Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 3, 2013

Research Article

COMPARATIVE ESTIMATION AND CHEMICAL STANDARDIZATION OF NEW AND OLD SAMPLE OF CHYAWANPRASH

ARKA GHOSH, DAMIKI LALOO, *NARENDRA KUMAR SINGH

Faculty of Ayurveda, Institute of Medical Sciences, Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur 231001. Email: narendra_pharma1982@rediffmail.com

Received: 02 July 2013, Revised and Accepted: 04 Aug 2013

ABSTRACT

Objective: The present study was carried out to compare the quality of new and 24 months old sample of Chyawanprash from the same brand.

Material and Methods: Total polyphenolics, total reducing sugar, ascorbic acid, hydroxymethyl furfural (HMF), and fibre content were measured in both the new and 24 months old sample of Chyawanprash.

Results: Results obtained reveal the reduction in the percentage of total polyphenolics, total reducing sugar and ascorbic acid content while high level of HMF in the old sample as compared with new sample of Chyawanprash.

Conclusion: The results indicate that chemical degradation might take place during storage and may result in the loss of therapeutic activity of Chyawanprash.

Keywords: Chyawanprash, Polyherbal formulation, Galic acid, Vitamin C, Reducing sugar, Hydroxymethyl furfural.

INTRODUCTION

According to Ayurvedic Pharmacopeial Index (API), Chyawanprash is a polyherbal formulation with a semisolid and sticky in nature. It is a chocolate brown coloured having sweet taste with non-specific pleasant odour [1]. In Ayurvedic texts, Chyawanprash is classified under the group of Rasayana, where the main purpose is to maintain the body's integrity for delaying the ageing process, enhance longevity and improves digestion [2]. Throughout the country, it is used as a household remedy, which has a popular nutritive value and in India it has been relished as a health food since ancient times with the enthusiasm for the past 4000 years. This polyherbal formulation has been regarded as one of the most respected anti-ageing ayurvedic tonic, long time back since before the clinical importance of vitamins, minerals and antioxidant was appreciated [3-4]. Chyawanprash is the main source for the treatment of the respiratory tract system such as bronchial spasm, cough, asthmatic breathing, tuberculosis and is also useful as immunomodulator and memory enhancer [5]. It is a polyherbal formulation comprising of more than 50 medicinal plants ingredients such as Amlaki (*Emblica officinalis),* Bilva (*Aegle marmelos*), Agnimantha **(***Premna Integrifolia),* Syonak (*Oroxylum indicum*), Kasmari (*Gmelina arborea),* Patala (*Stereospermum suaveolens),* Bala (*Sida cordifolia),* Salaparni (*Desmodium gangeticum*), Prsniparni (*Uraria picta),* Mudgaparni (*Phaseolus trilobus),* Mashparni (*Teramnus labialis),* Pippali (*Piper longum),* Goksura (*Tribulus terrestris),* Brhati (*Solanum indicum*), kantakari (*Solanum surattense),* Srngi (*Pistacia integerrima),* Bhumyamalaki *(Phyllanthus amarus*), Draksha (*Vitis vinifera),* Jeevanti (*Leptadenia reticulate),* Puskaramul (*Inula racemosa),* Agaru (*Aquilaria agallocha),* Haritaki *(Terminalia chebula),* Guduchi *(Tinospora cordifolia*), Rddhi (*Habenaria intermedia*), Jivaka (Malaxis acuminate), Rsabhaka (*Malaxis muscifera*), Sati (*Hedychium spicatum*), Mustak (*Cyperus rotundus)*,Punarnava (*Boerhaavia diffusa)*, Meda (Polygonatum cirrhifolium), Ela (*Elettaria cardamomum*) Candan (*Santalum album*), Utpala (*Nymphaea stellata*), Vidari (*Pueraria tuberose*), Vrsamula (*Adhatoda vasica*), Kakoli (*Lilium polyphyllum*) and Kakanasika (*Marytynia annua*) [4]. All these ingredients have been well scientifically validated individually for their health care benefits [6]. One of the main active ingredients of Chywanprash is Indian goose berry fruit (*Emblica officinalis*) which is a rich source of Vitamin C and polyphenolics including flavonoids. It is also scientifically reported to have potent antioxidant and free radical scavenging activity [7]. Many of the natural antioxidants, hence, especially flavanoids exhibit a wide range of biological effects, including antibacterial, antiviral, and anti-inflammatory, anti allergic, antithrombotic, and vasodilatory actions [8].Vitamin C present in amla is one of the main factors that can help to retrieve or refill the energy lost by body [9]. Vitamin C is released into the body due to an inherent mechanism and is mainly conjugated to gallic acid and reducing sugars thus resulting to the formation of complex synergistic effect with other phytoconetituents [10]. In the preparation of Chyawanprash, honey is used as a sweetening ingredient and also works as 'a carrier of herbs' called Yogavahi and probably helps in the absorption of various herbs deep into the tissues [11-12]. All these active ingredients on long storage can results in chemical changes thereby can cause deterioration which probably affects the efficacy of the formulation.

