferulic acid is one of the most abundant hydroxycinnamic acid in the plant kingdom and occurs mainly in the cell wall of cereal plants. Ferulic acid is found in the seeds of coffee, apple, artichoke, peanut and orange as well as in both seeds and cell walls of commelinid plants (such as rice, wheat, oats, the chinese water chestnut and pineapple), often in the form of chlorogenic acid., which is covalently linked to lignin with ether bonds [1]. Ferulic acid is a very important component for the structure and the biology of cellwall as it can cross link polysaccharide chains through dimerisation reaction [2]. It is the precursor of coniferyl alcohol, a monolignol which contributes to lignification in the conifers and angiosperms. It is mainly conjugated with mono- and oligosaccharides, polyamines, lipids and polysaccharides and seldom occurs in a free state in plants. Ferulic acid has been researched to have many functions including antioxidant [3], antimicrobial, antiinflammatory, antithrombosis, anticancer activities and antibiotic properties [4]. Ferulic acid is widely used in pharmaceutical, food and cosmetic industries [5]. Production of ferulic acid is highly recommended by using cheap by-products and waste substrates. The main problem in producing ferulic acid is to find raw materials which are environmental friendly and cheap. Most of the materials used for ferulic acid production are sugar based feedstock. There were several researches on ferulic acid production from waste such as wheat bran, paddy straw [6] and wheat straw. According to Papagianni, chemical purity is mainly depends on the essential part in the fermentation medium especially when cheap materials are being used [7]. Apart from there, many researchers had reported that ferulic acid is released by using pure culture of microorganisms. The co-production of enzyme by microorganisms is very important to increase the production of ferulic acid. The main objective of this current study was to extract ferulic acid using

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 3, March 2017

Staphylococcus aureus from pineapple peel by solvent extraction. It was confirmed by TLC and HPLC. The antioxidant capacity was performed by DPPH and FRAP assay and anticancer of extracted ferulic acid was evaluated against HeLa cell line.

II. RELATED WORK

1. Preparation of Substrate: Pineapple wastes were collected from the vegetable market near Lifeteck Research Centre, Chennai, Tamilnadu. The pineapple peels were processed for the production of ferulic acid. The pineapple peels were cut, and cleansed with 100ml of 1.5% of Tween 20 solution along with water. The washed pineapple peels were autoclaved for 20 minutes at 121 °C to inactivate any endogenous enzymes and proteinaceous inhibitors and dried in hot air oven. The dried pineapple peels were used as a carbon source for the production of ferulic acid.

2. Microorganism: Pure cultures of *Staphylococcus aureus* strain was obtained from Lifeteck Research Centre, R&D division, Chennai, Tamilnadu. It was maintained on a mixed medium containing both beef extract and peptone and the cultures were incubated at 37 °C and pH 7.2.

3. Production of Ferulic Acid: Ferulic acid is a secondary metabolite which is produced by *Staphylococcus aureus* using minimal essential medium (Muheim & Lerch 1999). The minimal essential medium was prepared to add the pineapple peels. The pH of the minimal medium was initially adjusted to 7.0 before autoclaving for 15 min at 121 °C. A loopful of *Staphylococcus aureus* was inoculated in the medium and incubated at room temperature for 6 days for the production of ferulic acid.

4. Extraction of Ferulic Acid by Acidification and Filtration: After 6 days of incubation, the broth was filtered to remove the suspended solid particles. The filtered medium was brought to the acidic pH (1-2) for effective extraction. Ferulic acid was then extracted from the medium by adding equal volumes of ethyl acetate. The solvent present in crude extract was then evaporated using the rotary vacuum evaporator. The obtained Ferulic acid powder was stored at -20°C for further analysis.

5. Quantitative Analysis of Ferulic Acid by TLC: Thin Layer Chromatography was performed to confirm the presence of Ferulic acid. The standard ferulic acid was prepared using Dimethyl sulfoxide (DMSO) at a concentration of 1mg/ml. The developed plate was dried in hot air stream and visualized in UV light at 254 nm. The band of the extracted ferulic acid was compared with the bands of the standard ferulic acid.

