Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2014, 3 (3): 1104-1107

(International Peer Reviewed Journal)

A Comparative Study on Quantitative Estimation of Tannins In Triphula by using Spectrophotometer

Pooja V. Jagasia

Department of Chemistry, B.N. Bandodkar College of Science, University of Mumbai, Thane-400601, Maharashtra, **INDIA**

Email: jagasiapooja10@gmail.com

Accepted on 10th April 2014

ABSTRACT

Triphula tablets were analyzed for the tannin content, which is responsible for antioxidant activity. The amount of tannins were analyzed by Folin Denis reagent. Tannic acid was used as a standard compound and the tannins were expressed as mg g^{-1} tannic acid equivalents (Standard curve equation; $Y=0.02873x+0.2411$, $R^2 = 0.948$).The total tannins varies from 59.33±0.58 to 48.6±1.16 in the extract of different solvents.

Keywords: Triphula, Tannins, Folin Denis reagent, Tannic acid.

INTRODUCTION

Triphula is one of the commonest and cheapest of herbal preparations available in India. It is composed of equal parts of three most valuable herbs, Indian Gooseberry (Emblica officinalis), Chebulic Myrobalan (Terminalia chebula), and Beleric Myrobalan(Terminalia belerica)[1,2]. These drugs have been evaluated for their comparative antidiabetic and antioxidant activities [3].Triphula is well documented as a rejuvenator and antioxidant[4-6]. It has been scientifically validated for its anti-inflammatory[7] and hypolipidemic effects.

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 [8], black caraway, carrot, cranberry, orange [9] tomato [10]against oxidative damage diseases such as arteriosclerosis, cardiovascular, coronary heart disease, aging, stroke and cancer[11-14]. Many plants have been studied and reported the importance of tannins and its variation.

MATERIALS AND METHODS

Principle: Tannin-like compounds reduce phosphotungstomolybdic acid in alkaline solution to produce a highly colored blue solution, the intensity of which is proportional to the amount of tannins. The intensity is measured in a spectrophotometer at 760nm. Tannin contents of plants were measured by Folin-Denis method[15,16].

Apparatus: All glassware used for the experimental purpose were made up of Pyrex or Borosil glass. The burette, pipette and standard flasks were calibrated by the method described by Vogel [17]. The absorption measurements were carried out on a spectrophotometer, model EQ-822, supplied by Equiptronics, Powai using 1-cm matched glass cells. The spectrophotometer was calibrated by measuring the absorption spectra of potassium chromate in potassium hydroxide solution and that of potassium permanganate in sulphuric acid solution [18]. A digital pH meter model EQ-610, supplied by Equiptronics, Powai having an accuracy of ±0.02 pH and resolution of 0.01 pH was used to measure the pH of the solutions. The pH meter was calibrated with standard buffer solutions of pH 7.0, 4.0 and 9.2. A single pan digital analytical balance of series CA-223, supplied by Contech, having sensitivity of 0.001 g was used for weighing chemicals, reagents and samples.

Reagents: All the chemicals used were of A.R. grade. Distilled water was used for preparing standard solutions as well as for all experimental work.

Preparation of standard tannic acid solution: Standard solution of tannic acid was prepared by dissolving 100 mg in 100 mL distilled water. Fresh solutions were prepared for each test.10 mL of the standard solution was diluted to 100 ml.1-10 mL aliquot were used for the analysis.

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

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

Preparation of the extracts of Triphula tablet: Extracts were prepared by using three different solvents.
A. Hydro alcohol extract: 0.750g of triphula tablet was extracted with 100 mL of 50% alcohol for 4 h.
B. Acetone water extract: 0.750g of triphula tablet was extracted with 100 mL of 70% acetone for 4 h.
C. Aqueous Extract**:** 0.750 g of triphula tablet was extracted with 100 mL of water, refluxed for about 4 h.

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 min at room temperature before the absorbance was at read at 760nm spectrophotometrically. All determinations were performed in triplicate. The Folin-Denis reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction. This blue colour is measured spectrophotometrically. Thus total tannin content can be determined.

Estimation of Tannins: An aliquot of sample extract containing not more than 0.5 mg of tannic acid was used and amount of tannin was determined.

