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# A REVIEW: STUDY ON TANNASE PRODUCED BY MICROBIAL SOURCES AND ITS INDUSTRIAL APPLICATION

[1*]Sadia Siddique, [2]Anjali Kumari, [3]Vinod Kumar Gupta

[1,2]UG Student, [3]Scientist

[1,2]Department of Biotechnology, Anugrah Narayan College, Patna, India

[3]Rapture Biotech International Private Limited, Noida, India

*Abstract:* Tannin acyl hydrolase is commonly referred as Tannase, hydrolyses ester and depside of hydrolysable tannins to produce Gallic acid , glucose and galloyl ester. Tannase plays a central role in bioconversion of hydrolysable tannins, a well known precursor for manufacturing of broad spectrum antibiotics, trimethoprim. Another important application of Tannase is the removal of haze formation and wine. Apart from that, they are extensively used in the food industry, especially in instant tea production where it enhances the extractability and cold water solubility of key compounds. As a potential drug and recent application in agriculture for crop protection make Gallic acid a molecule of huge market potential. In view of growing demand, it is important to isolate high productive strain an develop. The study reviews on bacterial source and Industrial application.

***Index Terms* - Gallic acid, food industry, antibiotics, Tannase, industrial applications, tea production**

## I. Introduction

Tannase is also known as tannin acyl hydrolases. This unique enzyme is discovered by Teighem in 1867 in an experiment of formation of Gallic acid into an aqueous solution of tannins .it catalyzes the hydrolysis of ester bond and depside bond present in hydrolysable tannins to form glucose and Gallic acid. Tannins are used as the natural substrate for Tannase enzyme.

Also tannins enzyme attack Gallic acid methyl esters, but it possesses high specificity towards the moiety of the substrate. Tannic acid hydrolyzed to form Tannase and this enzyme used for the production of Gallic acid (3,4,5- tri-hydroxy benzoic acid ) and it found to be natural phenolic substrate for the Tannase enzyme. Tannase is used in the beer and wine industries to remove chill haze formation in beer and wine (1).

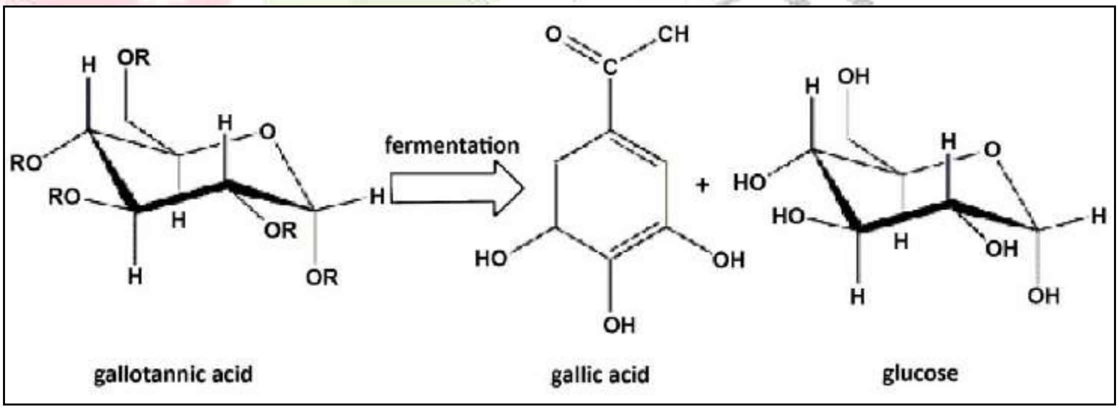


**Fig 1- Hydrolysis of Tannase by tannic acid**

Tannase is also used in manufacturing of instant tea and the production of Gallic acid, a substrate for propyl gallate production and trimethoprim synthesis (2, 3).

