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Estimation of total phenol and tannin content present in the leaf, bark and fruits of an endemic semi-evergreen tree species *Terminalia pallida* Brandis

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Abstract
Terminalia pallida species was endemic to Seshachalam hills with a good source of phytochemicals. The leaf, bark and fruits of the tree were having high-quality phytochemicals in large quantities hence these parts used to cure various diseases by the local tribal people. The present study was about to estimate the total phenols, non- tannin phenols, hydrolysable tannins and condensed tannins present in the three parts of this endemic tree and their applications. The results were shown that the total phenol and hydrolysable tannins (HTs) were present in the higher quantities, whereas the non-tannin phenols and condensed tannins (CTs) were in very low quantity. As the leaf, bark and fruits were showing phytochemicals in higher concentration, they can be used in the production of high-quality drugs from individual parts as well as by combining them in proper percentages.

Keywords: *T. pallida*, endemic, estimation of phenols and tannins

1. Introduction
Fragrance, essential oils and wax have given the highest importance in the cultures of the Mesopotamian and Egyptian societies and their production has done with the complete business point of view. The same type of information has collected in the surrounding regions of Baghdad dated back to 3500 BC. Primary and secondary metabolites were collectively known as phytochemicals. Secondary metabolites help the plants in the acclimatization process and also play the major role in the protection of a plant (Rahini. V, 2014) [1], (Ruby Tiwari & C.S. Rana, 2015) [2] & (S.B. Nasrin Fathima, 2015) [3]. Phytochemicals were present in all parts of a tree (Sharma Swaati, 2014) [4], from the past secondary metabolites were used for profitable purposes and they were categorized into three groups they were "Essential oils", these include a combination of terpenoids used as flavoring agents, perfumes and solvents. "Glycosides", these include flavonoids, saponins, phenolics, tannins, cyanogenic glycosides and mustard oils all were used as dyes, food colours and medicines (Eg. hormones, antibiotics etc.). "Alkaloids" a highly different bunch of compounds with over 4000 structures and all the naturally present alkaloids were of plant origin (Rahini. V, 2014) [1]. Among the phytochemicals most important were phenols and tannins (Sytar Oksana *et al*., 2012) [5].
Phenols were secondary metabolites with one aromatic ring in their structure C_6 along with one or more hydroxyl groups. Phenols were synthesized from cinnamic acid, this was formed from phenylalanine by the act of l- phenylalanine ammonia-lyase pal (Ec 4.3.1.5), and this was an enzyme present in between primary (Shikimate pathway) and secondary metabolism phenylpropanoid. Phenols were differentiated based on the carbon atoms attached to the primary structure of phenol (Simple phenols, benzoic acids, phenylpropanoids and flavonoids), (A. Michalak, 2006) [6] & (Amita Bhattacharya, 2010) [7]. They were two groups "Simple phenols" and "Highly complex phenols" having a number of aromatic rings bonding together. Simple phenols include among others, p-hydroxybenzoic acid, o-hydroxybenzoic acid (salicylic acid), caffeic acid, gallic acid, vanillic acid, syringic acid, coumaric acid or cinnamic acid. Highly complex phenols with aromatic ring were formed in the shikimic acid pathway from amino acid phenylalanine. In the process of formation of phenolic compounds, the release of the amino group –NH2 from phenylalanine has easily taken place and this form the cinnamic acid with the help of a catalyst enzyme Phenylalanine ammonia-lyase (PAL). This cinnamic acid used in the synthesis of complex phenolic compounds. Phenols and phenolic compounds had chelate property in the case of heavy metal ions. Phenols also have antioxidant properties and show good free radical reactions.

