

Short Communications

Spectrophotometric Estimation of Total Tannin Content in Some Ayurvedic Eye Drops

PRIYANKA SONI*, VISHAL SONI, LAJJA PATIDAR, NIKITA RIJHWANI

Department of Herbal Drug Research, B.R. Nahata College of Pharmacy, Research Centre, Mhow Neemuch Road, Mandsaur 458001, India

ARTICLE DETAILS*Article history:*

Received on 24 June 2015

Modified on 20 September 2015

Accepted on 23 September 2015

Keywords:

Ayurvedic eye drops,

Tannins,

Folin-denis reagent,

Spectrophotometer

ABSTRACT

Ayurvedic eye drops preparation contains aqueous extracts of different herbs. Ethnobotanical survey shows that plants used in Ayurvedic eye drops formulation are rich source of tannin and tannin like compounds. Tannins are responsible for antimicrobial and antioxidant properties of Ayurvedic eye drops. The present study was designed with the aim to determine the tannin content in 3 different brands of Ayurvedic eye drops, by colorimetric method using Folin-denis reagent. The tannin content of all the three brands was found to be A-825.23, B-656.00 and C-558.49 µg/ml. The results obtained are reproducible with coefficient of variation less than 0.99 %. The present approach can be used as one of the parameters for the standardization of Ayurvedic eye drop preparations.

© KESS All rights reserved

INTRODUCTION

Eye is one of the most sensitive organs of human body. It gets affected by different environmental agents. The common ocular diseases are glaucoma, conjunctivitis, cataract, ocular allergies, ocular inflammation etc. Ophthalmic problem afflicts a substantial segment of the population; some of these can be managed with antibiotics and steroids. However prolonged use of these drugs may have potential side effects. Now-a-days, people increasingly prefer ayurvedic eye drops because it is safe and relatively free from side effect and adverse reactions [1]. Ayurvedic eye drops preparation contains aqueous extracts of different herbs. Ayurvedic eye drops are known to show anti-inflammatory, antioxidant and antimicrobial activity due to their tannin content in aqueous extract [2]. Therefore in the present study an attempt has been made to determine the tannin and tannin like compounds present in ayurvedic eye drops, using Folin-Denis method and estimation was done on UV/Vis spectrophotometer [3].

Estimation of tannins was carried out by using Folin-Denis reagent. The method is based on oxidation of the molecules containing a phenolic hydroxyl group. Tannins like compound reduce phosphotungstomolybic acid in alkaline solution to produce highly blue coloured solution, the intensity of which is proportional to the tannin content. This intensity is measured at 775 nm using visible spectrophotometer [4,5]. Three eye drops were (Patanjali eye drop, Himalaya eye drop and Dey's Medical Stores Mfd. Ltd.) procured from local market of Mandsaur. All the brands contain herba rich in tannin content.

MATERIALS AND METHODS

These three brands were evaluated for estimation of total tannin content against tannin acid as standard solution by using UV/Vis spectrophotometer. One centimeter matched quartz cell was used for the study [7, 8]. All the chemicals used were of AR grade. Folin-Denis Reagent and saturated sodium carbonate solution were prepared in laboratory. Working standard solution of tannic acid which was procured from Hi-Media was prepared by dissolving 100 mg tannic acid in 100 ml distilled water in volumetric flask. One millilitre of this solution was diluted to 100 ml in distilled water, in another volumetric flask to give 10 µg/ml tannic acid solutions. Calibration curve from standard solution of tannic acid was prepared

***Author for Correspondence:**

Email: soni_priyanka21@rediffmail.com

and with the help of this curve the tannin contents of ayurvedic eye drop was estimated. The method was validated for precision and accuracy. A series of calibrated 10 ml volumetric flask were taken and appropriate aliquots of the working standard solution of the tannic acid were added. To each flask was added Folin-Denis reagent (0.5 ml), sodium carbonate solution (1 ml) and distilled water (up to 10 ml). The absorbance for so formed blue coloured complex was measured at absorption maxima 775 nm within 30 min of the reaction against the reagent blank prepared in similar manner without the tannic acid. The absorption maxima and Beer's law were noted. The linear correlation between these concentration (x-axis) and absorbance (y-axis) were graphically presented and the slope (b), intercept (a) and coefficient correlation(r) were calculated out for linear equation ($Y = bx + c$) by regression analysis using the least square method [9,10].

The appropriate aliquots from ayurvedic eye drop of each brand were withdrawn in 10 ml volumetric flask separately. The blue coloured complex was developed in the similar manner as in calibration curve studies, replacing the tannic acid with ayurvedic eye drop and the absorbance for aliquots of each brand was noted at 775 nm. The corresponding concentration of tannins against respective absorbance was determined as tannic acid using the calibration curve. The statistical analysis for checking the uniformity in different brands was also performed.

The method was validated for precision and accuracy by performing the recovery studies at two levels by adding known amount of tannic acid to herbal eye drop (1.0 ml) of which the tannin content had been estimated previously (A-825.23, B-656.00 and C-558.49 µg/ml). The data obtained were recorded and recoveries were calculated.

The calibration curve for tannic acid was found to be linear in the range of 5-30 µg/ml. The correlation coefficient (0.99) indicates the good linearity between the concentration and the absorbance.

Recovery studies were performed at two levels by taking known quantities of tannic acid with estimated quantity of tannic acid in ayurvedic eye drop. The method was validated for precision and accuracy by repeating the experiment three times at both the levels. The average percentage recoveries of three brands

are (99.02, 99.01, and 99.11) is also satisfactory indicating the good accuracy of the method.