Realizing the immense potential of bacterial Tannase made it worthwhile to optimize the production process of this Tannase to achieve maximum yields. Conventional methods for the optimization of medium and fermentation conditions involve varying one parameter at a time while keeping the other constant. The process is time consuming and expensive. It also does not take into account combine interaction between various physicochemical parameters (4, 5).

Studies have shown that the tannin is responsible for reducing the amount of food intake, in other sense an anti nutritional factor. As a result of lowering the food efficiency and the net energy of metabolism and reduce the digestive protein because of its effect on the digestive enzyme, then lowering digestion of an amino acid as well as preventing degradation of the starch inside alimentary canal and cause damage to the cell lining and stomach and does influence in the degree of absorption of the rest food elements and its ability on the linkage with protein to form complex protein tannin for precipitate in the form of gel in water solution, so the foods which have level of tannin regards a low nutritional value of food, as well as tannin has a negative impact on the iron absorption being associated with iron in the digestive system thereby reducing of its vital provides and absorption. (6,7,8) Tannin can be obtained from plant, animal and microbial sources from plant sources, tannin rich enzyme is present in vegetables mainly in their fruits, leaves , branches and barks of tree like, *Konnam* and *basil* . Microbial way is the most important sources through which we can obtain the enzyme because the produce enzymes are more stable than similar once obtained from other sources. It does not require expensive extraction method; it can be easily extracted (9).

Tannins are also abundant plant constituents, similar to cellulose and lignin. Tannin is water soluble polyphenols. the connotation of the term tannic acid as well as hydrolysable gallotannins, I.e., those which yields Gallic acid on hydrolysis .hydrolysable gallotannins may be obtained from many sources including *Caesalpinia spinosa* (Tara), *Caesalpinia digyna*(Teri) and many others. Tannase was widely used for bioconversion of hydrolysable tannins to simple phenolic such as Gallic acid.

### 1.1 TANNINS – NATURAL SUBSTRATE FOR BIOCONVERSION TO GALLIC ACID

Of the major classes of secondary metabolites , phenolic are most commonly synthesized in plant in response to herbivores attack, pathogens, UV light cold or nutrient deficiencies . Plant phenolic comprise a diverse group of defender molecules ranging from low molecular weight phenolic such as coumarins ,stilbenes, flavonoids to complex polymers like lignin and tannins.

As reported tannins are widespread in the plant kingdom which includes pteridophytes , gymnosperms and angiosperm are generally accumulated in large amount roots, bark, leaves, and fruits. But they were found to be most abundant in the parts such as leaves or flowers which are more likely to get damaged or to eaten by herbivores (10).

## II. SOURCE OF TANNASE

Tannins are high molecular weight secondary phenolic metabolites of plant origin that have been traditionally considered anti-nutrients due to their capacity to bind and precipitate protein (11,12). This group of chemicals can be found in berries, fruits and chocolate among other dietary components.

Recently, Tannase have also been isolated from bacteria that populated environments rich in vegetable content, although just a few of the genes encoding bacterial Tannase have been described and even fewer have been characterized biochemically. The most studied Tannases Tannase are present in the bacteria isolated from the rumen, gutmicrobiota or soils with abundant vegetation (13, 14, 15). An inducible enzyme produced in the presence of tannic acid by a number of fungi (16) and bacteria. It hydrolyzes the ester and depside linkages of tannic acid to produce glucose and Gallic acid. The enzyme has wide applications in food, beverage, brewing, cosmetics, and chemical industries (17). It is mainly used for the preparation of Gallic acid, coffee flavored soft drinks, instant tea, high grade leather tannin, clarification of fruit juice and bear, food detannification, and to clean up highly polluting tannin from the leather industry effluent (18, 19). Beside this, Gallic acid (3, 4, 5 tri-hydroxy benzoic acid), the enzymatic product of hydrolytic cleavage of tannic acid, has several applications in chemical and pharmaceutical industries for the production of propyl gallate, pyrogallol, trimethoprim, semiconductor resin, etc. (20)

Tannase is now known to be an ever-present enzyme of the microbial world and has wide spread occurrence in various fungi. Most of the reported Tannase producing organisms are fungi and only a few are bacteria. All the above known isolates are from the sources like plant material, soil, vegetable liquor, and from animal feces. Yet the search continues for organisms which are better sources of Tannase. (21)

### 2.1 Fungal source

In nature a variety of Tannase producers have been identified which includes bacteria, fungi, yeast and plant sources. Among then many research proved that fungi are the most prominent producers of Tannase. In which *Aspergillus* and *Penicillium* was found to be most promising.

