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Spectrofluorimetric Determination of Anthraquinone Glycoside from *Terminalia chebula* and its *churnas*

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A simple and sensitive spectrofluorimetric procedure was developed for the estimation of total anthraquinone glycoside in *Terminalia chebula* (*Harde*). The method was based on the reduction of anthraquinone glycoside with sodium dithionite solution in presence of phosphate buffer, which shows strong fluorescence in sodium borate solution having excitation and emission wavelength 385 and 495 nm respectively. Linear relationship for the fluorescence intensity was obtained in the range of 80-400 ng/ml. The method was statistically validated and was applied successfully to determine the anthraquinone glycoside in *T. chebula* fruits and its six marketed formulations.

Fruits of *Terminalia chebula* (Family: *Combrataceae*) are reputed in Ayurveda as a safe and effective purgative and are used alone or in combination with other plant drugs by ayurvedic physician in their routine practice[1]. It is generally available in local market under the name of *Harde (Haritaki, harara, myrobalan). T. chebula* contains mainly 30-32% tannins, 13-16% non-tannins, anthraquinone glycosides and lipids. The anthraquinone derivative is responsible for the observed purgative activity of *T. chebula*[2]. *Triphala churna* is a mixture of equal portion by weight of *Harde (T. chebula), Bihara (T. belerica)* and *Amla (P. emblica)*, widely used as an effective laxative. The purgative action of *Triphala churna* is due to the presence of *T. chebula*[3].

A literature survey revealed that only a few methods have been reported for the measurement of the purgative activity of *T. chebula* and *triphala churna*[1,4-6] but no method is reported for quantification of anthraquinone glycosides present in *T. chebula*. The Pharmacopoeial methods[7,8] for the estimation of hydroxy anthracene glycoside expressed as sennoside B from senna, is not suitable for *T. chebula* because of the large amount of tannins, which interfere in the estimation. Therefore, it was thought of interest to develop a specific, simple, precise and accurate method for the quantitative estimation of anthraquinone glycoside attributed to purgative activity of *T. chebula*. In the present work, USP 2000[9] spectrofluorimetric method is modified for quantitative estimation of anthraquinone glycosides present in *T. chebula* fruits and in six *churnas* (one *harde churna* and five *triphala churna*).

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MATERIALS AND METHODS

Reference Ca-Sennoside (20 % w/w) was obtained from Cipla Ltd, Bangalore. *Harde* fruits were obtained from commercial sources. They were authenticated as *T. chebula* in the Pharmacognosy department. Fruits were powdered to 60 # size in our laboratory. *Churna* formulations were procured from the local market. Sodium borate was obtained from Samir Tech-chem., Vadodara. Dibasic sodium hydrogen phosphate and monobasic potassium dihydrogen phosphate were obtained from Loba Chemie, Mumbai. All chemicals were of analytical reagent grade. Triple distilled water was used in the study.

Fluorescence spectrophotometer with single quartz cell of 1cm path length (Hitachi, F-2000) was used to measure fluorescence intensity of the resulting solutions. Buffer pH

was measured and adjusted using a Digital pH meter (Systronic). A sonicator (Frontline FS-4) and constant temperature water bath (Labtronic) were also used in the study.

Preparation of solution:

Phosphate buffer, pH 7 was prepared by mixing 61.1 ml of dibasic sodium hydrogen phosphate solution (0.95 %w/v solution of Na_2HPO_4 in water) with 38.9 ml of monobasic potassium dihydrogen phosphate solution (0.90 %w/v of KH_2PO_4 in water). Sodium borate solution (3.8 %w/v) was prepared in water and filtered through Whatman filter paper No. 41. Sodium dithionite solution (1.5 %w/v) was prepared fresh in water.

Standard stock solution of sennoside was prepared by transferring accurately weighed reference Ca-sennoside powder (equivalent to 25 mg of sennoside) to a 25 ml volumetric flask and mixing with phosphate buffer. The flask was sonicated for 30 min, solution was filtered and volume was adjusted up to the mark with phosphate buffer. Filtrate (0.5 ml) was further diluted to 100 ml with sodium borate solution.

Sample solutions were prepared by mixing 1.6 g of *T. chebula* fruit powder or its *churna* or 5 g of *triphala churna* with 20 ml of phosphate buffer. The flask was sonicated for 30 min, solution was filtered and remaining residues were further washed with phosphate buffer to make volume 25 ml. The solution (5 ml) was further diluted to 100 ml with sodium borate solution. To prepare respective blanks, standard stock solution or sample solution was pipetted in a 25 ml volumetric flask and diluted up to the mark with sodium borate solution.