Suitable functional groups like hydroxyl and carboxyl in phenols help to chelate iron and copper ions. It was noticed that these valuable properties of phenols were due to the presence of nucleophilic aromatic rings rather than the occurrence of definite functional groups (Kamila Kulbat, 2016) [8]. Phenols antioxidant property shows inhibition of membrane lipid peroxidation by catching alkoxyl radicals and this was due to the structure of molecules and the number of position of hydroxyl groups. Phenols stabilized membranes by controlling the diffusion of free radicals and reduce the peroxidation of membrane lipid by decreasing their fluid nature. It proves the ability of the phenols to bind to essential membrane proteins and phospholipids and this is the reason for the "polar character" of the compounds and permit suspension in water. Under the various environmental factors and stress conditions, phenols were showing improvement of phenylpropanoid metabolism and the total phenolic compounds in plants (A. Michalak, 2006) [6].

Polyphenols were the largest groups with 8000 compounds out of these few were having simple structures (e.g. phenolic acids) to the polymeric substances like tannins. These include forming of insoluble complexes with proteins, polysaccharides, nucleic acids or alkaloids and also exhibit other characters such as bioactivities related to the antioxidant activity (Vit Koleckar *et al*., 2008) [9]. Tannins were polyphenolic biomolecules, astringent with a bitter taste. The tannin compounds were widely distributed in many plant species commonly found in both gymnosperms and angiosperms (tannic acid, gallic acid, catechins & chlorogenic acid) (Himanshu Jaiswal *et al*., 2018) [10]. Phloroglucinol is belonged to tannins and plays an important role in the ripening of the fruit. Plant tannins have molecular weights ranging from 500 to over 3000 (Praveen Kumar Ashok 2012) [11]& (Archana A. bele *et al*., 2010) [12]. Several researchers had classified tannins into variously on their molecular basis, structural formation etc. Based on their chemical structure and biological activities tannins were classified into hydrolysable tannins and condensed tannins (Balamurugan *et al*., 2018) [13]. Based on the polyphenol groups in their molecules tannins were previously classified into two groups they are "Pyrogallol type tannins" and "Catechol type (or catechin type) tannins", recent advancement in tannin chemistry has given remaining two groups to "hydrolyzable tannins" and "condensed tannins" (Takuo Okuda, 2011) [14]. Other classifications accepted about tannins were three main groups "Hydrolysable tannins", "Proanthocyanidins" (Condensed tannins) and "Phlorotannins". Phlorotannins were structurally most simple tannin group present in "Phaeophyceae" members (Juha-Pekka Salminen, 2011) [15]. Generally, Tannins were divided into "True tannins" and "Pseudo tannins". True tannins were again differentiated into Hydrolysable, Condensed and Complex tannins. Hydrolysable tannins once again differentiated into "Gallitannins (GTs) and Ellagitannins (ETs)" (Himanshu Jaiswal *et al*., 2018) [10]. Hydrolysable tannins were also called gallotannins when these contain a central core of glucose or another polyol esterified with gallic acid, whereas hydrolysable tannins when contains hexahydroxydiphenic acid or its dilactone form and ellagic acid then these called as ellagitannins (Takuo Okuda, 2011) [14]. Metabolism of hydrolysable tannins contains pentagalloylglucose (PGG) as a basic unit and other molecules are derived from these tannins. The structural variety in all these compounds formed by different oxidative linkages and oligomeric compounds with molecular weight between 2000 to 5000 Dalton were formed by intermolecular oxidation reactions (Vit koleckar *et al*., 2008) [9]. Based on the structure ETs were divided into six subgroups they were hexahydroxydiphenoyl (HHDP) esters, dehydro-HHDP esters, nonahydroxytriphenoyl (NHTP) esters, flavonoellagitannins the remaining two are oligomers with both varying degrees of oligomerization types of bonds between the monomers and it was noticed that modified dehydro-HHDP esters were gathered in "Combretaceae and Euphorbiaceae" families (Juha-Pekka Salminen, 2011) [15]. Condensed tannins (Muhammad Aslam Shad *et al*., 2012) [16] & (Martin Heil *et al*. 2002) [17]. Proanthocyanidins are another name given to the condensed tannins was polymers of 2 to 50 (or more) flavonoid units those were joined by two carbon bonds and not susceptible to being cleaved by hydrolysis. These condensed tannins were abundantly present in fruit and their products, it was a distinctive group of phenols and it was associated with the astringency and colour of fruits (S.B. Nasrin Fathima, 2015) [3]. More importantly condensed tannins and gallotannins (GTs) present over ellagitannins (ETs), since CTs and GTs have generally much higher PPC (protein precipitation capacity) than ETs. It was given that most of the plant cells concurrently doesn't produce both the GTs and ETs, because the production of ETs shows a direct complete adverse effect on CTs and synthesis of flavonoids. Due to this reason, both types of tannins do not present in high concentrations in the same tissue material. Hence these contents formed in opposite seasons and perform their functions. It was reported that ETs present in the peak stage in young tissues whereas CTs were most abundantly present in old leaves (Juha-Pekka Salminen, 2011) [15]. Although hydrolysable tannins and most condensed tannins were water soluble, few very large condensed types of tannin are insoluble (S.B.Nasrin Fathima, 2015) [3].