### 2.2 Bacterial source

Many gastrointestinal bacteria of adopted domesticated and wild animals have been found to produce Tannase.

### 2.3 Yeast source

The major source for Tannase is considered to be fungi and bacteria. Recently, much attention has been paid on the production of these enzymes from yeast sources by conventional and molecular biology approach, because of its shorter generation time and convenience towards molecular genetics.

**Table 1: Microorganisms of Tannase**

| Microorganisms | References |
|---|---|
| **Bacteria -** | |
| ***Achromobacter sp.*** | (22) |
| ***Bacillus Pumilis***<br>***Corynebacterium sp.***<br>***Klebsiella planticola***<br>***Bacillus polymyxa***<br>***Pseudomonas solanacearum*** | (23) |
| ***Lactobacillus plantarum***<br>***Lactobacillus paraplantarum***<br>***Lactobacillus pentosus*** | (24) |
| ***Bacillus cereus KBR9*** | (25) |
| ***Streptococcus bovis*** | (26) |
| **Fungi -** | |
| ***Aspergillus flavus*** | (27) |
| ***Pencillium chrysogenum*** | (28) |
| ***Pencilliya crellanumi***<br>***Trichoderma sp.***<br>***Helicostylumsp*** | (29) |
| ***Aspergillus awamori*** | (30) |
| ***FusariumSolani***<br>***Mucor sp.*** | (26) |
| **Yeast -** | |
| ***Pichia sp.***<br>***Debaryomyces hansenii*** | (23) |

