

RESEARCH ARTICLE

A Study on Quantitative Estimation of Tannins in *Mussaenda erthyrophylla* using Spectrophotometer

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ABSTRACT

Mussaenda erthyrophylla being an ornamental plant is still not fully explored. The most characterized compounds in *Mussaenda* are the iridoids, tannins triterpene and saponins. Very few species have been explored for chemical and biological studies. No pharmacognostic profile of the plant is available; its many allied species have been investigated for isolation and pharmacological activities. Saponins, a number of triterpenoids and glycosides were reported. *Mussaenda* genus viz., contains *mussaendosides* U(1) and V(2), *mussaendosides* G(1) and K(2) are two new triterpenoid saponins, *mussaendosides* A-C, M and N with cyclolanostene type aglycone and aureusidin, iridoid glycosides. This has been adopted from ethnobotanical leaflets. Leaf, root contains tannins, astringents, and mucilage. The study aims to estimate the amount of tannins present in the drug using the folin-denis method. *M. erthyrophylla* was crushed to coarse powder and extract with 50% aqueous methanol by cold maceration for 24 hours and make up volume upto 100 mL. Tannin concentration was determined by the standard graph of tannic acid solution and was found to be 25.5 mg/g, respectively. The concentration curve for tannic acid was determined, and the correlation coefficient was calculated and was found to be 0.996, indicating the good linearity between the concentration and absorbance. It has been found that the tannins form 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: *Mussaenda erthyrophylla*, Gallic acid, Folin-Denis Reagent.

How to cite this article: Yadav G, Saxena V. A Study on Quantitative Estimation of Tannins in *Mussaenda erthyrophylla* using Spectrophotometer. Int. J. Pharm. Edu. Res. 2022;4(2):18-19.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Tannins and tannin-like substances are widespread in nature and are probably present in all plant materials.^{1,2}

These are poly-phenolic and divided into two main groups hydrolysable and condensed. Hydrolysable tannins contain 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 hydrolysed to simple components.³⁻⁷ The therapeutic properties of herbal drugs are due to the presence of secondary metabolites, which varies according to age and maturity. Polyphenols (flavonoids, phenolics, condensed and hydrolysable tannins) are major.^{8,9}

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 765 nm. Tannin contents of plants were measured by Folin-Denis method. Tannins content was determined by Folin Denis method.^{8,9}

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 hours and volume was made to one liter with distilled water.

Preparation for Test Solution:

Weight accurately 0.5 g of powdered drug and extract with 50% aqueous methanol by cold maceration for 24 hours and make up volume upto 100 mL.

Preparation of Standard Curve for Gallic Acid

Weight accurately 10 mg of gallic acid and dissolve in 100 mL of distilled water in a volumetric flask (100 µg/mL). From the above stock solution, pipette out aliquots of (0.5 to 2.5 mL) into 25 mL volumetric flask. Add 10 mL of distilled water and 1.5 mL of Folin Ciocalteu reagent to dilute it according to label and specification to each of the above volumetric flask. After 5 minutes, add 4 mL of 20% sodium carbonate solution and volume make up

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Table 1: Conc. of standard gallic acid ($\mu\text{g/mL}$) and its absorbance

S.No.	Conc. of standard gallic acid ($\mu\text{g/mL}$)	Absorbance (at 765 nm)
1.	2	0.015
2.	4	0.141
3.	6	0.222
4.	8	0.323
5.	10	0.363
6.	12	0.469
7.	14	0.519

to 25 mL with distilled water, incubate for 30 minutes. The absorbance was recorded at 765 nm and a graph of standard curve was plotted for absorbance versus concentration (Table 1).

Procedure

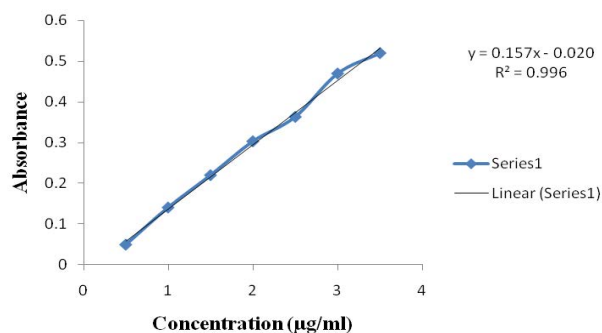
From the above extract pipette out 100 μL into 25 mL volumetric flask and above procedure was followed for developing colour, using folin reagent. The amount of tannin content was calculated using the calibration curve of gallic acid (Figure 1). Tannins content was determined by Folin Denis method. UV took the standard curve of gallic acid at, 765 nm.¹⁰

RESULT

By standard calibration curve, sample concentration was found to be 25.5 mg/g tannin determination was found to be 25.5 mg/g which is gram equivalent to gallic acid (Figure 1).

DISCUSSION

Tannins are complex secondary metabolites having various medicinal properties but are difficult to isolate in pure form. Tannins and polyphenols greatly influence the nutritive value of human and animal foodstuffs. Most of the ayurvedic formulations are lacking in defined quality control parameters. FDA has made 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 traditional medicine practitioners. Recent interest in phenolic compounds due to their protective role, through the utilization of fruits and indigenous vegetables such as apple, black caraway, carrot, cranberry, orange tomato against oxidative damage diseases such as arteriosclerosis, cardiovascular, coronary heart disease, aging, stroke and cancer.^{3,11} Many plants have been

**Figure 1:** Standard calibration curve of gallic acid

studied and reported the importance of tannins and its variation.¹²

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