Terminalia pallida (Shaik Althaf Hussain *et al*., 2017) [19] is a rare species and endemic to Seshachalam hills of Chittoor district (Kummari Rajasekhar *et al*., 2013) [20]. *Terminalia* was the "Second largest genus" of Combretaceae family distributed in the subtropical and tropical regions such as Egypt, India etc. The family consists of 20 genera and 475 species out of the 200 species were belongs to Terminalia, about 30 species were found in Africa and this genus has great diversity in their morphological, anatomical and karyotypical way. This genus has been known for rich phytochemicals such as phenols, flavonoids, alkaloids, triterpenoids, tannins and other compounds. Due to this *Terminalia* species have multiple biological, pharmacological and medicinal activities (Hanaa Mohamed El-Rafie, 2014) [21]. Phytochemical extracts from *Terminalia* species have been known for their antioxidant and antimicrobial properties. They are used in the management of cardiovascular diseases, myocardial infarction, degenerative neurological diseases, cancer, amyloidosis, acute pancreatitis, arthritis, atherosclerosis, inflammatory bowel disease, diabetes, senile dementia, retinal degeneration and senile cataract particularly in humans because of outstanding antioxidation potential (S. Ramya *et al*., 2008) [22]. The leaf, bark and fruits had the medicinal value hence highly used as anti-inflammatory, analgesic, fever, cold, cough, diabetic, dysentery and diarrhoea (T. Bharathi *et al*., 2015) [23]. Fruits have been using in the treatment of ulcers, diarrhoea and in venereal disease (Malaya Gupta *et al*., 2002) [24]. The fruit and bark of this tree has been using from the Vedic period (1500–500 BC) in the treatment of cardiac disorders (D. V. Singh *et al*., 2002) [25]

and also as a diuretic, to stop swelling, abdominal disorders, bacterial infections, sore throats, conjunctivitis, dysentery, gastric ulcers, headaches, fever, heart diseases, hookworms, hypertension, jaundice, leprosy, nosebleed, edema and pneumonia (Fahmy *et al*., 2015) [26]. The fruits produced in *Terminalia* species were having 100% potential antioxidants (Bhatnagar *et al*., 2013) [27]. To cure fissures, cracks in feet and in veterinary medicine they used the fruit (dry) paste mixed with turmeric. The dried fruit powder used as a remedy to burns by applied externally and with water as decoction control diabetes and dry pickles were made by fruits (M.Siddaiah and K.Ravindra Reddy, 2015) [28] & (T. Bharathi *et al*., 2015) [23]. Fruits as in dry powder form used as antipyretic, purgative, diuretic, against cold, cough and to prevent diarrhoea the dry fruit powder decoction used orally. Terpenoids were generally found in fruits and are considered to be natural antioxidants (Bhatnagar *et al*., 2013) [27]. The fruit of this tree was used in the treatment of hepatic disorders and the treatment of diabetes by tribal people. *T. pallida* fruit extract showed a significant anti-diabetic on rats at a dose of 0.50g / kg BW. A notable antiulcer activity against indomethacin, histamine and at the same time Swiss albino rats by enhancing the antioxidant state of the gastric mucosa thereby reducing mucosal damage was achieved by the Ethanolic fruit extract of *T. pallida* (Fahmy NM *et al*., 2015) [26].

