NATURAL POLYPHENOLICS

Dr. N. Lokeswari &
Lenin Kumar Bompalli

Natural Polyphenolics

First Edition

Dr. N. Lokeswari
Lenin Kumar Bompalli

Publishing Partner:
International Journal of Scientific and Research Publications (ISSN: 2250-3153)
IJSRP Inc

Preface

NATURAL POLYPHELONICS is a brief introduction and classification of the natural tannins. It provides basic information about polyphenolic tannins. This Monograph is based mainly on the research work of *ANACARDIUM OCCIDENTALES* L. (Cashew tree), is the source for extracting tannins. Cashew tree psudo fruit are on the front and back cover. We are very greatful to the authors, a list of which is given in the bibliography.

Authors express their heartful gratitude to their parents and other family members whose love, care and moral support always in their daily lives. Every effort is made to prepare the monograph as accurately as possible, mistakes may occur. Readers are requested to communicate any errors in this monograph. We are very much appreciating criticisms, suggestions from my readers, which will be gratefully acknowledged. We are thankful to International Journal of Scientific and Research Publications (IJSRP) for publishing this monograph.

Dr. N. Lokeswari

Lenin Kumar Bompalli

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Authors

Dr. N. Lokeswari, Assistant Professor, Department of Biotechnology, Dr. B. R. Ambedkar University, Etcherla, Srikakulam, Andhra Pradesh, India and Principal Investigator, Department of Science & Technology, Under Ministry of Science & Technology, New Delhi, India is the co- author of this book. She has ten years research experience and seven years teaching experience in biotechnology subject. She has published twenty research papers in National and International journals and presented 25 research papers in different International and National conferences. Her research area includes bioactive compounds, microbial enzymes, strain improvement and fermentation technology. She was Reviewer in International Journal – African Journal of microbiology.

Lenin Kumar Bompalli, Research Scholar, Department of Biotechnology, Dr. B. R. Ambedkar University, Etcherla, Srikakulam, Andhra Pradesh, India is the Author of this book. He has four years research experience and two years teaching experience in biotechnology subject.

You can get in touch with authors by emails lokeswari.n@hotmail.com and leninbiotechnology2020@gmail.com

Dedicated to

Who Could Not See Today

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1. INTRODUCTION

The word phenol is extremely recent and reflects a standard technology. "Tanning" (waterproofing and preserving) was the word wont to describe the method of remodeling animal hides into leather by exploitation plant extracts from completely different plant components of various plant species. Plant parts containing tannins include bark, wood, fruit, fruit pods, leaves, roots, and plant galls.

The term "tannin" was wont to describe substances in vegetable extracts used for changing animal skins into stable leather (Seiguin, 1796; Alwala *et al.,* 2014).

Tannins aswell broadly action in accepted aliment such as cashew nut, hazelnut, walnut, banana, strawberry, raspberry, blackberry, grape, and mango and so on. Vegetable tannins can be classified into hydrolysable and nonhydrolysable, getting tannic acerbic the alot of adumbrative hydrolyzable tannins. Tannic acerbic is one of the a lot of abounding assets abstracts of plants (Rodriguez *et al.*, 2008).

Tannins accredit to the assorted accumulation of actinic compounds in wine that may accept an aftereffect the color, crumbling adeptness and arrangement of the wine. Whereas tannins cannot be smelled or tasted, they can be perceived during wine tasting by the concrete dehydration awareness and faculty of acerbity that they can leave in the mouth. This is due to the addiction of tannins to acknowledge with proteins, such as the ones begin in saliva. In aliment and wine pairing, foods that are top in proteins (such as red meat) are generally commutual with tannic wines to abbreviate the acidity of tannins. However, abounding wine drinkers apprehend the acumen of tannins to be a absolute trait-especially because it relates to mouthfeel. The

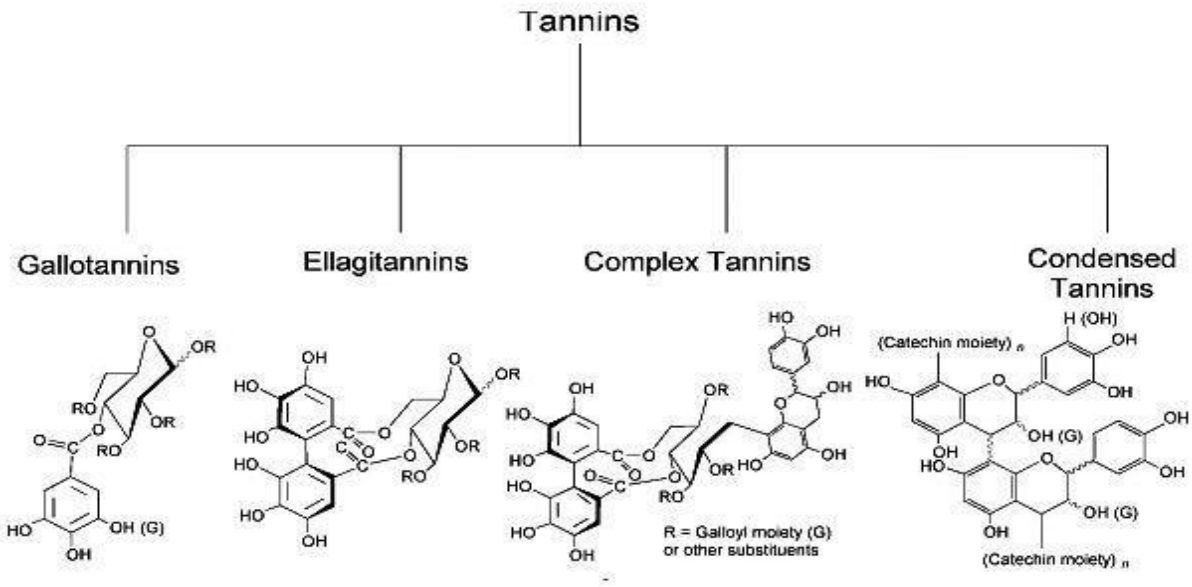
administration of tannins in the winemaking action may be a key allotment with in the after quality.