Fluorimetric method:

Standard stock solution (0.4-2.0 ml, 80-400 ng/ml) or sample solution (2 ml) was pipetted in 25 ml volumetric flask. Then 7.5 ml of each sodium borate solution as well as freshly prepared sodium dithionite solution was added to each flask and mix. The mixture was heated for 30 min on boiling water bath, cool to room temperature and volume was adjusted up to the mark with sodium borate solution. The fluorescence intensity of resulting solution was measured at emission wavelength 495 nm keeping excitation wavelength 385 nm. The calibration curve was prepared by plotting concentration of sennoside *Vs* corrected fluorescence intensity of the respective solution. The correction in fluorescence intensity is made by subtracting the native fluorescence of blank solution from observed fluorescence intensity of standard or sample solution.

Factors affecting development of fluorescence:

Effect of Sodium dithionite concentration was evaluated by taking 2 ml solution of *T. chebula* fruit powder in a series of 25 ml volumetric flasks, treated with 7.5 ml of varying concentration of sodium dithionite solution (0.5-2.5 %w/v) and 7.5 ml of sodium borate solution. The solution was analyzed as described under fluorimetric method. Optimum time required to develop fluorescence was determined by taking 2 ml of stock solution of *T. chebula* in a series of 25 ml volumetric flasks and treated similarly as described under fluorimetric method except heated at different time interval and analyzed. The stability of developed fluorophore was determined by measuring the fluorescence intensity of the treated sample up to 90 min. Effect of tannins on excitation and emission maxima of sennoside was also evaluated. For that, mixture of sennoside and tannic acid, in the ratio of 1:9, was prepared and analyzed as described under fluorimetric method. Proposed method was validated in terms of accuracy, precision, linearity, specificity, limit of detection and limit of quantification.

RESULTS AND DISCUSSION

Various methods were reported for the estimation of hydroxy anthraquinone derivatives from different plant materials and formulations[10-13] (The wonders of *Triphala*: Ayurvedic formula for Internal Purification, www.webmaster@planetherbs.) They are generally based on oxidative cleavage of dianthrone followed by hydrolysis. These methods cannot be useful in case of *T. chebula* due to presence of large amount of tannins, which interfere in estimation of anthraquinone glycoside. The USP 2000[9] describes fluorimetric estimation of sennoside after its reduction to anthranol. Basically anthrones are non-fluorescent compound but after reduction they converted to anthranol that gives strong fluorescence in sodium borate solution. Earlier it was reported that anthraquinone glycoside in *T. chebula* is similar to sennoside A[2]. Therefore, it was thought of interest to apply USP fluorimetric method for the estimation of anthraquinone glycoside present in *T. chebula.*

In present work the anthraquinone glycoside present in *T. chebula* is treated with sodium dithionite in presence of borate solution. The reaction mixture showed excitation and emission wavelength at 385 and 495 nm respectively. Reported excitation and emission wavelengths for sennoside are 395 and 505 nm respectively. The shift in wavelength from 505 to 495 nm in case of *T. chebula* is due to the presence of large amount of tannins. To confirm this, standard sennoside solution is treated with tannic acid and a fluores-

cence spectrum was recorded. Comparison of the overlay spectrum of sennoside with tannic acid in the ratio of 1:9 and sennoside alone showed the shift of emission maxima of sennoside from 505 to 495 nm on addition of tannic acid. Similarly an excitation maximum was also shifted from 395 to 385 nm. Tannic acid showed a native fluorescence in borate buffer. Therefore, fluorescence intensity of sennoside and tannic acid mixture was higher than that of sennoside alone. After subtraction of native fluorescence of tannic acid from the fluorescence of mixture of sennoside and tannic acid, the resulting fluorescence intensity is identical with that of the fluorescence intensity of sennoside alone. Therefore, difference in the fluorescence intensity of the sample solution and the blank was representing the amount of anthraquinone glycoside present in *T. chebula*.

These results confirm that the fluorescence spectra obtained with *T. chebula* is due to the presence of anthraquinone glycoside, which is similar to sennoside. Therefore, USP method is suitable and correctly reflects the amount of anthraquinone glycosides in terms of sennoside in *T. chebula*. Thus sennoside can be used as a reference standard for the estimation of anthraquinone glycoside in *T. chebula*. It gave linear response in the range of 80-400 ng/ml and linear regression equation was $y=0.1899x-0.4198$, where 'y' is corrected fluorescence intensity and 'x' is concentration of anthraquinone glycoside in terms of ng/ml. Correlation coefficient was found to be 0.9991 for linear regression analysis.