2. Material and Methods

2.1 Collection of leaf, bark and fruit material of *Terminalia pallida*

The three parts of this endemic species were collected from Japali teertham of Tirumala hills. The leaves and fruits are washed with tap water and then with distilled water to remove any foreign particles which can change the accuracy of all parameters in the secondary metabolites. The three parts were separately dried in a cool and shade environment for 20 days after that those parts powdered stored in three airtight jars for future use. The dry powder of leaf, bark and fruits were taken into conical flasks with standard procedure estimated the total phenols and Tannins.

2.2 Preparation of leaf, bark and fruits crude extract samples for estimation of phenol &tannin content

For the estimation of total phenols and tannins in leaf, bark and fruits the powder samples were taken 400mg each in three conical flasks and to each sample added 40 ml Diethyl ether containing 1% acetic acid and mixed to remove the pigment material. To these solvents containing test tubes added 20 ml of 70% aqueous acetone and kept in the electrical shaker for 2 hrs for extraction. Finally, these were filtered through Whatman No. 1 filter paper and samples were kept in a refrigerator at 4 ºC, to that extract folin phenol reagent and Na_2CO_3 solution was added after 40 min. at room temperature, the O.D values were taken at 725nm in spectrophotometer and concentrations were estimated from the standard curve for three samples. Total phenol was estimated as tannic acid equivalent and expressed on a dry matter basis. For the estimation of non-tannin phenols present in leaf, bark and fruit samples, 200mg PVP has taken into 3 test tubes each, added 2.0 ml of distilled water and 2.0ml tannin extract. The mixtures were vortexes and filtered through Whatman No.1 filter paper and filtrates were taken for estimation of non–tannin phenol. Three filtrates were taken into the three test tubes and volume was made to 1.0 ml with distilled water then added folin phenol reagent 20% Na_2CO_3 after 40 min. at room temperature, the O.D values were taken at 725nm in spectrophotometer and concentrations were estimated from the standard concentration of non–tannin phenols and were calculated from the standard curve.

2.3 Tannins: Total tannins were calculated by subtracting non-tannin phenol from total phenol. For the estimation of condensed tannins in leaf, bark and fruit samples, 0.5 ml of tannin extracts were taken in three test tubes with 3.0 and added Butanol, Hcl and 0.1 ml of ferric reagent. These test tubes were vortexes and mouths of the test tubes were covered with glass marble and boiled them for 60 min. Similarly, blanks were prepared for each sample but without heating the reagent. The test tubes were cooled to room temperature and readings were taken at 550nm using Spectrophotometer and condensed tannins as leucocyanidin equivalent was calculated.

$$\text{Percentage of Condensed Tannins} = \text{A550nm} \times 78.26 \times \frac{\text{Dilution factor}}{\text{\% of Dry matter}}$$

Hydrolyzed tannins were obtained by the subtraction of condensed tannins from total tannins.

3. Result and Discussion

3.1 Results showing Total Phenol and Tannin Content present in Leaf, Bark and Fruits of *Terminalia pallida*

Table 1: Results of Estimated Total Phenol and Tannin content

S. No.	Secondary Metabolites	Quantitative Estimation (mg/g DW)		
		***T. pallida* Leaf**	***T. pallida* Brak**	***T. pallida* Fruit**
1.	Total Phenol content	291.41	447.92	573.08
2.	Non - Tannin Phenols	68.67	68.67	68.85
3.	Condensed Tannins	2.614	2.637	0.19
4.	Hydrolysable Tannins	288.78	445.28	572.89

