

## A COMPARATIVE STUDY ON QUANTITATIVE ESTIMATION OF TANNINS IN *TERMINALIA CHEBULA*, *TERMINALIA BELERICA*, *TERMINALIA ARJUNA* AND *SARACA INDICA* USING SPECTROPHOTOMETER

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### ABSTRACT

Ashoka, arjuna baheda and harde are constituents of digestive churna from old age civilization. These drugs principally contain tannins which are widespread in plant kingdom. It is a poly-phenolic compound which is proven as antioxidants and possesses a chemo-protective potential. They are found in leaves, flowers, fruits and bark. The aim of study is to estimate the amount of tannins present in these drugs using folin-denis method. *Terminalia chebula* (combretaceae) (harde), *Terminalia belerica* (combretaceae) (bahera), *Terminalia arjuna* (combretaceae) (arjuna) and *Saraca indica* (leguminaseae) (ashoka) were crushed to coarse powder. The alcoholic extracts of the drugs were prepared for photometric determination of tannins using folin-denis method. The tannin concentration was determined by the standard graph of tannic acid solution and was found to be 99.55456mg/gm, 9.95568mg/gm, 54.96288mg/gm, and 57.4869 mg/gm for harde, arjuna, baheda and ashoka respectively. The concentration curve for tannic acid was determined and the correlation coefficient was calculated and was found to be 0.998 which indicates the good linearity between the concentration and the absorbance. It has been found that the tannins form the stable complexes with protein, starch and metal chelates by disturbing the metabolic activity of the bacterial enzymes nutrient availability, and functionality of biological membranes. The present study can be used as one of the parameters for standardization of medicinal plants.

**Keywords:** *Terminalia chebula* (combretaceae) (harde), *Terminalia belerica* (combretaceae) (bahera), *Terminalia arjuna* (combretaceae) (arjuna) and *Saraca indica* (leguminaseae) (ashoka)

### INTRODUCTION

Tannins and tannin-like substances are widespread in nature and are probably present in all plant materials. These are poly-phenolic compounds divided into two main groups - hydrolysable and condensed. Hydrolysable tannins contain a polyhydric alcohol usually, if not always, glucose esterified with gallic acid or with hexahydroxydiphenic acid. Condensed tannins are mostly flavonols and are probably polymers of flavan-3-ol (catechin) and these cannot be hydrolyzed to simple components. *Terminalia chebula*, *Terminalia beherilica*, *Saraca indica* and *Terminalia arjuna* are important medicinal trees being used in several herbal formulations. Among different chemical constituents polyphenols (flavonoids, phenolics, condensed and hydrolysable tannins) are major bioactive compounds responsible for the prevention of chronic diseases and health care [1, 2]. The therapeutic properties of herbal drugs are due to the presence of secondary metabolites which varies according to their age and maturity. Polyphenols (flavonoids, phenolics, condensed and hydrolysable tannins) are major

### Materials and methods

#### Principle

Tannin-like compounds reduce phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution, the intensity of which is proportional to the amount of tannins. The intensity is measured in a spectrophotometer at 700nm. Tannin contents of plants were measured by Folin-Denis method [11, 12]

#### Preparation of Folin-Denis reagent

Sodium tungstate (100 g) and phosphomolybdic acid (20 g) were dissolved in 750 ml distilled water and later 50 ml phosphoric acid was added into the solution. Mixture was refluxed for 2 h and volume was made to one liter with distilled water.

#### Preparation of carbonate solution

bioactive compounds responsible for the prevention of chronic diseases. They have been reported to exert anti-inflammatory, antimicrobial, antioxidant, anti-carcinogenic and body mass reducing activities. *Terminalia* is a genus of large trees of the flowering plant, family Combretaceae, comprising around 100 species distributed in tropical regions of the world. This genus gets its name from latin word terminus, Refers to the fact that the leaves appear at the very tips of the shoots. Trees of this genus are known especially as a source of secondary metabolites, e.g. cyclic triterpenes and their derivatives, flavonoids, tannins, and other aromatics. Phenolic compounds are a unique category of phytochemicals especially in terms of their vast potential health-benefiting properties [3,4,5]. They have multiple biological effects and also act as antioxidants by preventing the oxidation of Low-Density Lipoproteins (LDL), platelet aggregation and damage of red blood cells. These chemical constituents (secondary metabolites) present in plant vary according to their age and maturity. Some of these substances have anti-fungal, anti-bacterial, anti-cancer and hepatoprotective effects [6,7,8,9,10.]