6. Quantitative Analysis of Ferulic Acid by HPLC: Separation and identification of ferulic acid was carried out by High Performance Liquid Chromatography (model). It was performed by using HPLC with a linear isocratic solvent system. The identified ferulic acid was confirmed by comparing retention times of external standards [8].

7. AntiOxidant Activity:

7.1. DPPH assay: DPPH (1, 1-diphenyl-2-picrylhydrazyl) is characterized as a stable free radical by virtue of the delocalisation. 3.7 ml of absolute methanol was added to sample test tube and 3.8ml of absolute methanol was added to another test tube kept as a standard. 100 µl of Butylated Hydroxy Toluene (BHT) was added to tube marked as standard and 100 µl of extracted ferulic acid samples to the sample test tube. Then 200 µl of DPPH reagent was added to all the test tubes. The samples were incubated at room temperature in dark condition for 30 minutes and the absorbance was read at 517 nm [9].

7.2. FRAP ASSAY: Total antioxidant activity was measured by ferric reducing antioxidant power assay (FRAP). 100 µl of ferulic acid sample and 3 ml of FRAP reagent were added to the tube marked as test. 3 ml of FRAP reagent was taken as a blank. Absorbance was measured (593 nm) at 0 minutes after vortexing. Samples were incubated at 37 °C in

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 3, March 2017

water bath and the absorbance (593 nm) was measured after 4 minutes. Ascorbic acid was used as the standard. FRAP value of ascorbic acid is 2 [10].

8. Anti cancer activity (MTT assay): The anticancer activity of ferulic acid was studied against *HeLa cell* line. *HeLa cell* line was obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS), penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO₂ at 37 °C. The cells were grown in 24-well plates and incubated at 37°C with 5% CO₂ condition. Once the cell reached the confluence, the ferulic acid samples were added to the cells at different concentrations and incubated for 24 hrs. After incubation, the samples were removed from the well and washed with phosphate-buffered saline (pH 7.4). 100 µl/well of 0.5% of (5mg/ml) 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. Then 1ml of DMSO was added in all the wells after incubation. The absorbance was measured with UV- Spectrophotometer at 570 nm using DMSO as the blank. Graph was plotted using the % of Cell Viability on Y-axis and concentration of the sample on X-axis [11].

III. EXPERIMENTAL RESULTS

Ferulic acid was extracted from pineapple peel using *Staphylococcus aureus* by solvent extraction method and it was characterize by TLC and HPLC. Further, the antioxidant studies are evaluated by DPPH and FRAP assay. Finally the anticancer activity was estimated based on percentage of cell viability using MTT assay by treating the sample against HeLa cells.

1. TLC: Thin layer chromatography was used to identify and separate compounds based on retention factor. The bands of the extracted ferulic acid were compared with the bands of the standard ferulic acid and the crude extract was identified as ferulic acid based on retention factor.

$$RF = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

The standard and extracted ferulic acid has RF value 0.986 and 0.9795. Hence the extract was conformed as ferulic acid. The quantitative analysis showed that TLC can be used for the identification of ferulic acid. Separation of crude extracts by TLC and visualization by UV light offers rapid method for detection of ferulic acid.

2. HPLC: HPLC system consists of compact LC and CE driver for the preparation of sample and it was controlled by EZ chrome elite software. The analysis of phenolic compounds is detected by HPLC detector system. The analysis of HPLC chromatogram was based on the retention time of both standard and extracted ferulic acids was represented in Figure 1 and 2.