Results have shown that (Table. 1) phenols and tannins in three parts of *T. pallida* were estimated as tot al phenols, non-tannin phenols, tannins as condensed tannins and hydrolysable tannins. It was confirmed that the total phenols were highest in fruit 573.08 mg/g DW, next in bark 447.92 mg/g DW and finally in leaf 291.41 mg/g DW (Fruit>Bark>Leaf). It was confirmed that the total hydrolysable tannins were highest in fruit 572. 89 mg/g DW next in bark 445.28 mg/g DW and finally in leaf 288.78 mg/g DW (Fruit>Bark>Leaf). The non-tannin phenols present in three parts were almost in equal concentration with negligible difference i.e., in fruit 68.82 mg/g DW, in bark 68.67 mg/g DW and in leaf 68.67 mg/g DW. The condensed-tannins present in three parts were very less concentration i.e., in bark 2.637 mg/g DW, in leaf 2.614 mg/g DW and in fruit 0.19 mg/g DW. Results of these estimated phytochemicals were shown by two type graphs.

3.2 Results showing total phenol and tannin content collectively present in leaf, bark and fruits of *Terminalia pallida*

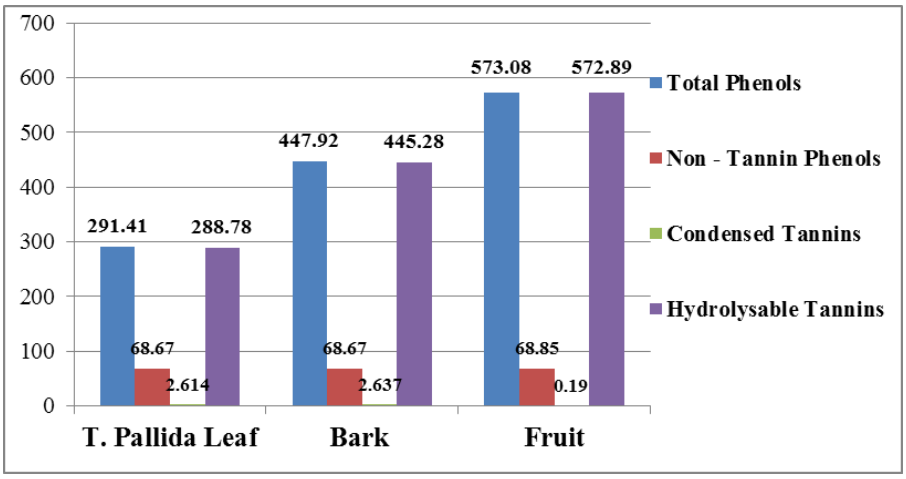

Fig 1: Graphical representation (Bar graph) of Estimated Total Phenol and Tannin content

3.3 Results showing total phenols and tannins present separately in leaf, bark and fruit of *Terminalia pallda*

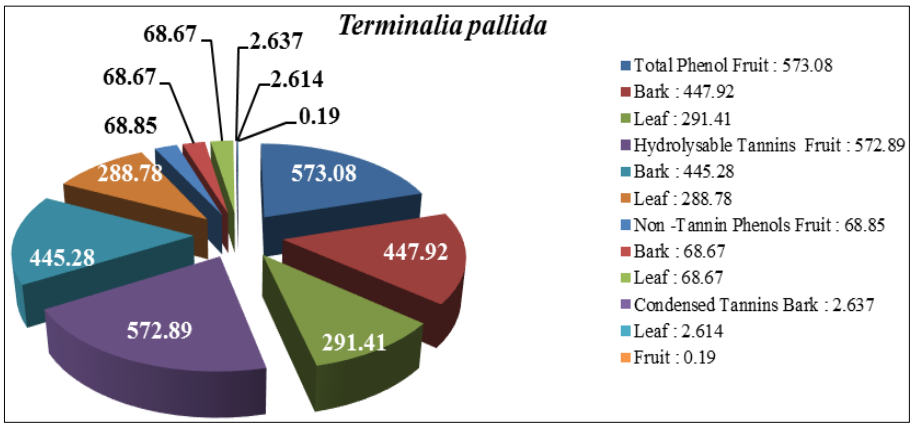

Fig 2: Graphical representation (Pie -chart) of Estimated Total Phenol and Tannin content

