

the pure drug sample and analysis time required is only few min.

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REFERENCES

1. Rao, G.R; Avadhunula, A.B. and Batsa, D.K., *Indian Drugs*, 1990, 27, 532.
2. Mathur, S.C. Sunder Lal; Murugesan, N; Sethi, P.D. Rathore, Y.K; *Indian Drugs*, 1990, 27, 398.
3. Shanbag, G.S; Thampi; P.P. and Thampi, G.S. *Indian Drugs*, 1991, 28, 279.
4. Chowdhary, K.P.R. and Rama Prasad, Y.V; *Indian Drugs*, 1993, 31, 277.
5. Sane, R.T. Patel, D.V; Dhumal, S.N; Nerurkar, V.R; Maninkar, P.S. and Gangal, D.P; *Indian Drugs*, 1990, 27, 248.
6. *United States pharmacopoeia*, Suppl. 1st; United States Pharmacopoeial Convention, Inc. MD; 1990, 963.
7. Sastry, C.S.P. Aruna, M and Vijay, D. *Indian J Pharm Sci.*, 1988, 49, 190.
8. Sethi, P.D. Chaterjee, P.K. and Jain, C.L; *J. Pharm. Biomed. Appl.*; 1988, 6, 253.
9. Shrinath, Vand Bagawant, G; *Indian Drugs*; 1986, 24, 173.
10. Sadana, G.S. and Gaonkar, M.V.; *Indian Drugs*; 1989, 26, 510.
11. Sanghavi, N.M. Joshi, N.G; and Dubal, K.S; *Indian Drugs*, 1981, 80, 354.
12. Sadana, G.S. and Gaonkar, M.V; *Indian Drugs*, 1989, 26, 121.
13. Menonkar, M; Cuermousche, S. and Guermouche, M.H; *J. Pharm. Belg*, 1987, 42, 243.
14. Ray, S. *Eastern Pharmacist*, 1989, 32, 125.
15. Pernarowski, M; Knevel, A.M. and Christainn, J.E; *J. Pharm. Sci.*, 1961, 50, 943.

Determination of Vitamin C Content of *Phyllanthus Emblica* and *Chyavanprash*

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Specific and sensitive O-Phenylene diamine fluorimetric method has been adopted for the determination of vitamin C content during various stages of *chyavanprash* preparation starting from major vitamin C containing fruit, *Phyllanthus emblica* (amla) and its dehydrated powder. The pericarp of both bigger and smaller varieties of amla fruit, and its freeze dried powder was found to contain 2.915 (± 0.1), 3.775 (± 0.15) and 23.24 (± 0.18) mg of vitamin C per gram of pulp/powder, respectively. Vitamin C was found to be exceptionally stable in fresh and dried Amla fruits. All the three market samples of Chyavanprash tested do not contain any vitamin C. It is probably destroyed during frying of amla pulp with ghee.

CHYAVANPRASH, a traditional polyherbal formulation, is widely used as tonic, rejuvenator, anabolic, immunomodulator and memory enhancer¹. Amla, *Phyllanthus emblica*, one of the rich-

est sources of vitamin C², constitutes the main ingredient (35%). Due to lack of suitable quality control standards of Ayurvedic drugs it is difficult to ensure uniformity of their composition and consequently

efficacy of final products³. Even though the official methods for quality assurance of chyavanprash⁴ do not include vitamin C content, there are conflicting reports on the presence of Vitamin C in chyavanprash^{5,6} probably due to adoption of less sensitive and nonspecific methods for its determination, such as titrimetric⁷ and spectrophotometric^{8,9}. In view of these conflicting reports a systemic study was carried out for the determination of vitamin C content in the fresh and dried amla fruits, boiled and fried amla pulp and also in the three market samples of Chyavanprash using highly sensitive and specific fluorimetric method¹⁰.

Phyllanthus emblica fruits (Amla), both bigger and smaller varieties, were collected from the local market in the month of January and stored in deep freezer. Three established brands of market formulation of Chyavanprash were procured from the local market. Standard ascorbic acid, Ortho phenylenediamine dihydrochloride, sodium acetate, boric acid, meta phosphoric acid sticks, glacial acetic acid, sodium bicarbonate were of analytical reagent grade.