## III. Types of Tannins

Tannins are divided in the following types:-

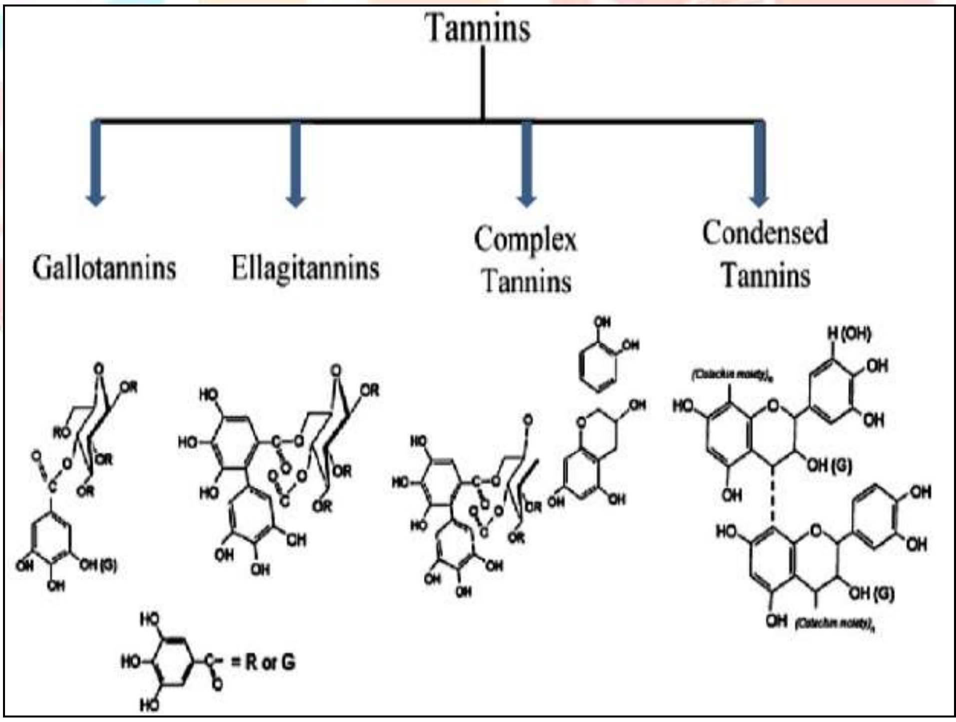


**Fig 2. Types of Tannins**

### 3.1 Gallotannins

Gallotannins is any of a class of molecules belonging to hydrolysable tannins. Gallotannins are polymers formed when Gallic acid, a polyphenol monomers, esterifies and binds with the hydroxyl group of a polyol carbohydrates such as glucose.

Tannic acid—Its chemical structure is given as C76H52O46, which corresponds with that of decagalloyl glucose, but in fact, it is in actuality, a mixture of polygalloyl glucoses or polygalloylquinic acid esters, with the number of galloyl moieties per molecule ranging from 2 up to 12 (depending on the plant source used for the extraction of the tannic acid). Tannic acid is usually extracted from *Caesalpina spinosa* (Molina) *Kuntze*, *Rhus semialata* Murray, *Rhus coriaria L.*, and *Quercus infectoria* Oliv Chinese gallotannin (*Rhus chinensis* Mill.) and Turkish gallotannin (*Quercus infectoria* Oliv.)—the number of galloyl moieties per molecule range from 8 up to 10, depending on the plant source. Turkish gallotannin contains penta-, hexa-, and heptagalloyl glucose, while Chinese gallotannin mainly holds decagalloyl glucose(31).

### 3.2 Ellagitannins

The ellagitannins are a diverse class of hydrolysable tannins, a type of polyphenol formed primarily from the oxidation linkage of galloylgroups are linked through c-c bonds, whereas the galloyl groups in Gallotannins are linked by depside bond (32).
Ellagitannins contain various members of hexahydroxydiphenoyl, units as well as galloyl units and / or sanguisprboyl units bounded to sugar moiety. In order to determine the quantity of every individual unit, the hydrolysis of the extracts with trifluoro acetic acid in methanol / water system is performed. Hexahydroxydiphenicacid, created after hydrolysis, spontaneously lactonized to ellagic acid, and sanguisorbic acid to sanguisorbic acid dillactone, while Gallic acid remairocycle, whereas gallotannins do not.

### 3.3 Complex tannins

Herein, a catechin unit is bound glycosidically to a gallotannin or to an ellagitann in unit .Oak complex tannins: Acutissimin A and eugenigrandin A—In these, the ellagitannin moiety of vescalagin is connected through a C–C bond to (+)-catechin and to (+)-gallocatechin, respectively. The acutissimins A and B were isolated from the bark of several *Quercus* species and the bark of *Castanea crenata* (Siebold & Zucc.) while *Eugenigrandin* A and *guajavin* B have also been found in the bark of *Psidium guujava* L(33).

### 3.4 Condensed tannins (Proanthocyanidins)

In such, all oligomeric and polymeric proanthocyanidins are formed by the linkage of the C-4 of one catechin (flavan-3-ol) with the C-8 or C-6 of the next monomeric catechin . While monomeric catechins and leuko-anthocyanidins have no tanning properties, when converted into oligomers and polymers, they do hold tanning properties by way of acidic and enzymatic action. Biosynthetically, the condensed tannins are formed by the successive condensation of the single building blocks, with a degree of polymerization between two and greater than fifty blocks being reached. Oligomers and polymers consisting of two to ten catechin units are also known as flavolans . Derivatisations as O-methylation, C- and O-glycosylation and O-galloylation are frequently reported, and a structural complexity is most prominently present in the rearrangement products of proanthocyanidins.
The variation in hydroxylation pattern brings about a classification of the proanthocyanidins into several subgroups: propelargonidins (3,4′,5,7-OH), procyanidins (3,3′,4′,5,7-OH), prodelphinidins (3,3′,4′,5,5′,7-OH), proguibourtinidins(3,4′,7-OH), profsetinidins (3,3′,4′,7-OH), prorobinetinidins (3,3′,4′,5′,7-OH), proteracacidins (4′,7,8-OH; found only as a synthetic), promelacacidins (3′,4′,7,8-OH), pro-apigeninidins (4′,5,7-OH), and proluteolinidins (3′,4′5,7-OH). Of these subgroups, procyanidins are the most common (33).