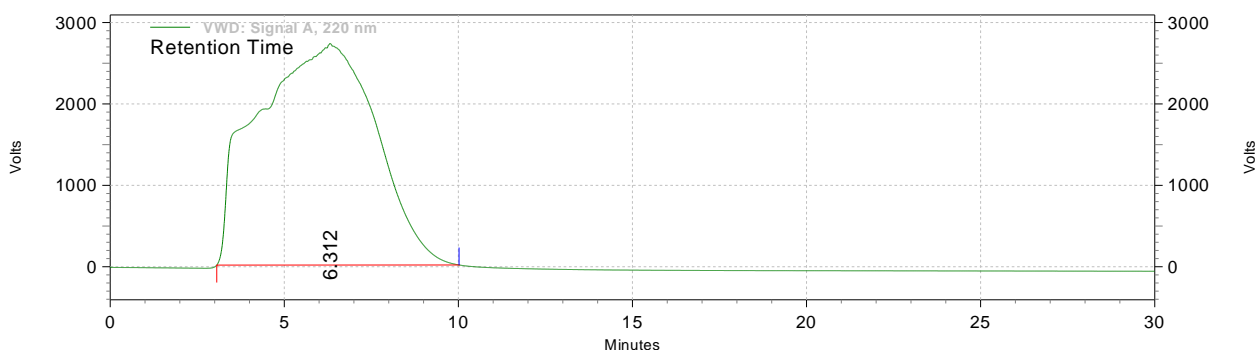


Figure 1. HPLC chromatogram of standard ferulic acid at 220 nm (Rt = 6.312).

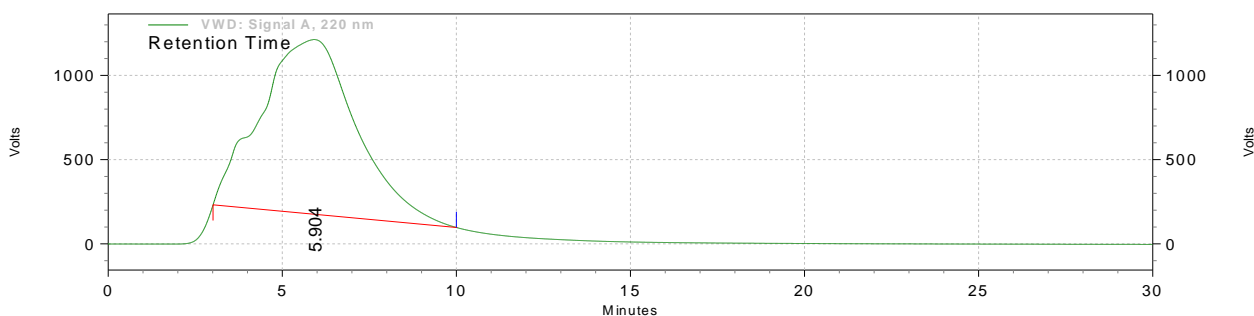


Figure 2. HPLC chromatogram of extracted ferulic acid at 220 nm (Rt = 5.904).

The analysis is time consuming and the quantity of solvents by linear isocratic solvent system. The intense peak was eluted at Tr = 6.3 and 5.9 minute indicating that ferulic acid as a major phenolic compound of hydrolyzed was extracted at 220 nm.

3. DPPH Assay: The Antioxidant activity of standard and extracted ferulic acid was calculated using DPPH Assay to delocalize the stable free radical. The antioxidant potential was expressed in Table 1.

Table 1-DPPH ASSAY

| S.No. | Sample | Concentration (µg/ml) | O.D. values | | | DPPH activity (%) |
|-------|--------------------------|--------------------------|-------------|-------|---------|----------------------|
| | | | I | II | Average | |
| 1 | Ferulic acid | 1000 | 0.145 | 0.151 | 0.148 | 81.92 |
| 2 | Ferulic acid standard | 1000 | 0.082 | 0.125 | 0.104 | 87.30 |

The antioxidant activity was determined by the formula

$$\% \text{ Antioxidant activity} = \frac{(\text{Absorbance at blank}) - (\text{Absorbance at test})}{(\text{Absorbance at blank})} \times 100$$

4. FRAP assay: The total antioxidant activity of sample and standard ferulic acid were determined using FRAP Assay and their values are expressed in Table 2.