To the best of our knowledge there is no scientific evidence so far reported in regards to the proper analytical testing related to the changes of the quantity of phytoconstituents present in Chyawanprash products on long storage. Hence, the present study was aimed to compare the new sample (within two months from the manufacturing date) and 24 months old sample of Chyawanprash from the same manufacturer to evaluate the changes in the concentration of some major phytoconstituents and other excipients that led to the loss of quality and efficacy of the products.

MATERIAL AND METHODS

Chemicals and instrumentation

Gallic acid, Vit C and hydroxymethylferfurol (HMF) were purchased from Sigma Aldrich, India. All other chemicals used were of analytical grade. UV absorbance was recorded using Varian UV-Visible spectrophotometer with Carry-100 software.

Sample collection

New sample (within two months from the manufacturing date) and 24 months old sample of Chyawanprash of the same brand were purchased from retail shop.

Estimation of total polyphenolic content

Total polyphenols content was determined by Folin-Ciocalteau methods [13-14]. 100 mg of each sample of Chyawanprash were taken and suspended in 50 ml of triple distilled water. 10 ml of 10 % lead acetate was added to the above suspension and filtered. Volume of the filtrate was adjusted up to 100 ml. 1ml of the above aqueous solution was transferred in a test tube. It was added by 8ml of triple distilled water, 0.5 ml of Folin-Cioattchu reagent and 1.5 ml of 20% of sodium carbonate solution. Test tubes were vortexed and absorbance of blue coloured mixtures were recorded after 40 min at the wave length 765 nm against blank. The amount of total polyphenols content was calculated equivalent to gallic acid

equivalent and expressed as mg of gallic acid/ gm of Chyawanprash. All measurements were done in triplicate.

Stock solution of gallic acid was prepared by dissolving 100 mg of gallic acid in 100 ml of triple distilled water. 2, 4, 8, 16 and 20 ml stock solution were transferred in separate volumetric flask and volume were adjusted up to 100 ml. 1 ml solution from each volumetric flask were transferred in separate test tubes and added with 7 ml of triple distilled water, 0.5 ml of Folin reagent and 1.5 ml of 20% sodium carbonate solution. Absorbance of blue coloured mixture was recorded after 40 min at the wave length 765 nm against blank.

Estimation of total hydroxymethyl furfural (HMF) content

Stock solution of HMF was prepared by dissolving 100 mg of furfural in 100 ml of water. Standard solutions were prepared from stock solution in the range of 2 to 20 µg/ml in water. The absorbance of HMF was measured at 283 nm against water as blank. Mean of three readings were taken for each standard solutions. 100 mg of each new and old sample were taken and diluted with 100 ml of distilled water in a 100 ml volumetric flask. The absorbance of sample solutions was measured at 283 nm against water as blank [15].

Estimation of total Vitamin-C content

Stock solution of the ascorbic acid was prepared by dissolving 100 mg of ascorbic acid in 100 ml of water. Standard solutions were prepared from stock solution in the range 0.5 to 2.5 µg/ ml in water. The ascorbic acid standard solutions were added with 1ml of each ferric chloride (1mM) and potassium ferricyanide (1mM) solution, mixed together by vigorous stirring for 10 min and volume adjusted up to 100 ml with water. The absorbance of ascorbic acid was measured at 709 nm against blank [16]. For the preparation of sample, 100 mg of each new and old Chyawanprash sample were taken separately and diluted with 100 ml of distilled water in a 100 ml volumetric flask. 1 ml of each sample were taken and added with 1ml of each ferric chloride (1mM) and potassium ferricyanide (1mM) solution, mixed together by vigorous stirring for 10 min and volume adjusted up to 100 ml with water. The absorbance of ascorbic acid was measured at 709 nm against blank.