Fig 1: Fermenting with the stem, seeds and derma will access the tannin agreeable of the wine. A abstraction in wine assembly and burning has apparent that tannins, in the anatomy of proanthocyanidins, accept a benign aftereffect on vascular health. The abstraction showed that tannins suppressed assembly of the peptide amenable for hardening arteries. To abutment their findings, the abstraction aswell credibility out that wines from the regions of southwest France and Sardinia are decidedly affluent in proanthocyanidins, and that these regions aswell aftermath populations with best activity spans.

Reactions of tannins with the phenolic admixture anthocyanidins creates addition chic of tannins accepted as bistered tannins which influences the blush of red wine. Commercial affairs of tannins, accepted as enological tannins, fabricated from oak wood, grape berry and skin, bulb gall, chestnut, quebracho, gambier and myrobalan fruits, can be added at altered stages of the wine assembly to advance blush durability.

a. Classification of tannins

Tannins are divided into four major groups: gallotannins, ellagitannins, condensed tannins, and complex tannins. **Tannins are advised to be the plant's accessory metabolic articles and play no absolute role in the plant's metabolism** (Augilar *et al.*, 2007).



b. Hydrolysable tannins

Hydrolysable tannins are polyphenolic bulb accommodation acquired from address – to **pentagalloyllated β**-D.glucopyranose

Kumar and Singh, 1984 reported hydrolysable tannins are present in a lot of of the residues from college plants and are polyphenolic compounds formed by the affiliation of sugars and gallic and ellagic acids via ester linkages.

i. Gallotannins

Gallo tannins are hydrolysable tannins which accommodate a axial amount of carbohydrates which are esterified by phenolic like gallic acid.

Snehal and Ramesh, 2013 declared that Gallo tannin which has esterified with gallic acid, is accepted that, gallo tannin will absolution those gallic acerbic residues in chargeless anatomy aloft hydrolysis like added hydrolysable tannins.

In the analysis of Mathur *et al.,* 1996; Bhattacharya *et al.,* 2002; Patel and Goyal, 2011; Snehal and Ramesh, 2013 gallo tannins begin in legumes, vegetables, fruits and beverages and are the a lot of abounding antioxidants in our diets. Gallotannins were appear to acquire assorted biological activities including anti cancer, anti oxidant, anti allergic, anti inflammatory, antihyperglycaemic, lipid blurred and anti microbial activities and their burning may accord to anticipate stroke, cardio vascular affection disease, neurodegenerative diseases.

ii. Ellagitannins

Pornsiri Pitchakarn *et al.*, 2013 reported ellagic acerbic is a polyphenolic admixture and present in fruits and berries such as blackberries, strawberries, raspberries and pomegranates.

Stoner and Gupta, 2001; Han *et al.*, 2006; Pornsiri Pitchakarn *et al.,* 2013 reported ellagic acerbic has anticarcinogenic, antioxidant and antifibrosis properties. Anti-carcinogenic aftereffect of eallagic acerbic was apparent in several blight types including esophageal, colon, skin, breast and prostate cancers.

c. Complex tannins

The structures of the circuitous tannins are congenital up from a gallotannin assemblage or an ellagitannin unit, and a catechin unit. Acutissimin A is circuitous tannin, accepting a flavogallonyl assemblage (nonahydroxytriphenoyl unit) apprenticed glucosidically to C-1, and affiliated via three added hydrolysable ester bridges to the D-glucose acquired polyol.

d. Condensed tannins

Condensed tannins are aswell accepted as proanthocyanidins (PAs), and abide of phenols of the flavon blazon flavonoids. They are aswell alleged flavolans because they are polymers of flavan-3-ols such as catechin or flavan-3, 4 - diols accepted as leucocyanidins. Biosynthetically the abridged tannins were formed by the alternating abstract of a individual architecture block, with a amount of polymerization amid two and greater than fifty blocks getting accomplished (Karamali and Teunis, 2001).

Liang-liang and Yi-ming, 2008 appear PAs, the oligomeric and polymeric flavan-3-ols, which are affiliated through C4-C8 or C4-C6 linkages. The assortment of abridged tannins is accustomed by the structural airheadedness of the monomer units (different hydroxylation patterns of the ambrosial rings A and B, and altered configurations at the chiral centers C2 and C3). The admeasurement of PA molecules can be declared by their degrees of polymerization (DPs). PAs are baptize acrid and can anatomy complexes with proteins and polysaccharides.

Santos-Buelga and Scalbert, 2000 reported because of their almighty antioxidant accommodation and accessible careful furnishings on beastly health. PAs are of abundant assimilation in diet and medicine. PAs accept antioxidant backdrop accompanying to their abolitionist scavenging accommodation (Ricarda Da Silva *et al*., 1991), and these backdrop accept been acclimated

adjoin affection ache through abbreviation lipid oxidation. It was accepted that the chargeless abolitionist scavenging backdrop of PAs may abate the accident of cardiovascular diseases, blight (Bagchi *et al.*, 2000) and claret clotting, and assertive types of trimeric PAs may assure adjoin urinary amplitude infections (Santos-Buelga and Scalbert, 2000).