## IV. APPLICATION OF TANNINS IN VARIOUS INDUSTRIES

Currently, hydrocarbon based raw materials are exploited in different petro-chemical industries ranging from fuel to cosmetology. It leads to the widespread deficiency of raw materials eventually that creates high inflations, environmental degradation, and adverse effect on human and animals' health. Tannins can be the best natural raw material for emerging and traditional industries. This is attributed to tannin unique natural properties, chemical structure, and commercial properties (34).

### 4.1 Food industry –

Tannins are the secondary metabolites present in a substantial amount in plant based food product due to their positive effect on the food as antibacterial and antioxidants, they are the major constituent of foods. Tannins are used as food preservatives packaging materials, and food enhancement. Which owe protective nature. Recently, a packaging material was prepared by introducing an tannins into cellulose nanofibrils in a single step process of mechanical fibrillation. This newly developed packaging film offers high density and enhanced surface hydrophobicity which resulted in almost six times improvement in air barrier and antioxidant properties. Simultaneously, nano cellulose based film are active packaging material which also provide a green sustainable non-toxic packaging source for food and pharmaceuticals product (35, 36) .
Microorganism, fungus, yeast, virus, pollens and chemicals are the biggest threat to food's shelf life in home as well as food markets. Biochemically proanthocyanidins and Gallic acid are flavonoids monomers by nature and our major food constituents isolated from pomegranate, strawberry, blackberry, raspberry, walnut, almonds and seeds. Various studies have proved that tannins prevent growth of microorganisms. Tannins are quite effective against the resistant methicillin- resistant *Staphylococcus aureus* (MRSA) (37, 38). Moreover, punicalagin, an ellagitannin, isolated from pomegranate peel show very strong antibacterial properties against S*taphylococcus aureus* and can be used to control contamination by *S. aureus* in food industry.

### 4.2 Wood industry -

Wood is the inseparable part of the furniture and several important industries. Wood contains organic acid tannins, and lignocellulosic materials which are most susceptible to biological, chemical, and physical decaying agents. Therefore, wood requires a large number of synthetic adhesive, glues, anti termite chemical, and other coating materials in order to protect it. However, these materials have tremendously benefitted the wood industry, but they adversely affect the environment conditions. Because synthetic phenolics , amino resins, and formaldehyde used in a wood industries are generally carcinogenic in nature. To overcome this problem, scientists are investigating natural materials of herbal or animal origins such as tannins that can be the best option for alternative material to be used in wood industries (39).

### 4.3 Medicinal industry -

Tannins are present in a variety of plants that are utilized as food and feed (40). Tannins seem to be a two-edged sword, since they are beneficial to health due to their chemo-preventive activities against carcinogenesis and mutagenesis, but simultaneously, they may be involved in cancer formation, hepatotoxicity, or anti-nutritional activity (41).Tannins are known as anti-nutrients i.e. they are able to reduce the effectiveness of converting digestible nutrients into new substances. The molar mass of tannin molecules affects the tannin characteristics directly. It has been found that the higher the molar mass of tannin molecules, the stronger the anti-nutritional effects and the lower the biological activities (40, 41).