RESULTS AND DISCUSSION

Calibration graph: The adherence to Beer's law was studied by measuring the absorbance value of the series of solutions containing different concentrations of tannic acid. A linear calibration graph drawn between absorbance and tannic acid concentration indicates that tannic acid may be determined in the range 0.2-5.0 μg cm^{-3} (Fig. 1). The amount of total tannin was determined with the Folin-Denis reagent. Tannic acid was used as a standard compound and the total tannins were expressed as mg g^{-1} tannic acid equivalent using the standard curve equation: $Y = 0.02873X+0.2411, R^2=0.948$, where Y is absorbance at 760nm and X is the total phenolic content in the different extracts of triphula expressed in mg g^{-1}. Tannin compounds are a class of antioxidant agents which acts as free radical terminators. Table shows the variation of mean absorbance with concentration of Tannic acid and the contents of total tannins that were measured by Folin-Denis reagent in terms of Tannic acid equivalent. The total phenol varied from 59.33±0.58 to 48.6±1.16 mg g^{-1} in the extracts. The maximum tannin content was found in the aqueous extract (92.4± 0.14 mg g^{-1}) of Triphula tablets

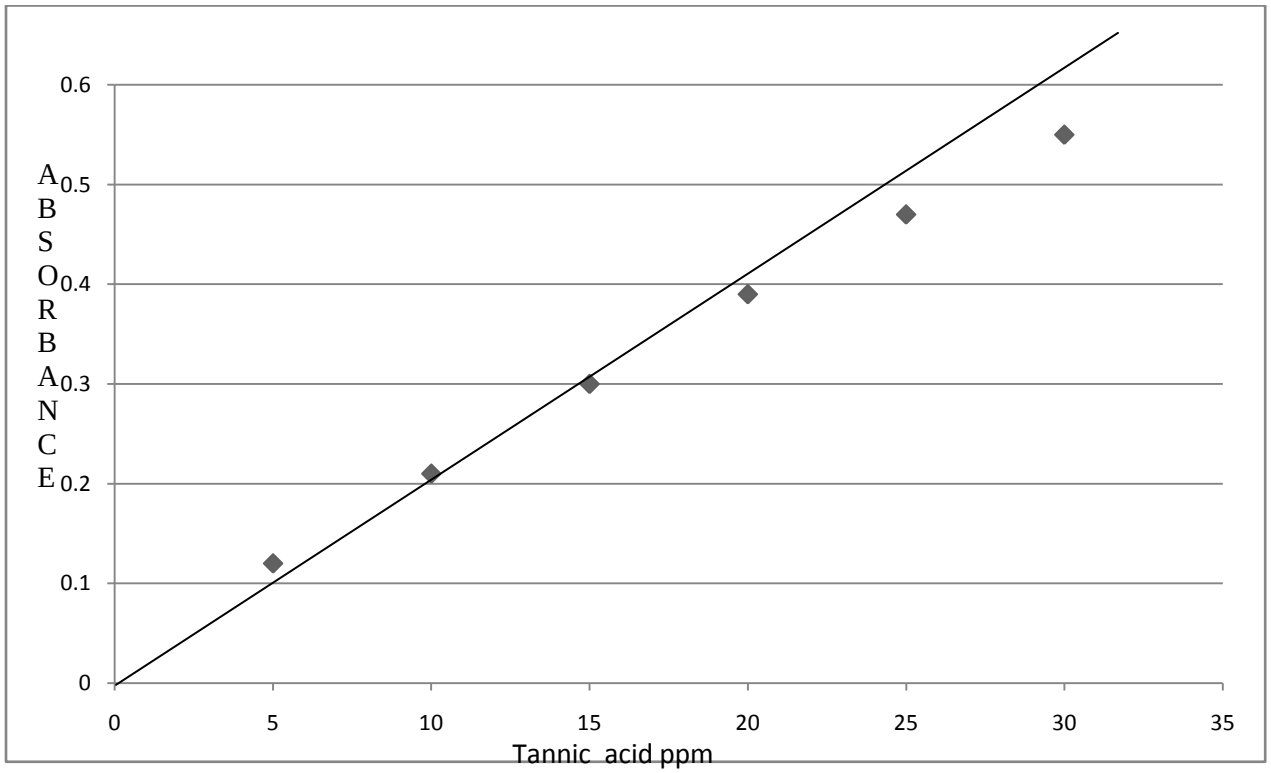

Fig. 1: Calibration curve for tannic acid.

