Spectrophotometric Estimation of Total Tannins in Some Ayurvedic Eye Drops

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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. Antioxidant and antimicrobial properties of ayurvedic eye drops are attributed to the presence of tannins and tannin like compounds. Therefore in the present study an attempt has been made to determine the tannin content in some ayurvedic eye drops, by using Folin-Denis method. A blue colored complex is formed by using phosphotungustomolybdic acid. Estimation was done on UV/Vis spectrophotometer. The tannin content of all the three brands was found to be 420, 918 and 270.49 µg/ml. The results obtained are reproducible with coefficient of variation less than 1.0% . Hence the present approach can be used as one of the parameters for the standardization of ayurvedic eye drop preparations.

Key words: Ayurvedic eye drops, tannins and spectrophotometer

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. Ayurvedic eye drops are known to show antiinflammatory, antioxidant and antimicrobial activity due to their tannin content in aqueous extract[1]. Therefore in the present study an

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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.

In addition to our work on standardization of ayurvedic eye drop, we decided to determine the tannin and tannin like compounds in our study. The tannin content in some ayurvedic eye drops was estimated spectrophotometrically by Folin-Denis method. The method is based on oxidation of the molecules containing a phenolic hydroxyl group. The tannin and tannin- like compounds reduce phosphotungungustomolybdic acid in alkaline solution to produce a highly coloured blue solution; the intensity of which is proportional to the amount of tannin and can be estimated against standard tannic acid solution at wavelength of 775 nm[2].

Three Ayurvedic eye drops branded (A, B, C) were procured from market. All the brands selected contain aqueous extract of *Haritaki* (*Terminalia chabula*), *Vibhitaki* (*Terminalia belerica*) and *Amla* (*Emblica officinalis*) as a principle source of tannin. These brands were estimated for their tannin contents against standard tannic acid solution on UV/Vis spectrophotometer (Shimadzu 2401). One centimeter matched quartz cell was used for the study. 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 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 (Table 1). 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[3].

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-420.00, B-918.00 and C-274.49 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 5-45 µg/ml. The correlation coefficient (0.991) 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

TABLE 1: OPTICAL CHARACTERISTIC, STATISTICAL REGRESSION DATA AND VALIDATION PARAMETER FOR TANNIC ACID COMPLEX

Parameter	Values
Absorption maxima	775 nm
Beer's law limit (µg/ml)	5-45
Regression equation Y= a+bx	Y= 0.0061x
Intercept (a)	0.00
Slope (b)	0.0061
Correlation coefficient (r)	R^2 = 0.9915
Precision (n=3, % RSD)	A= 0.31
	B= 0.20
	C= 0.97
Accuracy (%)	A= 99.10
	B= 99.08
	C= 99.30

TABLE 2: ESTIMATION OF TANNIN CONTENT OF HERBAL EYE DROP PREPARATIONS AND RECOVERY STUDIES

Sample	Tannin content (µg/ml) *±SD	Tannin added µg/ml	% Recovery	RSD %
Brand –A (Eye tone, Himayala)	420.00±1.391	100	99.10	0.084
Brand –B (Itis, Dey's Medical)	918.00±2.639	100	99.41	0.067
Brand –C (Opticare, Ozone Ayurvedics)	270.49±0.782	100	99.21	0.071

*Mean±SD of three determinations

repeating the experiment three times at both the levels. The average percentage recoveries of three brands are (99.10, 99.41, and 99.21) is also satisfactory indicating the good accuracy of the method (Table 2).

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[4]. 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.

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