Estimation of the total reducing sugar

5 gm of sample was taken in a 100 ml volumetric flask and added with 10 ml of neutral lead acetate solution. Volume was made up to 100 ml with water and filtered. Filtrate was added with potassium oxalate solution in drop wise until there was no further precipitation. Solution was mixed properly and filtered through Whatman No. 1 filter paper. Filtrate was filled in the burette and titrated with 5 ml of each Fehling solution A and B using methylene blue as an indicator at 80 °C until blue colour turn to brick red colour. [17]

Estimation of the fibre content

3 gm of the sample was taken and added with 20 ml of 0.255 N sulphuric acid. The sample was boiled for 30 minutes and filtered. Residue obtained was washed with hot water until it was free from sulphate. The remaining residue was then transferred to the beaker and added with 20 ml of 0.313 N NaOH solution. It was boiled for 30 minutes and filtered through Whatman No. 1 filter paper. The remaining residue was washed with hot water until it is free from alkali. Finally, the residue was dried at 100°C to constant weight. The residue obtained was wrapped in the filter paper and incinerated in the furnace. [17]. The % of fibre content was calculated using formula-

$$\% \text{ of fibre content} = \frac{\text{Weight of residue} \times 100}{3}$$

Weight of residue = Total weight of residue – [weight of filter paper + weight of ash]

RESULTS

Estimation of total polyphenolics content

Total polyphenols content in the new sample of Chyawanprash was found to be 5.23 ± 0.04 % equivalent to gallic acid while in the old sample 3.75 ± 0.02 %.

Estimation of hydroxymethyl furfural content

Hydroxymethyl furfural content in the new and old sample of Chyawanprash was found to be 0.56 ± 0.002 % and 1.65 ± 0.04 % respectively.

Estimation of Vitamin C (Ascorbic acid) content

Vit-C content in the new and old sample of Chyawanprash was found to be 0.0512 ± 0.0003% and 0.0253 ± 0.0001 % respectively.

Estimation of total reducing sugar content

Total reducing sugar content in the new and old sample of Chyawanprash was found to be 54.46 ± 0.62 % and 51.32 ± 0.54 % respectively.

Estimation of fibre content

Fibre content in the new and old sample of Chyawanprash was found to be 9.66 ± 0.08 % and 8.52 ± 0.1% respectively.