Marwa *et al.,* 2013 appear that abridged tannins affect comestible accumulation to the beastly by complexing with digestive enzymes, comestible and autogenous proteins. The affection of tannins, a altered actinic acreage is to bind to augment proteins and thereby abate boundless breakdown of protein in rumen and access availability of top superior protein for assimilation in the lower gut of ruminants.

2. TANNINS OCCURRENCE

Esmaeil *et al.,* 2013 appear tannins action by itself in the bulb commonwealth and are by itself occurring polyphenolic compounds with capricious atomic weights. These phenolic compounds alter from others by accepting the adeptness to accelerate proteins from solutions.

Kumar and Vaithiyanathan, 1990 appear the actuality of tannins in the leaves of 46 altered breed of fodder copse and declared that about timberline leaves and browse accommodate both types of tannins (hydrolysable and condensed).

Alwala *et al.,* 2014 appear the capital families of dicotyledons, which accommodate tannins, cover Aceraceae, Actinidiaceae, Anacardiaceae, Bixaceae, Burseraceae, Combretaceae, Dipterocarpaceae, Ericaceae, Grossulariaceae and Myricaceae while those of monocotyledons cover Najadaceae and Typhacea

Tannins accept advanced alignment furnishings on animals and bacilli (Waterman and Mole, 1994). Hagerman, 1989 appear tannins are polyphenolic accessory metabolites of plants

which anatomy hydrogen bonds in solutions, consistent in the accumulation of tannin-protein complexes.

Hina Iqbal and Ashima Kapoor, 2012 appear tannins are present in ample amount of augment and forages. The accumulation of complexes of tannins with nutrients, such as carbohydrates, proteins and minerals, has abrogating furnishings on their utilization.

Tannins are begin in the skin, stems, and seeds of wine grapes about may aswell be alien to the wine through the use of oak barrels and chips or with the accession of tannin powder. The accustomed tannins begin in grapes are accepted as proanthocyanidins as a aftereffect of their adeptness to absolution red anthocyanin pigments if they are acrimonious in an acerb solution. Grape extracts are mainly affluent in monomers and baby oligomers (mean amount of polymerization <8). Grape berry extracts accommodate three monomers (catechin, epicatechin and epicatechin gallate) also as procyanidin oligomers. Grape derma extracts accommodate four monomers (catechin, epicatechin, gallocatechin and epigallocatechin), as able-bodied as procyanidins and prodelphinidins oligomers. The tannins are formed by enzymes during metabolic processes of the grapevine. The abundance of tannins begin by itself in grapes varies depending on the array with Cabernet Sauvignon, Nebbiolo, Syrah and Tannat getting 4 of the a lot of tannic grape varieties. The acknowledgment of tannins and anthocyanins with the phenolic admixture catechins creates addition chic of tannins accepted as bistered tannins which access the blush of red wine. Commercial affairs of tannins, alleged enological tannins, fabricated from oak wood, grape berry and skin, bulb gall, chestnut, quebracho, gambier and myrobalan fruits, can be added at altered stages of the wine assembly to advance blush durability. The tannins acquired from oak access are accepted as "hydrolysable tannins" getting created from the ellagic and gallic acerbic begin aural the wood.

a. Plant resources of tannins

Madhavakrishna *et al.*, 1960 appropriate that aloft growth, plants amalgamate ample amounts of gallic acid, chebulinic acerbic and hexahydroxyphenic acid, and as the plants aftermath fruit, the bake-apple ripens and it was envisioned that these acids adeptness become esterified with glucose with the advice of tannase to anatomy circuitous tannins. Aloft abscission of the bake-apple the esterase action in the tannase may accord to the hydrolysis of the preformed tannins.

Tannins are broadcast in breed throughout the bulb kingdom. They are frequently begin in both gymnosperms as able-bodied as angiosperms the administration of tannin in 180 families of dicotyledons and 44 families of monocotyledons (Cronquist). A lot of families of dicot accommodate tannin-free breed (tested by their adeptness to accelerate proteins).

The best accepted families of which all breed activated accommodate tannin are: Aceraceae, Actinidiaceae, Anacardiaceae, Bixaceae, Burseraceae, Combretaceae, Dipterocarpaceae, Ericaceae, Grossulariaceae, Myricaceae for dicot and Najadaceae and Typhaceae in Monocot. To the ancestors of the oak, Fagaceae, 73% of the breed activated (N = 22) accommodate tannin. For those of acacias, Mimosaceae, alone 39% of the breed activated (N = 28) accommodate tannin, a part of Solanaceae amount drops to 6% and 4% for the Asteraceae. Some families like the Boraginaceae, Cucurbitaceae, Papaveraceae accommodate no tannin-rich species. The alot of abounding polyphenols are the abridged tannins, begin in around all families of plants, and absolute up to 50% of the dry weight of leaves. Tannins of close dupe tend to be of a cathetic attributes rather than of the gallic blazon present in abstemious woods.

There may be a accident in the bio-availability of still added tannins in plants due to birds, pests, and added pathogens.

b. Localization in plant parts

Tannins are begin in leaf, bud, seed, root, and axis tissues. An archetype of the area of the tannins in axis tissue is that they are generally begin in the advance areas of trees, such as the accessory phloem and xylem and the band amid the case and epidermis. Tannins may advice adapt the advance of these tissues.

c. Cellular localization

In all vascular plants advised so far, tannins are bogus by a chloroplast-derived organelle, the tannosome. Tannins are mainly physically amid in the vacuoles or apparent wax of plants. These accumulator sites accumulate tannins alive adjoin bulb predators, but aswell accumulate some tannins from affecting bulb metabolism while the bulb tissue is alive; it is alone afterwards corpuscle breakdown and afterlife that the tannins are alive in metabolic effects.