RESULT AND DISCUSSION

The tannin concentration in three eye drops (Patanjali, Himalaya and Dey's Medical Stores Mfd.) was determined using standard calibration curve of tannic acid [11]. The linear correlation between these absorbance (x-axis) and concentration (y-axis) were graphically presented and the slope (b), intercept (a) and coefficient correlation(r) were calculated out for linear equation ($Y = bx + c$) by regression analysis using the least square method. The calibration curve for tannic acid was found to be linear in the range of 5-30 µg/ml. The correlation coefficient (1) indicates the good linearity between the concentration and the absorbance [12,13].

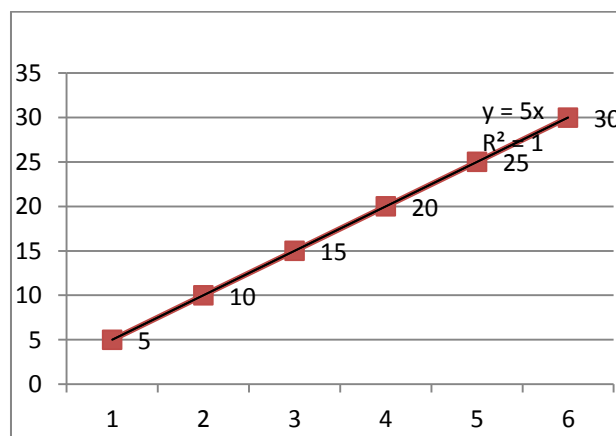


Figure 1: Standard curve of tannic acid

Table 1: Estimation of total tannin content herbal eye drops preparation and their recovery study

Sample	Tannin content (µg/ml) ± SD	Tannin added (µg/ml)	Recovery %	RSD %
Brand A (Patanjali Drishti eye drop)	825.23	100	99.02	0.085
Brand B (Himalaya eye drop)	656.00	100	99.01	0.067
Brand C (Itone, Dey's Medical Stores Manf. Ltd.)	558.56	100	99.11	0.082

*Mean ± SD of three determinations

Now a day's 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, now these preparations have to be tested for the identity, purity, potency, safety and efficacy so that they would gain universal acceptance. In the light of the above, the present study can be used as one of the parameters for standardization during the routine quality control of Ayurvedic eye drops.

They are divided in two groups-hydrolysable and condensed [6]. Therapeutic action results due to its astringent property. It acts as protective, antiparasitic, antidiarrhoeal, antimicrobial and antioxidant [7, 8]. People apply tannic acid directly to the affected area to treat cold sores and fever blisters, diaper rash and prickly heat, poison ivy, ingrown toenails, sore throat, sore tonsils, spongy or receding gums, and skin rashes; and to stop bleeding. Tannic acid is also taken by mouth and applied directly for bleeding, chronic diarrhea, dysentery, bloody urine, painful joints, persistent coughs, and cancer. Vaginally, tannic acid is used as a douche for white or yellowish discharge (leucorrhea) [9]. Many tannin molecules have also been shown to reduce the mutagenic activity of a number of mutagens. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation. The generation of superoxide radicals was reported to be inhibited by tannins and related compounds [10].

CONCLUSION

Herbal eye drops consisting of herbal extract of different drugs are the rich source of tannins and similar compounds, which acts as antimicrobial agent. The total tannin content above 5% is often considered as lethal. Tannin content was determined using folin denis reagent and sodium carbonate solution by spectrophotometer. As per result the tannin content was found as Patanjali Drishti eye drop > Himalaya eye drop> Dey's Medical Stores Manf. Ltd.

ACKNOWLEDGMENTS

The authors thank to the Director B. R. Nahata College of Pharmacy, Mandsaur College of Pharmacy and Management for their kind support.

REFERENCES

- [1] Sandhu PS, Singh B, Gupta V, Bansal P & Kumar D. Potential herbs used in ocular disease. *Int J Pharm Sci Res* 2011; 4(3):1127-11140.
- [2] Hangerman AE, "The Tannin Handbook", Mimani University, Oxford, OH 45050, 2002; 2: 689.
- [3] Polshettiwar SA, Ganjiwale RO, Wadher SJ, Yeole PG. Spectrophotometric estimation of total tannins in some ayurvedic eye drops. *Indian J Pharm Sci* 2007; 69:574-6.
- [4] Anonymas <http://www.ansci.cornell.edu/plants/toxicagents/tannin.html#ref>
- [5] Kadam SS, Salunkhe DK, Chavan JK. Dietary tannins: consequences and remedies. Boca Raton: CRC Press, 1990 p. 177.
- [6] Kirtikar KR, Basu BD. *Indian Medicinal Plants*. Lalit Mohan Basu, Allahabad India, 1956.
- [7] Anonymous www.docstoc.com/docs/38089655/tanninsppt.
- [8] Scalbert A. Antimicrobial properties of tannins. *J Phytochem* 1991; 30(12):3875-3883.
- [9] Covington TR, et al. *Handbook of Nonprescription Drugs*. 11th ed. Washington, DC: American Pharmaceutical Association, 1996.
- [10] Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. *Crit Rev Food Sci Nutr*. 1998; 38(6):421-64.
- [11] Saxena V, Mishra G, Saxena A & Vishwakarma K. A comparative study on quantitative estimation of tannins in *Terminalia Chebula*, *Terminalia Belerica*, *Terminalia Arjuna* & *Saraca Indica* using spectrophotometer. *Asian J Pharm Clin Res* 2013; 6(3): 148-149.
- [12] Beckett A.H., Stenlake J.B. "Practical pharmaceutical chemistry" fourth edition – part two, published by- CBS publishers and distributors, New Delhi, page no. 275.
- [13] Connors KA. *A Textbook of Pharmaceutical Analysis*, 3rd edition, A Wiley Interscience Publication, John Wiley and Sons, New York, 1995, 595.