

**UTILIZATION OF NATURAL TANNINS FROM *ANACARDIUM OCCIDENTALIS* TESTA FOR PRODUCING THE INDUSTRIALLY IMPORTANT GALLIC ACID THROUGH SUBMERGED FERMENTATION**

**Lokeshwari Nallabilli\***

Assistant Professor, Department of Biotechnology, Dr. B. R. Ambedkar University, Etcherla,  
Srikakulam Dist., Andhra Pradesh, India.

Article Received on  
01 June 2016,

Revised on 21 June 2016,  
Accepted on 12 July 2016

DOI: 10.20959/wjpr20168-6731

**\*Corresponding Author**

**Dr. Lokeshwari Nallabilli**

Assistant Professor,

Department of

Biotechnology, Dr. B. R.

Ambedkar University,

Etcherla, Srikakulam Dist.,

Andhra Pradesh, India.

**ABSTRACT**

Gallic acid has enormous application in many fields and is regarded as a non toxic substance to man. Furthermore, considerable health promoting effects have been ascribed to gallic acid. This work demonstrated the ability of microorganism to utilize cashew testa and crude intermediate product to produce gallic acid in a submerged fermentation system. Under optimal time, extraction of tannins experiment was carried out and estimation of concern byproduct was estimated.

**KEYWORDS:** Microorganism, Gallic acid, Submerged Fermentation and Tannins.

**INTRODUCTION**

Tannins are a large class of complex phenolic compounds, comprising hydrolysable, condensed and complex tannins. Hydrolysable tannins are constituted of several organic acids, such as gallic and ellagic, which are usually linked by an ester-like bond with a glucose molecule. On acidic, basic or enzymatic hydrolysis, gallotannins produce both glucose and gallic acid (Janeth Ventura *et.al.* 2008). One of the major commercial applications of tannase is the hydrolysis of tannic acid to gallic acid, a key intermediate required for the synthesis of an antibiotic drug, trimethoprim (Hadi *et.al.* 1994).

Gallic acid is used in the pharmaceutical industry for the synthesis of antibacterial drugs and also in the food industry as a substrate for the chemical synthesis of food preservatives,

whereas propyl gallate is an important food additive to prevent food oxidation. (Darah *et.al.* 2011). Gallic acid is also used in the enzymatic synthesis of gallic acid esters, e.g., propyl gallate, which is used mainly as an antioxidant in fats and oils, as well as in beverages (Hadi 1993). Ethanolic extracts (gallic acid and ellagic acid) of the leaves and stems of Banaba (*Lagerstroemia speciosa* L.), has been used as traditional medicines and are effective in controlling diabetes and obesity and inhibits HIV-1 infection (Nutan *et.al.* 2013). The present work is exploring towards the utilization of waste cashew testa into useful industrial product gallic acid by using microbial fermentation.

## MATERIALS AND METHODS

**Sample Collection:** *Anacardium occidentale* testae were collected from the cashew industries in Visakhapatnam.

**Sample Preparation:** Samples were collected fresh and they were conserved, freeze-drying is the gentlest method of preservation and is recommended instead of freezing and air or oven drying.

**Sample handling:** After collecting the samples, Samples were stored in the absence of heat, and placed in a dark container.

**Sample extraction:** The dried testa of cashew husk was crushed using ball mill to get the particular size below 5mm. 4g of the powder was added to a bottle and extracted by reflux in 50ml 60% methanol for 2 hours. The mixture was filtered, and the filtrate was collected. The extract was then Concentrated to dryness by rotary vaporization at 30°C under reduced pressure and a light brown powder was obtained.

**Submerged Fermentation:** Obtained extracted powder (sample) was used in submerged fermentation by using Czapek's Dox medium.

**Estimation of Total Tannins:** Tannin estimation was done by following the protein precipitation method for the quantitative determination of tannins as described by Hagerman and Butler. 1978

**Estimation of Gallic acid:** Spectrophotometric method was used by using methanolic rhodanine for the estimation of gallic acid at 520nm by spectrophotometer (Shimadzu UV-1800) (Sharma *et.al.* 2000).