Tannins are classified as ergastic substances, i.e., non-protoplasm abstracts begin in cells. Tannins, by definition, accelerate proteins. In this condition, they accept to be stored in organelles able to bear the protein precipitation process. Idioblasts are abandoned bulb beef which alter from adjoining tissues and accommodate non-living substances. They accept assorted functions such as accumulator of reserves, excretory materials, pigments, and minerals. They could accommodate oil, latex, gum, adhesive or pigments etc. They aswell can accommodate tannins. In Japanese persimmon (Diospyros kaki) fruits, tannin is accumulated in the corpuscle of tannin cells, which are idioblasts of parenchyma beef in the flesh.

Table 1: Tannin rich material:

Sugarcane bagasse	Lekha and Lonsane, 1994
Wheat bran	Sabu *et al.,* 2005.
Tamarind seed	Hina Iqbal and Ashima Kapoor, 2012.
Palm kernel cake	Sabu *et al.,* 2005.
Chestnut bark	Deschamps *et al.,* 1983.
(*Caesalpinia spinosa*) Tara tannins	Pourrat *et al.,* 1985.
(*Quercus infectoria*) Gall nuts	Barthomeuf *et al.,* 1994.
(Rhus coriaria) leaves	Barthomeuf *et al.,* 1994.
Barbatimao Stem bark	Kelly Ishida *et al.,* 2009.
(*Terminalia chebula)* fruits	Banerjee *et al.,* 2005.
(*Caesalpinia digyna)* pod cover	Banerjee *et al.,* 2005.
(Canarium album) twigs, leaves and stembark	Liang-liang and Yi-ming, 2008.
(*Phyllanthus emblica*) Amla leaves, bark and fruits	Hina Iqbal and Ashima Kapoor, 2012.
(*Syzygium cumini*) Jamun leaves	Hina Iqbal and Ashima Kapoor, 2012.
Creosote bush leaves	Trevino-Cueto *et al.,* 2007.
(*Anogeissus latifolia*) Dhawa leaves	Dinesh Prasad *et al.,* 2012.
(*Caesalpinia coriaria*) Divi Divi pods	Dinesh Prasad *et al.,* 2012.
(*Psidium guazava*) Guava bark	Hina Iqbal and Ashima Kapoor, 2012.
(*Psidium guazava*) Guava leaves	Mailoa *et al.,* 2014.

(*Cassia fistula*) Amaltash leaves	Hina Iqbal and Ashima Kapoor, 2012.
(*Eucalyptus glogus*) Eucalyptus leaves and bark	Hina Iqbal and Ashima Kapoor, 2012.
(*Acacia nilotica*) Keekar leaves	Hina Iqbal and Ashima Kapoor, 2012.
(*Magnifera indica*) Mango leaves	Hina Iqbal and Ashima Kapoor, 2012.
(*Morus macroura*) Mulberry leaves	Hina Iqbal and Ashima Kapoor, 2012.
(*Punica granatum*) Pomegranate rind	Hina Iqbal and Ashima Kapoor, 2012.
Coffee pulp	Roopali *et al.,* 2013.
(*Terminalia bellirica* Roxb.) Bahera seeds	Sahu Bharti and Koche Vijaya, 2012.
(*Terminalia bellirica* Roxb.) Bahera dried fruits	Arijit *et al.,* 2012.
(*Vigna subterranea*) Bambara nuts	Difo *et al.,* 2013.
(Anacardium occidentale) Cashew husk	Lenin Kumar *et al.* 2015.
(*Anacardium occidentale*) Cahew leaves	Yogini Jaiswal *et al.,* 2013.
(*Garcinia Atroviridis*) Asam gelugor leaves	Ainnie *et al.,* 2013.
(Ficus religiosa Linn.) Leaves	Lakshmi HimaBindu *et al.,* 2013.
(Quercus infectoria) fruits	Vaibhav Vaidya *et al.,* 2013.

Madhavakrishna *et al.,* 1960 hypothesized that the condensed tannins are formed as intermediates or precursors that would later be transformed into complex tannin molecules. The tannin content of the plant material may also serve as a defence mechanism by which the plant

may be able to protect itself against microbial invasion. They also suggested that tannase does not only protect the plant against microbial invasion, but also against attacks from herbivores. When the plant leaves are under attack from herbivores the cells lose compartmentation, which brings the tannase into contact with the tannin substrate in the leaves. The substrate is then hydrolysed into harmful low molecular weight phenolic degradative compounds.

3. APPLICATIONS OF TANNINS

The world market of fruit juice is around US $ 5 billion/year. Cashew apple juice is a vitamin C rich by-product of cashew production. Sale of this juice in the world is hampered by its astringency and instability caused by the presence of tannins (Gustavo *et al.,* 2001).

Mahdi Haroun *et al.,* 2013 reported some groups of tannins are used in treatments fostering wound healing and were acting on arachidonic acid metabolism in leucocytes with important roles in reversing inflammations.

Because of phenol group, tannins can be used as an antibacterial, and have properties like alcohol is an antiseptic that can be used as an antimicrobial component (Mailoa *et al.,* 2014).

The convergent evolution of tannin-rich plant communities has occurred on nutrient-poor acidic soils throughout the world. Tannins were once believed to function as anti-herbivore defenses, but more and more ecologists now recognize them as important controllers of decomposition and nitrogen cycling processes. As concern grows about global warming, there is great interest to better understand the role of polyphenols as regulators of carbon cycling, in particular in northern boreal forests.

Leaf litter and other decaying parts of a kauri (Agathis australis), a tree species found in New Zealand, decompose much more slowly than those of most other species. Besides its

acidity, the plant also bears substances such as waxes and phenols, most notably tannins, that are harmful to microorganisms.