Table 2 – FRAP ASSAY

| S.No | Name of the Sample | FRAP(µM) |
|------|-----------------------|----------|
| 1 | Ferulic acid sample | 2315 |
| 2 | Ferulic acid Standard | 3985 |

FRAP value of the sample was calculated by the formula:

$$\text{FRAP value of sample } (\mu\text{M}) = \left(\frac{\text{Change in absorbance of sample from 0 to 4 minute}}{\text{change in absorbance of standard from 0 to 4 minutes}} \right) \times \text{FRAP value of standard } (1000 \mu\text{M})$$

The antioxidant activity of ferulic acid was calculated by DPPH and FRAP assay. It was expressed in percentage of DPPH molecules reduced by one molecule of the reductant. DPPH characterize the stable free radical by decolorization and it was estimated for standard and extracted ferulic acid from pineapple peel was 87.30% and 81.92% respectively. FRAP value was used to measure the total antioxidant capacity and it was expressed in µM for standard and extracted ferulic acid from pineapple peel was 2315 and 3985. Similarly, the antioxidant potential was expressed in µM for

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 3, March 2017

isolated and pure ferulic acid from sugar beet pulp was 0.39 ± 0.01 and 0.55 ± 0.01 respectively [12]. It offers a speedy procedure for calculating the antioxidant potential of biological fluids.

5. Anticancer Activity Using MTT assay: The anticancer activity of extracted ferulic acid was expressed in percentage of cell viability using MTT assay in HeLa cells. The cell viability with different concentrations was shown in Table 3. The % cell viability was calculated using the following formula:

$$\% \text{ Cell viability} = \frac{A570 \text{ of treated cells}}{A570 \text{ of control cells}} \times 100$$

Table 3 - Anticancer effect of Ferulic Acid on HeLa cell line

| S.No | Concentration (µg/ml) | Dilutions | Absorbance (O.D) | Cell Viability (%) |
|------|-----------------------|-----------|------------------|--------------------|
| 1 | 1000 | Neat | 0.332 | 19.98 |
| 2 | 500 | 1:1 | 0.510 | 30.70 |
| 3 | 250 | 1:2 | 0.622 | 37.44 |
| 4 | 125 | 1:4 | 0.798 | 48.04 |
| 5 | 62.5 | 1:8 | 0.987 | 59.42 |
| 6 | 31.2 | 1:16 | 1.163 | 70.01 |
| 7 | 15.6 | 1:32 | 1.375 | 82.78 |
| 8 | 7.8 | 1:64 | 1.522 | 91.63 |
| 9 | Cell control | - | 1.661 | 100 |

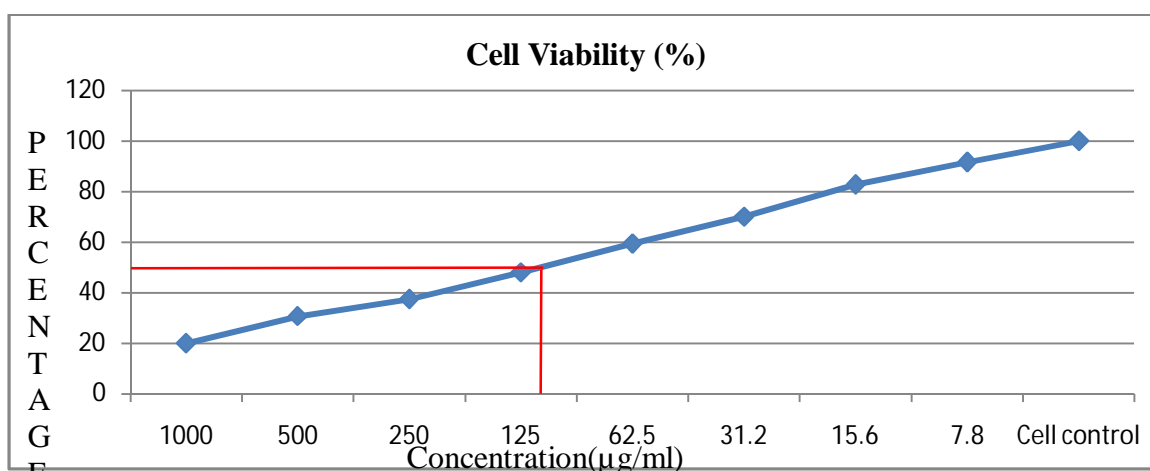


Figure 5. Viability of HeLa cells:

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 3, March 2017

Graphs are plotted using the % of Cell Viability on the Y-axis and concentration of the sample on the X-axis. The optimum cell viability was **48.04%** at a concentration of **125 µg/ml**. The maximum cell viability was **91.63%** and was obtained at the concentration of **7.8 µg/ml**.