Metaphosphoric acid-acetic acid solution was used as an extracting solution (30 g methaphosphoric acid + 80 ml acetic acid and water to 1000 ml). A Hitachi F-2000 Spectrofluorimeter was used. Fresh pericarp (60 g) of both the varieties of amla fruit was first crushed in a mortar with small volume of meta phosphoric acid-acetic-acid extracting solution. The solution was decanted and the crushed pulp was homogenized twice with 200 ml extracting solution, each time, for 1 h. The solutions were combined and volume made upto 500 ml with the extracting solution. Filtered aliquot was analyzed by fluorimetric method.

Freeze dried and room temperature dried amla powder (10 g) were extracted with extracting solution as described above and volume was made up to 250 ml with extracting solution. A suitable aliquot was analyzed as above. Chyavanprash sample

(100 g) was shaken with n-hexane to remove fats, and then homogenized in a homogenizer with 200 ml of extracting solution for one hour. This was again repeated with further 200 ml of extracting solution. The solutions were combined and volumes was made upto 500 ml. Aliquots were analyzed by fluorimetric method.

Fresh fruit (200 g) of both the varieties of amla were boiled in 600 ml of distilled water for 80 minutes. Seeds were removed. Pulp obtained was 166 g while aqueous extract after boiling was 210 ml. Pulp and aqueous extract left after boiling were analyzed as described in analysis of fresh amla fruit. Above boiled pulp (100 g) of smaller variety amla was fried with 5 g ghee (clarified butter) till the pulp turned faint red. The fried pulp was cooled to room temperature and analyzed as described in analysis of chyavanprash.

Recovery experiments were carried out in all the three market samples of Chyavanprash. The recovery of the added standard ascorbic acid in Chyavanprash was studied at two different levels. 2.5 and 5 mg of ascorbic acid/g of Chyavanprash were added in the form of a concentrated aqueous solution, mixed thoroughly and analyzed after 48 hrs as described in the analysis of chyavanprash.

Fluorimetric method¹⁰ is specific and determines both forms of vitamin C i.e. Ascorbic acid and dehydroascorbic acid. The excitation and emission spectra of standard vitamin C and vitamin C from fresh fruits of amla and their blanks are identical, thereby confirming the presence of vitamin C in amla fruits. Aqueous solutions of Vitamin C are very unstable and hence extraction and analysis were performed in metaphosphoric acid- acetic acid solution.

The linearity of the relative fluorescence intensity versus concentration of vitamin C at their excitation and emission wavelengths was found to be in the range of 1 to 24.0 ug. Accurate measurements can be made for as little as 0.14 ug vitamin C per ml.

Table 1: Recovery of Vitamin C from Chyavanprash

Sample	Amount of Vitamin C in chyavanprash		Total amount Recovered mg/g *	% Recovery
	Found In mg	added In mg/gm		
Chyavanprash I	0.0	2.5	2.42	96.8
	0.0	5.0	4.88	97.6
Chyavanprash II	0.0	2.5	2.39	95.6
	0.0	5.0	4.92	98.4
Chyavanprash III	0.0	2.5	2.45	98.0
	0.0	5.0	4.81	96.2

Values are mean of 5 experiments.

The pericarp of both bigger and smaller varieties of amla was found to contain 2.915 (± 0.1) mg and 3.755 (± 0.15) mg of vitamin C per gram of fruit pulp, respectively. While freeze dried and room temperature dried amla powder were found to contain 23.24 (± 0.18) and 21.04 (± 0.11) mg of vitamin C per g of dehydrated powder.

The boiled amla pulp of smaller variety was found to contain 2.87 (± 0.14) and water extract contain 0.80 (± 0.21) mg of vitamin C per gm of fruit pulp. Vitamin C remains stable even after boiling the amla fruit in plain water for 80 min., indicating thereby that amla fruit and its dehydrated powder are highly stable natural sources of Vitamin C.

To validate the fluorimetric method and establish its accuracy and precision the recovery studies were conducted. The recovery of added vitamin C in Chyavanprash after 48 h of addition was about 97% (Table 1). The results indicate that the fluorimetric method adopted is suitable for the determination of total vitamin C in polyherbal chyavanprash. The method is accurate, precise and specific¹⁰. The recovery study indicates that vitamin C does not complex with tannins and other constituents present in Chyavanprash. Further, 3% metaphosphoric acid-

acetic acid solution is suitable for the extraction of vitamin C from Chyavanprash.