The bar graph (Fig.1) have shown the results of estimated phytochemicals in each part i.e. total phenols, tannin types in leaf, bark and fruits whereas the Pie-Chart (Fig.2) have shown the comparative results of total phenol content present in the order from higher to the lower concentration (i.e., fruit, bark and leaf). In the same way, hydrolysable tannins, non-tannin phenols and condensed tannins results were given. As the phytochemicals were higher in concentration, hence the parts of this tree species have the ability to cure diseases caused by microorganisms.

Among the secondary metabolites, phenols were the compounds with the highest organic group in controlling a number of diseases and disease-related negative conditions such as anti-apoptosis, anti-septics, anti-carcinogen, anti-aging, anti-inflammation, anti-atherosclerosis, improvement of endothelial function and cardiovascular protection, also able to control the angiogenesis, cell proliferation activities were the anti-oxidative or free radical scavenging activities with accurate calculation of degenerative diseases were reported previously (Sharma Swaati1 *et al*., 2014) [4]. Phenols were the compounds related to quality and nutritional value mostly deals with modifying colour, taste, aroma and flavor hence positive effects to health. Especially these phenol compounds used in defense process against Reactive Oxygen Species (ROS) in such a way helping the plants by causing molecular damage to microorganisms, insects, pests and herbivores. Phenols were acts as both repelling and attracting agents about organisms. They also act as protective agents, inhibitors, natural animal toxicants and pesticides against invading organisms i.e. herbivores, nematodes, phytophagous insects, fungal and bacterial pathogens (Amita Bhattacharya *et al*., 2010) [7] & (Abdul Rasheed War *et al*., 2018) [30]. Phenols in plant parts with a high concentration of ETs oxidize in the insect mid-gut with more efficiently compared to phenols in plant parts with a high concentration of CTs (Juha-Pekka Salminen, 2011) [15].

Tannins were used in controlling diarrhoea, hemorrhoids, to treat wounds as bactericides, other poison and antidotes these tannins also includes condensed tannins these especially used in foods. Tannins were having the property of general antimicrobial and antioxidant activities; the reports have also proved that tannins have potential value as cytotoxic and antineoplastic agents; tannins play a major role as antihemorrhagic agent and show to have immense significance as anti-hyper cholesterol; hypotensive and cardiac depressant properties; tannins were used in the treatment of a sore throat, wound healing, anti-diarrhoea, anti-hemorrhagic agent and also used as antimicrobial degradation of dietary proteins of semen. Condensed tannins are also part of the polyphenolic compound with bitter taste mostly used in foods (S.B. Nasrin Fathima, 2015) [3] & (Sharma Swaati, 2014) [4]. Tannins of both types were talented in forming strong complexes with a certain type of proteins decreasing the rate of their digestion (Balamurugan V *et al*., 2018) [13].

These tannins work against carbohydrates of a bacterial cell wall and reducing digestibility of cell wall by forming indigestible complexes by binding to bacterial enzymes and CTs shown high antioxidant (Muhammad Aslam Shad *et al*., 2012) [16] & (Juha-Pekka Salminen, 2011) [15]. The poliovirus, herpes simplex virus and various enteric viruses were inactivated when CTs nurture with red grape juice and red wines (Himanshu Jaiswal *et al*., 2018) [10], free radical scavenging, antimicrobial, gastro protective, anti-ulcerogenic activities, as potent inhibitors of lipid peroxidation in heart mitochondria and possess anti fibrotic effects (Muhammad Aslam Shad *et al*., 2012) [16]. In the treatment of various diseases to improve human health tannins were using.

4. Conclusion

The Present study regarding the estimation of phenols and tannins has shown that the parts of this endemic tree species *Terminalia pallida* have the highest amounts of phenols and tannins compared to the other *Terminalia* species. This proved that this endemic species is medicinally very important to the production of improved novel drugs of different kinds from phytochemicals.

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