## RESULTS AND DISCUSSION

Currently in India, about 960 million tones of solid waste is being generated annually as by-products during industrial, mining, municipal, agricultural and other processes. In India more than 40% of solid waste generated annually is from organic and agricultural sources. There is a growing concern for these accumulating wastes as they are either being dumped in landfills, burnt, or left to rot in the open, leading to severe environmental pollution. A sustainable solution would be to utilize these solid wastes as an 'economical' alternative to costly raw materials and produce industrially important products of practical utility (Nandini *et.al.* 2013). Tannase has now been extensively used in different biochemical industries. The selected bacterium used in this study is able to synthesize high amounts of tannase through fermentation of crude tannin of *A. Occidentale*. Exploitation of these plant extracts could be a source of cheaper substrate for industrial production of microbial tannase.

**Table 1: Optimizing of the time period for the extraction of tannins from cashew testa.**

Time period (min.)	Tannin (mg/ml)
15	4.79
20	4.82
25	4.99
30	5.14
35	5.47
<b>40</b>	<b>5.82</b>
45	5.65
50	5.41

Obtained tannin extract was used for the production of gallic acid by using microbial fermentation. By using this method 30.12mg/ml gallic acid was obtained for 36 hours of fermentation with 120 rpm agitation speed at room temperature.

The worldwide annual demand of gallic acid is about 8,000 tons. Conventionally gallic acid is produced by acid hydrolysis of tannins, but this process releases a large amount of toxic effluent that causes environmental hazard. It also involves high production cost, low yield and purity (Aguilar *et.al.* 2001, Paranthaman *et.al.* 2009). Thus this gallic acid production from this waste cashew testa would help the country to produce gallic acid from cheap resources. Plenty of such efficacious raw materials ensured the possibility for exploitation of this microbial fermentation in large-scale production of gallic acid.

**REFERENCES**

1. Aguilar C. N, Augur C, Favela T. E and Viniegra G. G. Production of tannase by *Aspergillus niger* As-20 in submerged and solid-state fermentation: Influence of glucose and tannic acid. *Jrnl. Industrial Micro. Biotech.* 2001; 26(5): 296-302.
2. Darah I, Sumathi G, Jain K, and Lim Sheh Hong. Involvement of Physical Parameters in Medium Improvement for Tannase Production by *Aspergillus niger* FETL FT3 in Submerged Fermentation. *Biotechnology Research International.* Article ID 897931. 2011; 7.
3. Hagerman A. E and Butler L. G. Protein precipitation method for the quantitative determination of tannins. *Jrn.l of Agricultural Food Chemistry.* 1978; 26: 809–812.
4. Hadi T. A, Banerjee R and Bhattacharyya B. C. Optimization of tannase biosynthesis by a newly isolated *Rhizopus oryzae*. *Bioprocess Eng.* 1994; 11: 239-243.
5. Hadi T. A. 1993. Optimization of extracellular tannase (tannin cacyl hydrolase) production by locally isolated *Rhyzopus oryzae* (RO, IIT, EJP), MTech. Thesis, IIT Kharagpur, India.
6. Janeth Ventural, Ruth Belmares, Antonio Aguilera Carbo, Gerardo Gutierrez Sanchez, Raul Rodriguez Herrera and Crisobal Noe Aguilar. Fungal Biodegradation of Tannins from Creosote Bush (*Larrea tridentata*) and Tar Bush (*Fluorensia cernua*) for Gallic and Ellagic Acid Production. *Food Technol. Biotechnol.* 2008; 46(2): 213–217.
7. Nutan, Manoj M, Tanvi G, Tiyasa D, Shweta M, Samiksha S, Ajay K. S. R, Sharad K. S, Rakesh T, Swadesh M and Satish K. G. Ellagic acid & gallic acid from *Lagerstroemia speciosa* L. inhibit HIV-1 infection through inhibition of HIV-1 protease & reverse transcriptase activity. *Indian J Med Res.* 2013; 137: 40-548.
8. Paranthaman R, Vidyalakshmi R, Murugesh S and Singaravadivel K. Accelerated bioconversion of agricultural by products by supplementation of tannic acid in tannase production by *Aspergillus oryzae*. *Global jrnl. of biotech & biochem.* 2009; 4(1): 19-24.
9. Nandini K. E, Apoorva G and Krishna S. S. The Suitability of Natural Tannins from Food and Agricultural Residues (FAR) for Producing Industrially Important Tannase and Gallic Acid through Microbial Fermentation. *Intrnl. Jrnl. of Agri. and Food Sci. Tech.* 2013; 4(10): 999-1010.
10. Sharma S, Bhat T. K and Dawra R. K. A Spectrophotometric Method for Assay of Tannase using Rhodonine. *Analytical Biochem.* 2000; 278: 85-89.