IV. CONCLUSION

Ferulic acid produced from pineapple peel using *staphylococcus aureus* led to a high amount of phenolic compound released by solvent extraction method. The results of extracted ferulic acid showed good correlation with the standard ferulic acid. The TLC results proved that extracted sample was ferulic acid by comparing the bands of both standard and sample ferulic acid and the RF values of standard and extracted ferulic acid were found to be 0.986 and 0.9795. Analysis based on HPLC shows retention time for standard and extracted ferulic acid, $T_r = 6.30$ and 5.904 respectively. The percentage of antioxidant potential of standard and sample were found to be 87.30% and 81.92%. The FRAP value was expressed in μM and the values obtained are 2315 and 3985. The optimum cell viability was determined at a concentration of $125 \mu g/ml$ by treating the sample against HeLa cells. Further, the future study can be done by applying its therapeutic and beneficial functions in food, pharmaceuticals and cosmetic industries to treat cancer.

V. ACKNOWLEDGEMENT

We thank Dr.I.Seethalakshmi,Ph.D., (Managing Director) for providing the opportunity to work at 'Lifetech Research centre', Chennai, Tamilnadu.

REFERENCES

- [1] MacAdam, J.W., and Grabber J.H., "Relationship of growth cessation with the formation of diferulate cross-links and p-coumaroylated lignins in tall fescue leaf blades", *Planta* vol.215, pp. 783-793, 2002.
- [2] Ishii, T. "Structure and functions of feruloylated polysaccharides", *Plant Science* vol.127, pp. 111-127, 1997.
- [3] Graf, E. "Antioxidant potential of ferulic acid", *Free Radical Biology and Medicine* vol.13, pp. 435-448, 1992.
- [4] Beschia, M., Leonte, A. and Oancea, I. "Phenolic components with biological activity in vegetable extracts", *Bull.Univ.Galati* vol. 6, pp. 59-63, 1982.
- [5] Ou, S.Y., and Kwork, K.C., "Review Ferulic acid :pharmaceutical functions preparation and applications in foods", *Journal of the science of food and agriculture* vol.84, pp. 1261-1269, 2004.
- [6] Noor Hasyierah, M.S., Mohamed Zulkali, M.D., Dachyar, A., Muhammad Syarhabil, A. and Ku Syahidah, K.I. "Optimization of alkaline hydrolysis of paddy straw for ferulic acid extraction", *Industrial Crops and Products* vol.34, pp. 1635-1640, 2011.
- [7] Papagianni, M., Noke, S.E. and Filer, K. "Submerged and solid-state phytase fermentation by *Aspergillus niger*: Effects of agitation and medium viscosity on phytase production, fungal morphology and inoculums performance", *Food Technol. Biotechnology* vol.39, pp. 319-326, 2001.
- [8] Prakash Kumar Sarangi and Hara Prasad Sahoo "Ferulic acid production from wheat bran using *Staphylococcus aureus*", *New York Science Journal* vol.3(4), pp. 79-81, 2010.
- [9] Molyneux, P. "The use of the stable free radical diphenylpicryl-hydrazyl(DPPH) for estimating antioxidant activity", *Songklanakarin J. Sci. Technology* vol. 26, pp. 211-219, 2004.
- [10] Benzie, F.F., and Strain, J.J., "Ferric Reducing/ Antioxidant Power Assay: Direct Measure of Total antioxidant Activity of Biological Fluids and Modified Version for Simultaneous Measurement of Total Antioxidant Power and Ascorbic Acid Concentration", *Methods in enzymology* vol. 299, pp. 15-23, 1999.
- [11] Mosmann, T. "Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays", *J.Immunol.Methods* vol.65, pp. 55-63, 1983.
- [12] Aarabi A., Honarvar M., Mizani M., Faghihian H. and Gerami A. "Extraction and purification of ferulic acid as an antioxidant from sugar beet pulp by alkaline hydrolysis", *Italian Journal of Food Science* vol.28, pp. 362-375, 2016.