Tannin rich fresh water draining into Cox Bight from Freney Lagoon, Southwest Conservation Area, Tasmania, Australia

The leaching of highly water soluble tannins from decaying vegetation and leaves along a stream may produce what is known as a blackwater river. Water flowing out of bogs has a characteristic brown color from dissolved peat tannins. The presence of tannins (or humic acid) in well water can make it smell bad or taste bitter, but this does not make it unsafe to drink.

Tannins leaching from an unprepared driftwood decoration in an aquarium can cause pH lowering and coloring of the water to a tea-like tinge. A way to avoid this is to boil the wood in water several times, discarding the water each time. Using peat as an aquarium substrate can have the same effect.

Many hours of boiling the driftwood may need to be followed by many weeks or months of constant soaking and many water changes before the water will stay clear. Adding baking soda to the water to raise its pH level will accelerate the process of leaching, as the more alkaline solution can draw out tannic acid from the wood faster than the pH-neutral water.

Softwoods, while in general much lower in tannins than hardwoods, are usually not recommended for use in an aquarium so using a hardwood with a very light color, indicating a low tannin content, can be an easy way to avoid tannins. Tannic acid is brown in color, so in general white woods have a low tannin content. Woods with a lot of yellow, red, or brown coloration to them (like southern yellow pine, cedar, redwood, red oak, etc.) tend to contain a lot of tannin.

4. ANALYSIS OF TANNINS

Major methods for tannin analysis can be classified into three groups: calorimetric methods, protein binding methods and other methods. Due to the complexity of tannins, several methods have been developed for their quantification and unfortunately, none of them are completely satisfactory (Alwala *et al.,* 2014).

a. Vanillin assay

Sun *et al.,* 1998 reported the Vanillin – HCl assay is specific for a narrow range of flavanols and dihydrochalcones that have a single bond at the 2, 3 positions and free m- oriented hydroxyl groups on the B- ring.

b. Folin Denis assay

This assay is the most widely used type for measuring total phenol content in plant products and beverages. The principle is based on the reduction of phosphomolybdic phosphotungstic acid (Folin Denis reagent) to a blue colour complex in alkaline solution by phenols (Folin and Denis, 1912). This assay is relatively non-specific as it also reacts with several other compounds including xanthine, proteins and some amino acids (Lowry *et al.,* 1951).

c. Prussian Blue assay

Price and Butler, 1972 reported this assay is based on the reduction of the ferric ion ($Fe3^{+}$) to the ferrous ion ($Fe2^{+}$) by tannins and other phenolic compounds to form ferro ferri cyanide (Fe(III)[Fe(II)(CN)6]"), which is known as Prussian blue. The absorption of this

complex can be measured at 720nm. Polyphenols with a varying hydroxylation pattern and degree of polymerization react differently in this assay.

d. Butanol – HCl assay

This assay is specific for PAs or condensed tannins. The method involves the HCl catalyzed depolimerization of condensed tannins in to butanol to yield a red anthocyanidin product that can be detected spectropotometrically. Problems arise in this method are of tannin polymers are cleaved into dimmers or trimers instead of monomers and this leads to an underestimation. The degree of polymerization of the PAs can be estimated by combining the Butanol-HCl assay with the vanillin assay. The acid butanol assay measures the total number of flavanoid residues present and the vanillin assay measures the number of molecules. The Butanol – HCl assay is also used to estimate the amount of insoluble tannins from extraction residues and all red pigments is not dissolved in resulting in tannin underestimation.

e. Rhodanine assay

This assay is specific for one type of hydrolysable tannins that are gallotannins. In this method, the sample is subjected to hydrolysis to release gallic acid. The reaction between gallic acid and the dye rhodanine produces an intense colour that is measured spectrophotometrically.

f. Wilson and Hangerman assay

This method is specific for ellagitannins (one type of hydrolysable tannins). In this assay, the sample is subjected to hydrolysis to release ellagic acid. The reaction between ellagic acid and the sodium nitrite produces colored solution that is measured spectrophotometrically.

5. TESTS FOR TANNINS

There are three groups of methods for the analysis of tannins: precipitation of proteins or alkaloids, reaction with phenolic rings, and depolymerization.

a. Ferric chloride ($FeCl_3$) test

Powdered plant leaves of the test plant (1.0 g) are weighed into a beaker and 10 ml of distilled water are added. The mixture is boiled for five minutes. Two drops of 5% $FeCl_3$ are then added. Production of a greenish precipitate was an indication of the presence of tannins. Alternatively, a portion of the water extract is diluted with distilled water in a ratio of 1:4 and few drops of 10% ferric chloride solution is added. A blue or green color indicates the presence of tannins.

b. Hide-powder method

400 mg of sample tannins are dissolved in 100 ml of distilled water. 3 g of slightly chromated hide-powder previously dried in vacuum for 24h over CaCl2 are added and the mixture stirred for 1 h at ambient temperature. The suspension is filtered without vacuum through a sintered glass filter. The weight gain of the hide-powder expressed as a percentage of the weight of the starting material is equated to the percentage of tannin in the sample.

c. Stiasny's method

100 mg of sample tannins are dissolved in 10 ml distilled water. 1 ml of 10M HCl and 2 ml of 37% formaldehyde are added and the mixture heated under reflux for 30 min. The reaction mixture is filtered while hot through a sintered glass filter. The precipitate is washed with hot water (5x 10 ml) and dried over $CaCl_2$. The yield of tannin is expressed as a percentage of the weight of the starting material.