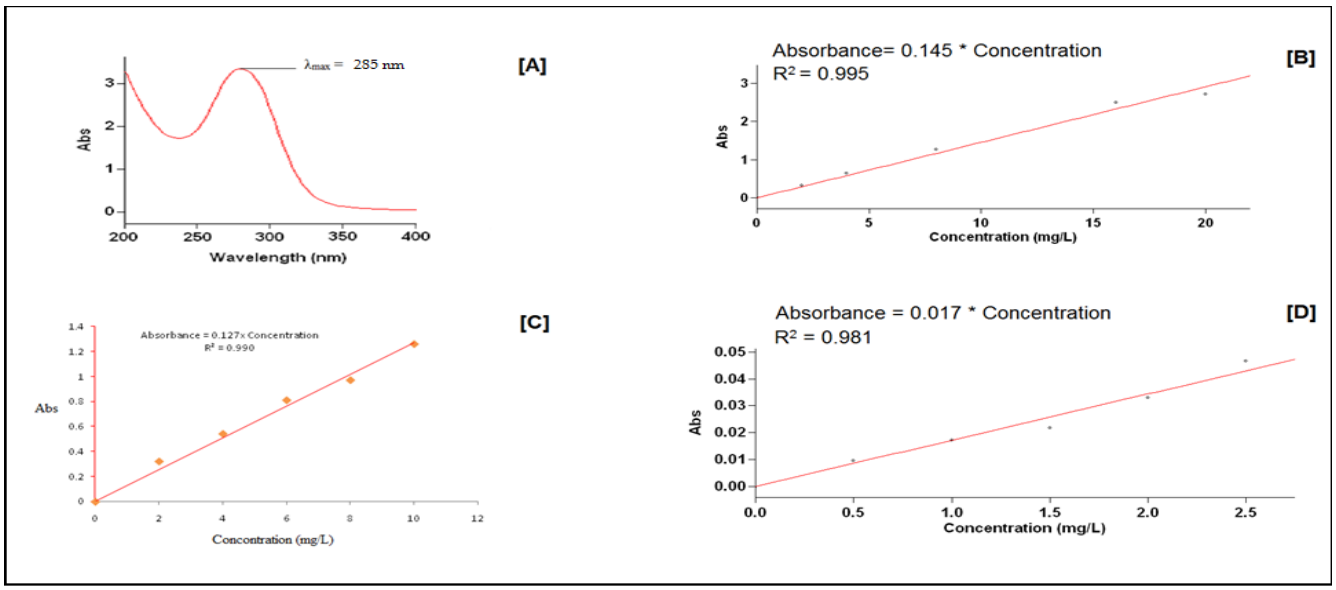

Fig. 1: [A] UV spectra of hydroxymethyl furfural [B] Calibration curve for hydroxymethyl furfural [C] Calibration curve for polyphenols [D] Calibration curve for ascorbic acid.

DISCUSSION

It has been well reported that Chyawanprash consist of various active and major phytochemicals which acts synergistically and are responsible for the therapeutic activity of the product. According to Trivedi et al. [18], Chyawanprash is made in amalaki (*Emblica officinalis*) base which is comprises the major percentage in the formulation and was also proven to contain the richest source of vitamin C (Ascorbic acid). Amalaki as well as ascorbic acid has been shown to be significantly effective as memory enhancer and potent antioxidant activity [2, 19]. It has been well documented that ascorbic acid is an unstable compound and on long storage it causes degradation [20-21]. The degradation of ascorbic acid during storage proceeds mainly *via.* anaerobic pathways and this generally leads to the formation of several decomposition reactive products [22-23] which therefore combine with amino acids, thus resulting in formation of brown pigments [24]. Hydoxymethyl furfural (HMF) is one of the decomposition products of ascorbic acid and suggested that it is the main precursor for the formation of brown pigments [21-22]. In addition, HMF is also one of the degradation products of honey and reducing sugars whereby a chemical ingredient furfural present in honey on long storage produce chemical degradation resulting to the production of hydroxymethyl furfural (HMF). The result of the present study signifies that the formation of degradable product HMF on storage increases in the old samples of Chyawanprash 1.65 ± 0.04 % as compared to the new samples of Chyawanprash containing 0.56 ± 0.002 %. Moreover, the percentage of ascorbic acid in the old samples (0.0253 ± 0.0001 %) was much lower than that of the new samples (0.0512 ± 0.0003%), thus signifying the chances of degradation on storage. Despite the high content of ascorbic acid, the fruit of *Emblica officinalis* and other ingredients of Chyawanprash also contain other active constituents such as polyphenolics (Tannins, phenolics and flavonoids) and reducing sugars which are responsible for balancing the therapeutic activity of the product. These phytoconstituents classes are also reported by many authors to be highly unstable and deterioriate on storage. Polyphenolics are regarded as the most abundant antioxidant in our diet and are widespread constituents of fruits, vegetables, cereals, olive, dry legumes, chocolate and beverages, such as tea, coffee and wine [25]. These are the classes of compounds which are highly unstable and extremely reactive and can undergo numerous reactions in the course of food processing and storage. Although the occurrence of such reactions and their roles in the development or degradation of food quality is well documented, the structures of the resulting products are still poorly understood and their concentrations in food are usually unknown. One of the main reasons for the decomposition of polyphenolics components is that these components are involving in reaction process either biochemically or chemically on storage, thereby resulting in browning of the products [26]. Polyphenols content in the old sample (3.75 ± 0.02 %) of chyawanprash decreases significantly as compared to new sample (5.23 ± 0.04 %). Our estimation results showed that the content of polyphenolics, ascorbic acid and reducing sugars are less in old samples while HMF content is more which can be predicted from the long storage of the products thereby leading to degradation.

CONCLUSION

From the present study, it was observed that there was a reduction in the quantity of various essential phytoconstituents in the old samples of Chyawanprash. This indicates that chemical degradation might take place during storage and may result in the loss of therapeutic activity of Chyawanprash products. Hence, from the overall results it can be concluded that phytochemical and analytical standardization of Chyawanprash should be done analytically on every batch so that to optimized and prevent the loss of various phytochemicals which probably has an impact on the therapeutic activity of the product.