Sodium carbonate (350 g) was dissolved in one liter water at 70°C. Solution was allowed to stand overnight and then it was filtered through glass-wool.

#### Preparation of standard tannic acid solution

Tannic acid (100 g) was dissolved in 100 ml distilled water.

#### Preparation of working solution

5 ml stock solution was diluted to 100 ml with distilled water. Each ml contained 50µg of tannic acid.

#### Collection and preparation of plant

*Terminalia chebula* (Combretaceae) (Harde), *Terminalia belerica* (Combretaceae) (Bahera), *Terminalia arjuna* (Combretaceae) (Arjuna) and *Saraca indica* (Leguminosae) (Ashoka) are collected

from garden of the college and the crude powder of the drug was used then the alcoholic extracts of the drugs were prepared. For photometric determination of tannins the standard procedure was followed by using Folin-Denis method.

### Method

#### Preparation of standard curve

10ml of standard solution was made up to 100ml distilled water. 1 - 10ml aliquots were taken in clear test tubes. 0.5ml of Folin-Denis reagent and one ml of sodium carbonate solution was added to each tube. Each tube was made upto 10 ml with distilled water. All the reagents in each tube were mixed well and kept undisturbed for about 30 minutes and read at 760 nm against reagent blank.[13, 14]

#### Extraction of Tannin

Accurately Weighed 0.5g of the powdered material was transferred to a 250mL conical flask. Add 75mL water. Heat the flask gently and boil for 30 min. Centrifuged at 2,000rpm for 20 min and collect the supernatant in 100mL volumetric flask and make up the volume. Transfer 1mL of the sample extract to a 100mL volumetric flask containing 75mL water. Add 5mL of Folin-Denis reagent, 10mL of sodium carbonate solution and dilute to 100mL with water and Shaken well. Read the absorbance at 700nm after 30 min.

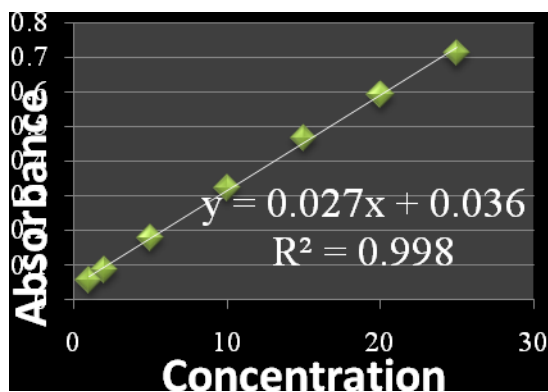
#### Estimation of sample

An aliquot of the sample extract containing not more than 0.5mg of tannic acid was used and the percentage of tannin was determined.

### RESULT

#### CALCULATION

The tannin concentration was determined by the standard graph of tannic acid solution and it was found to be 99.55456mg/gm,9.95568mg/gm,54.96288mg/gm,57.48659mg/gm for Harde, Arjuna, Baheda, Ashoka, respectively of powdered plant material



The concentration curve of the tannic acid

### DISCUSSION

Tannins are complex secondary metabolites having various medicinal properties but difficult to isolate in pure form. Tannins are polyphenols, have a large influence on the nutritive value of humans and animals foodstuff. Most of the ayurvedic formulations are lacking in defined quality control parameters. FDA has made the quality control and GMP mandatory for ayurvedic formulation, which has been implemented from 1st January 2003. Hence, there is no thorough scientific investigation on most of the claims made by the traditional medicine practitioners. Recent interest in phenolic compounds due to their protective role, through utilization of fruits and indigenous vegetables such as apple 15, black caraway, carrot,

cranberry, orange 16 tomato 17 against oxidative damage diseases such as arteriosclerosis, cardiovascular, coronary heart disease, aging, stroke and cancer 18, 19 20. Many plants have been studied and reported the importance of tannins and its variation. Further

study is necessary to understand the factors which may affect the production of tannins.

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