6. EXTRACTION OF TANNINS

There is no single protocol for extracting tannins from all plant material. The procedures used for tannins are widely variable. It may be that acetone in the extraction solvent increases the total yield by inhibiting interactions between tannins and proteins during extraction or even by breaking hydrogen bonds between tannin-protein complexes.

Tannins are phenolic compounds that precipitate proteins. Tannins are the fourth most abundant plant constituent after cellulose, hemicelluloses and lignin. They are composed of a very diverse group of oligomers and polymers. Tannins can be complex with proteins, starch, cellulose and minerals (Mailoa *et al.,* 2014).

Bhat *et al.,* 1998 reviewed Tannins are naturally occurring water soluble polyphenols with molecular weight ranging from 0.3-5 kd. They are classified into three groups, hydrolysable tannins which consist of polyhydric alcohol esterified with gallic acid, condensed tannins formed from monomeric flavan-3-ol and recalcitrant to hydrolysis and catechin gallates which occupy an intermediate position sharing the properties of hydrolysable and condensed tannins.

Mueller-Harvey *et al.,* 1987 reported after lignin, tannins are the second most abundant group of plant phenolics. The large amount of phenolic hydroxyl groups allows the tannins to form

complexes with proteins and to a lesser extent with other macromolecules like cellulose and pectin.

Marco *et al.,* 2009 reported one of the major characteristics of tannins is their ability to form strong complexes with protein and other macromolecules such as starch, cellulose, and minerals.

Roopali *et al.,* 2013 reported tannins are toxic and bacteriostatic properties making non-reversible complex with proteins.

7. METHODOLOGY

a. Plant sampling

A sample of cashew husk was collected from February to June 2011 in the A.P zone of Visakhapatnam. The collected samples were placed in black polyethylene plastic bags, and dried in an oven at 60 °C, for a period of 7 to 10 days. After that the dried husk were powdered in a Ball mill, and stored in plastic bottles in a dark place.

b. Extraction of crude tannins

Collected cashew husk from cashew industries near to Visakhapatnam, were grained into small particles, and dried in hot air oven at 60°C for 24 hrs. Tannins are extracted using water and acetone. Optimal yields are obtained from fresh tissues or freeze-dried tissues. Optimal yields are not obtained from dried tissues (tannins are irreversibly combined with other polymers). After eliminating the acetone (distillation), the pigments and lipids are removed from the aqueous solution by a solvent extraction.

c. Determination of moisture content

Moisture content of the sample was determined by the direct oven method of association of official Agricultural chemist (AOAC, 1960). One gram of the Cashew husk were taken in a porcelain crucible and kept in an electric oven at 90°C for about 4 – 5 hours (until constant weight), cooled in desiccators and weighed. From the loss in the weight of the sample, moisture content was determined.

$$\%\,\text{moisture content (W/W)} = \frac{\text{loss in weight}}{\text{Total weight}} \times 100$$

d. Extraction of Tannins

The Cashew husk were dried and milled using ball mill to get the particle size below 0.5 mm. Water is preferred as solvent in view of its high saturation limit of the dissolved solids, inherent safety and ease of separation. Tannins are extracted by using pressure autoclaving method. The Cashew husk was collected from the Costal area of Andhra Pradesh, India. The Cashew husk were dried and milled to get the particle size below 5mm. Small particle size achieve high extraction efficiency. Water is preferred as solvent in view of its high saturation limit of the dissolved solids, inherent safety and ease of separation. However the water used for extraction (leaching) should be soft and should not contain iron. The extraction efficiency depends on process factors like temperature, time and pressure. The material is extracted under pressure on autoclaving process at 10 PSI for 30 min and obtained extract was evaporated with vacuum filter and the obtained powder form was used for the entire experimentation. This process generally yields more extract than ordinary open vat extraction. It is generally accepted; **that pressure extraction yields a higher amount of "extract" than is obtained by the open leach**

method, but the product is darker in color and contains a higher proportion of non-tannin substances.

e. Detection of tannin by paper chromatography

Presence of tannin in the cashew husk extract was confirmed through paper chromatographic analysis. A descending mode of solvent system containing n – butanol, acetic acid and water (4:1:5) was used for the study. Detection of the spot was made by FeCl3 (0.1 g% in 30% methanol) as coloring spray reagent and confirmed after comparing it with standard tannic acid. (Lenin Kumar and Lokeswari. 2012)

f. Microorganism and inoculum

i. Microorganism:

The non-pathogenic tannase – producing strain of *Aspergillus oryzae* obtained from the NCIM Pune, was used in the present study. They were propagated on Malt extract Agar. The spores were collected using Tween 80 (0.01 %). Czapek minimal medium was prepared using the plant extracts as the sole carbon source (pH=5.0). *Aspergillus oryzae* spores were inoculated in this medium at a concentration of 2·107 spores/mL.

ii. Culture conditions

Batches of ten Erlenmeyer flasks (250 mL) with 3 g of polyurethane foam (cubes of 0.5 cm3) sterilized and impregnated (at 70 % humidity) with 7 mL of the inoculated medium were used. Reactors were covered with brown paper and incubated at 30 °C. Kinetics of the SSF process was monitored by collecting samples at 0, 24, 48, 72 and 96 h of the process. Collected samples

were washed using 25 mL of distilled water; and then the fermentation liquid was recovered by compression using a sterilized 60- mL syringe. Cotton was plugged inside the syringe to avoid the passage of particles and the collected samples were stored in small plastic bottles covered with aluminium foil at freezing temperature until further analysis.