### 4.3.1 Tannin as preventive medicine

Tannins (from French "tanner" is tanning of the skin) - water-soluble phenolic products, which are capable to precipitate proteins from aqueous solutions. Tannins are the second of the most widespread in nature group of phenols (after lignin) and these organic compounds are considered to be secondary metabolites of plants. Tannins are found in bark, wood, leaves and (or) fruits (sometimes in seeds, roots, tubers) of many plants. Tannins give a tart taste to leaves and fruits. They have a certain range of biological properties that provide mechanisms for protecting plants from pests and diseases caused by bacteria and viruses. Tannins suppress the growth of a number of microorganisms and these compounds are resistant to microbial action (40).

Tannins are a group of complex substances that are built from oligomeric chains. These chains are characterized by the presence of polyphenolic compounds. Molecular weight of tannins is 500 to 20,000 Dalton. One of the defining properties of tannins is the ability to form strong complexes with proteins and other macromolecules, such as starch, cellulose and minerals (42). There are hydrolyzed and condensed (non-hydrolysable) tannins. According to modern classification, four classes of tannins are distinguished: Gallotannins, Ellagatannins, condensed tannins and complex (hydrolyzed tannins) (40, 43, 44). Tannins have mechanisms to protect against diseases caused by pathogenic fungi, bacteria and viruses. The bitter taste of tannins helps protect plants from insects and herbivores. Food industry and it inhibits enzymatic reactions in brewing. The increased content of tannins in animal feed reduces the digestive efficiency and, consequently, the productivity of farm animals. There are reports that a reduction of tannin leads to an intensification of nitrogen assimilation in ruminant animals and, accordingly, to an increase of milk production. It is established that tannins contained in foodstuffs participate in the development of some types of cancer (45).

The presence of polyphenol compounds often determines the medical properties of plants. Therefore, since ancient times, tannin-rich plant extracts have been used in medicine. For example, tea extract is traditionally used in China and Japan as an anti-inflammatory, diuretic, antiseptic, styptic agent, and in cancer treatment (46). Tannins, as phenol compounds, are effective chelators for metal ions, so they can be used in the treatment of heavy metal poisoning (47). There are reports of a potential inhibitory effect of tannins on HIV-1 (48).

### 4.3.2 Immuno modulatory activities of Tannase

As mentioned above, tannins are known as anti-nutrients. However, at the same time, there are many reports of positive effects of tannins on human health: antitumor effects, the ability to lower blood pressure and modulate various types of immune response. Probably, these effects are associated with antioxidant properties of tannins (40). It has been reported that ellagic acid is effective antioxidant tannin with anti-tumor properties. Proanthocyanides, contained in grapes and olives, represent another type of tannins with extremely high antioxidant properties. Thus, tannins present in food products of plant origin in various concentrations have a visible effect on human health. It is also important to note that it is not necessary to take high amounts of tannins, since they can be included in the process of formation of malignant tumors and interfere with normal processes of digestive system. However, consumption of adequate amounts of correct type tannins beneficial to human health due to their effect on metabolic enzymes, immuno-modulation and other functions (40). Products of anaerobic decomposition of many tannins formed in the intestinal tract can also form compounds with beneficial effects to human health. For example, such degradation products are derivatives of propionic or phenyl acetic acids (48). These compounds have an anti-inflammatory effect when absorbed in the gastrointestinal tract (GIT). These substances, along with other products of decomposition of tannins, also have a wide range of antimicrobial activity in GIT, suppressing the development of pathogenic microorganisms.

## V. Conclusion :-

Tannin has the potential for a wide range of applications but due to higher cost & lower yield, their use is quite limited. Tannin play important role in food industry, wood industry and medical industry but there are several limitations to its uses as it binds to every molecule such as protein and starch and hence gives a reduced yield. This paper discusses about the various types of tannins and their sources; at the same time this paper also shows its uses in medical industry in detail. For future, in view of growing demand for the Tannase for industrial application, it is very important to develop high yielding & cost effective process to increase its usage in the biotechnical industry.

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