Table: Tannin content of extracts of triphula tablet in different solvents

Solvents	Concentration(ug/mL)	*Mean±SD
Hydro alcohol	60	59.33±0.58
Acetone water	52	51.00±0.574
Aqueous	50	48.60±1.157

*Average of three analysis

APPLICATIONS

The proposed method in the established optimum conditions was satisfactorily applied for the determination of tannins in synthetic mixtures, Herbal formulations and real samples.

CONCLUSIONS

This method describes a simple, rapid, sensitive spectrophotometric procedure for the determination of tannins in various samples. The simplicity of instrument, ease of handling, lack of need for consumables, and almost no maintenance have caused spectrophotometry to remain a popular and inevitable technique, particularly in the laboratories of developing countries with a limited budget. The amounts of total tannins were determined with the Folin-Denis reagent. Tannic acid was used as a standard compound and the total tannins were expressed as mg g^{-1} tannic acid equivalents. The maximum tannin content was found in the aqueous extract (92.4± 0.14 mg g^{-1}).

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Principal, Dr. (Mrs.) M.K. Pejaver, B. N. Bandodkar College of Science for providing the necessary facilities to carry out the present work.

REFERENCES

[1] T. Vani, M. Rajani, S. Sarkar, C.J. Shishoo, *International Journal of Pharmacognosy*, **1997**, 35 (5), 313-317.
[2] Ayurvedic Pharmacopoeia Committee. The Ayurvedic Formulary of India, Part I. 2 nd English ed. New Delhi: Controller of Publications; **2003**.
[3] M.C.Sabu, R.Kuttan, *J Ethnopharmacol*, **2002**, 81, 155-60.
[4] G.C.Jagetia, M.S.Baliga, K.J.Malagi, S.Kamath, *Phytomedicine*, **2002**, 9, 99-108.
[5] R.Srikumar, N.J.Parthasarathy, S.Manikandan, G.S.Narayanan, R.Sheeladevi, *Mol Cell Biochem* **2006**, 283, 67-74.
[6] N. Kumari, P.Kumar, D.Mitra, B.Prasad, B.N.Tiwary, L.Varshney, *J Food Sci* **2009**, 74, M109-13.
[7] M. Rasool, E.P.Sabina, *Phytother Res.*, **2007**, 21,889-94.
[8] J. Michalowski, P. Halaburda, *Talanta*, **2001**, 55, 1165-1171.
[9] A.N. Assimopoulou, D. Boskou, M.A. Anagnostopoulou, P. Kefalas , V.P. Papageorgiou, *Food Chem.*, **2006**, 94, 19-25.
[10] C.H. Chang, H. Lin, C.Y. Chang, Y.C. Liu, *J. Food Eng*, **2006,**77, 478-485.
[11] H. Tanizawa, Y. Ohkawa, Y. Takino, A. Ueno, T. Kageyama, S. Hara, *Chem. Pham. Bull.*, **1992**, 40, 1940-1942.
[12] O.I. Aruoma, *J. Am. Oil Chem. Soc.*, **1998**, 75, 199-212.
[13] S.Abdi, *Cancer Lett.*, **1999** ,142, 1-9.
[14] Sandhya T. Lathika, B.N.Pandey, K.P.Mishra, *Cancer Lett*., **2006**, 231,206-14.
[15] P.R. Ram, B.N.Mehrotra, Compendium of Indian Medicinal Plants, New Delhi, **1993**, 2, 453.
[16] R.T. Sane, *Indian Drugs*, **2002**, 39,184.
[17] G.H. Jeffery, J. Bassett, J. Mendham, R.C. Denney, A.I. Vogel's Text Book of Quantitative Inorganic Analysis including Elementary Instrumental Analysis, ELBS and Longman, New York, **1978**, 4th ed., 47-82.
[18] E.B. Sandell, Colorimetric Determination of Traces of metals, Interscience Publishers, New York, **1965**, 3rd ed.