g. Total tannins

Folin-Ciocalteu method (FAO/IAEA, 2000) was used for the analysis of tannins (*13*). In this assay, 800 mL of the sample were put into a test tube and mixed with the same volume of Folin-Ciocalteu (Sigma-Aldrich) reagent, shaken and left for 5 min. Then this solution was diluted with 5 mL of distilled water and analyzed in a UV-Visible spectrophotometer at 725 nm for the determination of total tannins and at 480 nm for hydrolysable tannins. The obtained absorbance values were analyzed against the standard curves prepared with tannic and gallic acid for total phenols and tannins, respectively.

h. Gallic acid

Citrate buffer (pH=5.0), methanolic rhodanine 0.67 % and KOH (0.5 mol/L) were needed for this assay and all reactants were pre-incubated at 30 °C for 5 min. An aliquot of 0.5 mL was mixed with 0.3 mL of methanolic rhodanine solution and incubated under the same conditions mentioned above. After that, 0.2 mL of KOH solution were added and incubated again. Finally, 4 mL of distilled water were added to the reaction mixture and incubated at 30 °C for 10 min and the absorbance was read at 520 nm.

i. Ellagic acid

Inside dark test tubes 10 mg of ellagic acid or 0.1 mL of the sample were placed, and then 0.1 mL of H2SO4 (2 mol/L) were added. The test tubes were frozen at –15 °C for 10 min, then sealed and the air was removed with a syringe. The test tubes with ellagic acid were incubated during 24 h at 100 °C. The test tubes were washed with 3 mL of pyridine and filtered. For determination, to 1mL of filtered sample, 1.1 mL of pyridine and 0.1 mL of HCl (37 %) were added, and then the mixture was shaken and incubated at 30 °C for 5 min. After incubation, 0.1mL of NaNO2 (0.01 %) was added and the absorbance was read at 538 nm. (Lokeswari and Lenin Kumar. 2012).

8. RESULTS AND DISCUSSION:

To evaluate antioxidant activity, the aqueous polyphenolic extracts were used as carbon source during the solid-state fermentation process using the fungal strain of *Aspergillus oryzae*. This fungus demonstrated its capacity to degrade hydrolysable tannins and the resulting monomers were either consumed or accumulated. Cashew husk extracts recorded the highest consumption of total phenols in the samples collected at 48 h of SSF process (Fig. 1). Initial concentration of total phenols in unfermented Cashew husk extracts was 7.29 mg/g of plant, and after 48 h of fermentation, it was 6.04 mg/g of plant. The obtained results demonstrated that *Aspergillus oryzae* degraded the hydrolysable tannin polymers present in phenolic extract of Cashew husk.

The monomers obtained by the hydrolysis of this kind of tannins were consumed by the fungus during the first 48 h of culture and then the hydrolysis products were accumulated.

However, it was observed that the monomers of condensed tannins were not consumed. The hydrolysable tannins present in the Cashew husk extracts were consumed (16 %) during the first 72 h of fermentation. At 96 h, the hydrolysable tannins were degraded and approx. 15 % of the monomers of phenolic acids were accumulated. The biodegradation of condensed tannins and the respective accumulation of catechin monomers were proportional to time (Table 1). The fungal strain recorded a similar behavior in the fermentation kinetics of substrate tested. The highest concentration of condensed tannins was reached at 96 h of fermentation process. During the fermentation, an increase of condensed tannins of 42 % was observed.

Table 2. Extracted Tannin content

Hydrolysable Tannins	
Time/h	**Cashew husk extract (mg/g)**
0	8.63
24	8.00
48	7.36
72	7.23
96	7.32
Condensed Tannins	
24	8.70
48	9.00
72	10.2
96	12.4

The accumulation of gallic acid indicated the depolymerization of gallotannins and after its release this substance could be used as a substrate. *A. oryzae* consumed nearly 72 % of free

gallic acid in the extract; the minimum concentration reported was 0.14 mg/g of cashew husk at 48 h of the process. After that, an accumulation of gallic acid was observed, indicating that the rate of gallotannin hydrolysis was faster than the consumption rate of gallic acid. In the fermentation of cashew husk extracts, the gallotannins were depolymerised after 48 h and the glucose and gallic acid were released. The highest level of gallic acid was reached at 96 h with a value of 0.08 mg/g of cashew husk.

The highest consumption of total phenols and hydrolysable tannins in the phenolic extracts of cashew husk was reached at 48 h of fermentation. This could be due to the fact that the phenolic extracts of cashew husk have complex polysaccharides, and moreover, the studied strain preferred to consume free monophenols and glycosides like gallic acid and glucose present in the extracts before the production of hydrolytic enzymes to degrade tannins.

Table 3: 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
40	5.82
45	5.65
50	5.41

Table 4: Optimizing the temperature for extraction of tannins from cashew testa

Temperature (ºC)	Tannin (mg/ml)
30	5.12
35	5.23
40	5.53
45	5.62
50	5.83
55	4.89
60	4.61

Some tannin-rich sources and several microorganisms have been used for gallic acid production and the hydrolytic enzyme responsible for its production is the tannase or tannin acylhydrolase. It had been reported earlier that tannase can also hydrolyse ellagitannins. But the results of the present study did not show this pattern and hence we consider that this enzyme is unable to degrade ellagitannins. Results obtained in this study are similar to those reported by Shi *et al.* 2005 for valonea tannins (79.2 % at 168 h). Comparing these results, the lower rate of hydrolysis in valonea tannins could be due to low protein levels in its phenolic extracts. However, Belmares-Cerda *et*al. 2006 reported better results using the same substrates as tested in this study. This could be explained by the fact that they used the cashew husk as a substrate, and this matrix has a high content of protein and tannin-protein complexes (Naczk et.al. 2000). Several fungal species such as Penicillium, Chaetomium, Fusarium, Rhizoctonia, Cylindrocarpon and Trichoderma (19) were reported to use the monomers of gallic acid as a substrate for the oxidative breakdown to a simple oxidative acid, which then enters the citric acid cycle (Bhat et.al. 1998) and is converted to pyrogallol. Finally, our results demonstrated the

possibility of considering that tested cashew husk in this study, could be employed in the microbial production of antioxidants due to their high tannin content.