The boiled amla pulp was deep fried with ghee (clarified butter) and analyzed for vitamin C at intervals of 0, 10, 30 and 45 days. Vitamin C content was found to be 2.23, 1.99, 1.88 and 1.87 mg per g of pulp respectively. There was a loss of 34.8% of vitamin C on frying the boiled amla pulp. The loss of vitamin C may be due to high temperatures of about 140 to 150° achieved during frying. Also such high temperature may destroy vitamin C as well as the constituents which may be otherwise responsible for its stability. When market formulations of Chyavanprash were analyzed, vitamin C was found to be absent in all the market samples of Chyavanprash (Table 1).

REFERENCES

1. **The Charak Samhita**, Vol. III, Shree Gulab Kunveraba Ayurvedic Society, Jamnagar, 1949, 1295.
2. Sastri, B.N, **The Wealth of India, Raw Material**, Vol. III, Council of scientific and Industrial Research Publications, New Delhi, 1952, 108.
3. Nitya Anand, in **Comprehensive Medicinal Chemistry**, Vol. I, P.D. Pergamon Press, New York, 1990, 113.

4. Pharmacopoeial Standards for Ayurvedic formulation, C.C.R.I.M.H. 15, 1976, 87.
5. Katiyar, C.K., *Eastern Pharmacist*, XXXVIII, 1995, 454, 4.
6. Narayanan, M.N., *Eastern Pharmacist*, XXXVIII, 1995, 451, 4.
7. Hall. W.L. and Deutsch M.J., *J. Assoc. Off. Anal. Chem.*, 1965, 48, 1236.
8. Thompson, J.C. and Mottola, H.A., *Anal. Chem.*, 1984, 56, 755.
9. Wahba, N., Yassa, D.A. and Labib, R.D., *Analyst*, 1974, 99, 397.
10. Patricia Cunniff, *Official Methods of Analysis of AOAC*, 16th Edition Vol. II, AOAC International, 1995, 45. 1. 15.

Acetylcholine Antagonistic action of Aqueous Extract of *Orthosiphon Thymiflorus*

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Effects of an aqueous extract of *Orthosiphon thymiflorus* (AEO) on acetylcholine-induced contractions of isolated frog rectus abdominis muscle were studied. AEO produced significant inhibitory effect on the skeletal muscle contraction which may be due to inhibition of the effect of acetylcholine at the receptor site.

DIFFERENT *Orthosiphon* aqueous extracts have been reported to be diuretic¹⁻⁴ and anti-inflammatory⁵⁻⁶. The present study focused on nicotinic antagonistic action of aqueous extract of *Orthosiphon thymiflorus*⁷ on isolated skeletal muscle preparation of frog. *Orthosiphon thymiflorus* was collected from Tirunelveli district of Tamil Nadu and confirmed in Central Siddha Research Unit, Tirunelveli, Tamil Nadu and found to comply with all specifications. The aqueous extract was obtained by macerating 5 kg of whole plant of *Orthosiphon thymiflorus* with 50 l of boiling water. The filtrate was reduced to about 4 l *in vacuo* at about 35° and freeze dried afterwards. The yield was about 750 g of freeze dried extract (17%).

Isolated frog-rectus abdominis muscle was mounted in frog-Ringer solution at room temperature.

The dose-response curves of acetylcholine HCl were obtained as described by Ghosh (1984)⁸. The experiment was repeated in the presence of aqueous extract of *Orthosiphon thymiflorus*, added in the reservoir at varying doses (50, 100, 200 and 400 µg/ml respectively). The average of five determinations was computed.

Aqueous extract of *Orthosiphon thymiflorus* in all the doses tested, produced significant dose-dependent inhibition of contraction by acetylcholine. The result (Figure -1) clearly shows that the aqueous extract of *O. thymiflorus* blocks the nicotinic action of acetylcholine that regulate the flow of ions through plasma membrane channels⁹. Acetylcholine causes the opening of an ion channel in the nicotinic acetylcholine receptor, which allows Na⁺ to diffuse down