TABLE 1: VALIDATION PARAMETERS FOR SPECTROFLUORIMETRIC METHOD.

Parameter	Range
Linearity range (ng/ml)	80-400
Detection limit (ng/ml)	50
Quantification limit (ng/ml)	80
Correlation coefficient (r)	0.9991
Accuracy (n=5)	99-101
Precision (%CV) (n=5)	1.21-3.88
Specificity	specific

The developed method was optimized using different parameters such as sodium dithionite concentration, heating time required for development of maximum fluorescence intensity and stability of developed fluorophore. Maximum fluorescence intensity was obtained with 7.5ml, 1.5%w/v sodium dithionite solution. On further increase in concentration of sodium dithionite fluorescence intensity get decreased. The minimum heating time required to get maximum fluorescence intensity was 30 min. No change in fluorescence intensity was observed on further heating. The developed fluorophore was stable up to 1hr at room temperature ($26\pm1^{\circ}$). After that fluorescence intensity diminished gradually. The optimized method was validated in terms of accuracy, specificity, precision, linearity, limit of detection

TABLE 2: ESTIMATION OF ANTHRAQUINONE GLYCOSIDE PRESENT IN *T.CHEBULA* AND ITS *CHURNA* FORMULATIONS.

Formulation	Varieties	Mean of Anthraquinone Glycoside found (%w/w) (n=3)	S.D.	%CV
T. chebula fruit	1	0.1060	0.032	3.039
	2	0.0784	0.017	2.212
	3	0.0433	0.190	4.355
T. chebula churna	1	0.0605	0.022	3.634
*Triphala churna**	1	0.0210	0.079	3.380
	2	0.0232	0.164	4.310
	3	0.0229	0.085	3.500
	4	0.0196	0.097	4.410
	5	0.0155	0.072	4.620

*It is a mixture of equal proportion of *T. chebula*, *T. belerica* and *P. emblica*.

and limit of quantification. The results are summarized in Table 1. The proposed method was successfully applied to determine the amount of anthraquinone glycoside in form of sennoside from three variety of *T. chebula* fruits and six marketed *churna* formulations (one *harde churna*, five *triphala churna*).

The different varieties of *T. chebula* showed variation in anthraquinone glycoside in the range of 0.0433-0.1060 %w/w. The variety bigger in size have high amount of anthraquinone glycoside. The pure *T. chebula* fruit powder i.e. *T. chebula churna* contain 0.0605 %w/w of anthraquinone glycoside. While *triphala churna*, which is a mixture of equal proportion of *T. chebula*, *P. emblica* and *T. belerica,* contain anthraquinone glycoside in the range of 0.0155-0.0232 %w/w (Table 2).

Thus, the developed method was specific, accurate, simple, precise and reproducible. The method can be directly applied for the determination of anthraquinone glycoside present in *T. chebula* fruits and its formulations in routine QC laboratories.

REFERENCES

1. Azim, A.M., Habib, A. and Nadia, A., **J. Nat. Prod.,** 1980, 43, 452.
2. Fairbairn, J.W. and Simic, S., **J. Pharm. Pharmacol.**, 1959, 12, 45T.
3. Green, M.W., **J. Amer. Pharm. Associ.**, 1936, 25, 107.
4. Inamdar, M.C., RajaramaRao, M.R. and Siddiqui, H.H., **Indian J. Pharm.**, 1962, 27, 87.
5. Kussmaul, W. and Becker, B., **Helv. Chem. Acta.**, 1947, 30, 59.
6. Lemli, J., **J. Pharm. Pharmacol.**, 1965, 17, 227.
7. Indian Pharmacopoeia, The controller of publications, Delhi, 1996, 675.
8. British Pharmacopoeia, Her Majesty's Office, London, 1993, 591.
9. United State Pharmacopoeia XXIV, National Formulary, XIX, US Pharmacopoeial convention Inc, Rockville, MD., 2000, 1516.
10. Lou, T.C., **J. Pharm. Pharmacol.**, 1949, 1, 673.
11. Patel, R.P., Derasari, H.R. and Parikh, S.H., **Indian J. Pharm.**, 1959, 21, 131.
12. Saini, T.S. and Gaind, K.N., **Indian J. Pharm.**, 1968, 30, 233.
13. Berned, V. and Latif, Z.A., **J. Pharm. Asso. Sci.**, 1949, 38, 589.