9. CONCLUSION:

Phenolic extracts of cashew husk can be used as a carbon source by A. oryzae. The gallic acid can be consumed by the fungus during the solid-state fermentation while ellagic acid is not used as a substrate. It is possible to decrease or increase the content of phenolic compounds by controlling the process of solid- -state fermentation. This study demonstrated the feasibility of the production of potent nutraceuticals. This is the first work about the use of aqueous phenolic extracts of cashew husk as a substrate for solid- -state fermentation and for the production of important nutraceuticals like gallic and ellagic acid. However, it is necessary to optimize the fermentation process.

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Appendix

ABBREVIATIONS USED IN THE TEXT

^{o}C	:	degree centigrade
%	:	percent
g	:	gram(s)
mg	:	milligram(s)
mm	:	millimeter(s)
cm	:	centimeter(s)
ml	:	milliliter(s)
L (or) l	:	liter(s)
hr	:	hour(s)
min	:	minute(s)
kd	:	kilo daltan
w	:	weight
v	:	volume
nm	:	nano meter(s)
β	:	beta

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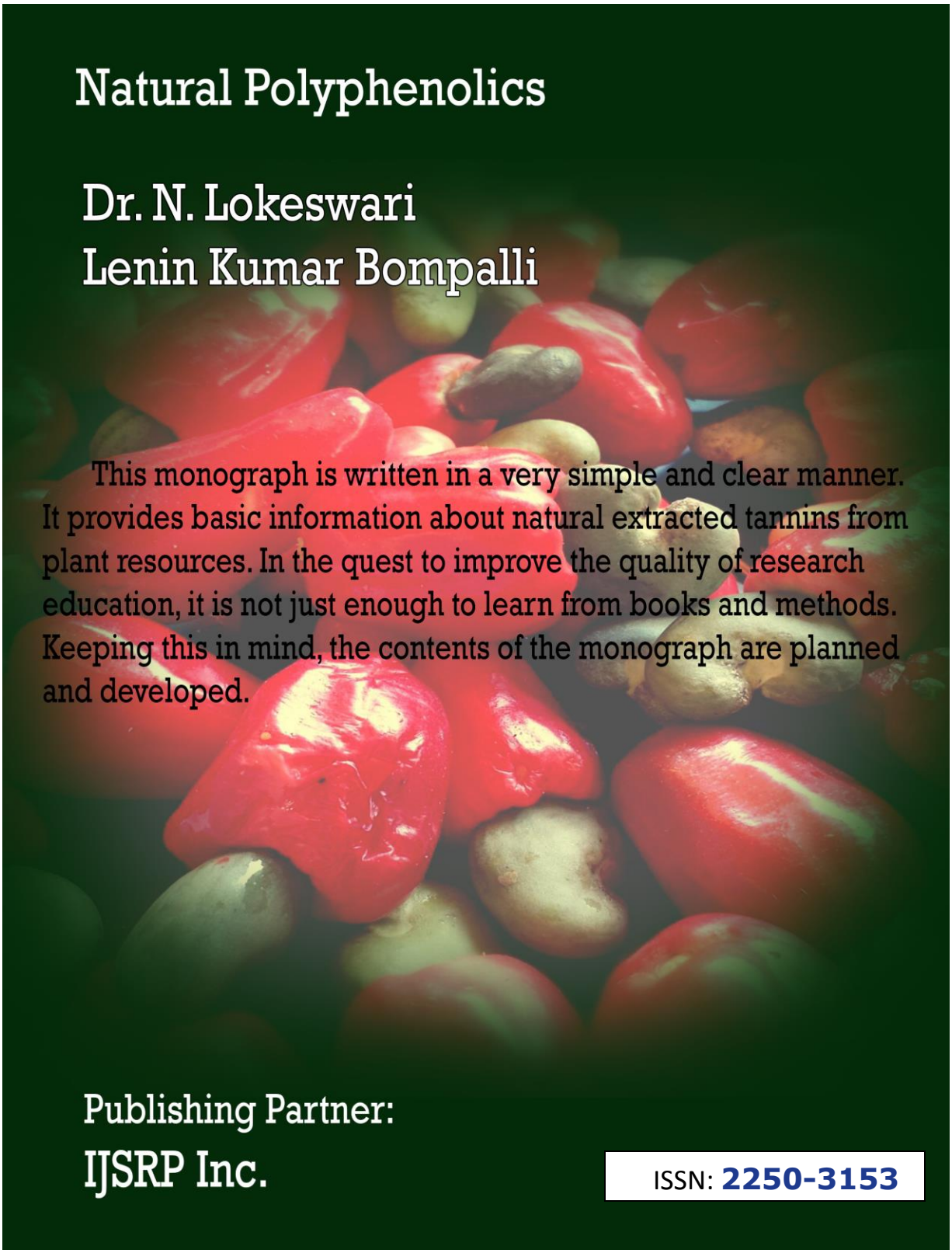
Natural Polyphenolics

Dr. N. Lokeswari
Lenin Kumar Bompalli



This monograph is written in a very simple and clear manner. It provides basic information about natural extracted tannins from plant resources. In the quest to improve the quality of research education, it is not just enough to learn from books and methods. Keeping this in mind, the contents of the monograph are planned and developed.

Publishing Partner:
IJSRP Inc.

ISSN: 2250-3153