ACKNOWLEDGEMENTS

We are extending our sincere thanks to the Department of Chemistry, Faculty of Science, Banaras Hindu University, Varanasi, for providing HMF for this study.

REFERENCES

1. Anonymous: Ayurvedic Pharmacopeia of India Part–II (Formulation): Department of AYUSH, Government of India. Volume – I, 1st Edition, 2007, p. 13-16.
2. Parle M, Dhingra D. Ascorbic acid: a promising memory enhancer in mice. J Pharmacol Sci 2003; 93(2): 129-135.
3. Kumar A, Kaur P, Rinwa P. Comparative study of various marketed brands of Indian Chyawanprash for their antianxiety and anti oxidant potential. Int. J. Pharm. Res. Bio-Sci. 2012; 1(4): 296-310.
4. Parle M, Bansal N. Antiamnesic Activity of an Ayurvedic Formulation Chyawanprash in Mice: Evid Based Complement Alternat Med. 2011:1-9.
5. Ojha J K, Khanna N N, Bajpay H S, Sharma N. A clinical study on Chyawanprash as an adjuvant in the treatment of pulmonary tuberculosis. J Res Indian Med 1975; 10: 11–14.
6. Rastogi S, Bala S, Govindrajan R, Rawat A K, Mehrotra S. Quantitative analysis of Chyawanprash: A well known Ayurvedic Formulation. Ind J Pharm Sci 2004; 66: 753-757.
7. Bhattacharya S K, Bhattacharya A., Sairam K, Ghosal S. Effect of bioactive tannoid principles of Emblica officinalison ischemia-reperfusion-induced oxidative stress in rat heart. Phytomedicine 2002; 9 :171-174.
8. Shah R, Kathad H, Sheth R, Sheth N. *In vitro* antioxidant activity of roots of Tephrosia purpuria Linn. Int J Pharm Pharm Sci 2010; 2(3):30-33.
9. Mirunalini S, Vaithiyanathan V, Krishnaveni M. AMLA: A novel Ayurvedic herb as a functional food for health benefits"- A mini review. Int J Pharm Pharm Sci 2013; 5(1): 1-4.
10. Singh V K, Palbag S, Singh N K. Comparative evaluation of furfural in branded and local honey sample by uv spectroscopy. Int J Pharm Pharm Sci Res 2012; 2(6): 95-96.
11. Chunekar K C. Bhav Prakash Nighantu, Chaukhambha Bharati academy, Varanasi, 2002, p. 788.
12. Tripathi R D. Ashthangsangrah. Chaukhambha Sanskrit Pristhan, Delhi, 2003, p. 106.
13. Hagerman A, Harvey M I, Makkar H P S. Quantification of tannins in tree foliage: a laboratory manual. FAO/IAEA, Vienna, 2000:4-7.
14. Singh N K, Sahu A N, Singh S K. Free radical scavenging and hepatoprotective activities of standardized methanolic extract of *maydis stigma*. Pharmacologyonline 2009; 2: 440-449.
15. Fallico B, Arena E, Zappala E. Degradation of 5-Hydroxymethylfurfural in Honey. J. Food Sci. 2008; 73 (9): 625-631.
16. Teepoo.S, Chumsaeng P, Jongjinakool S, Chantu K, Nolykad W. A new simple and rapid colorimetric screening test for semi qualitative analysis of vitamin c in fruit Juices Based on prussian blue. J Appl Sci 2012; 12 (6): 568-574.
17. Anonymous. The Ayurvedic Pharmacopoeia of India, Ministry of Health & Family Welfare, Govt. of India, Part – II, Volume – I, 1st Edition, New Delhi, 2007, p. 240-241.
18. Trivedi R P. Bhaishajay Kalpana, Dhanvantari Karyalaya, Vijayagarh, Aligarh, 1951, p. 260.
19. Vasudevan M, Parle M. Effect of Anwala Churna (*Emblica officinalis*): an Ayurvedic preparation on memory deficit rats. Yakugaku Zasshi 2007; 127(10): 1701–1707.
20. Johnson J R, Braddock R J, Chen C S. Kinetics of ascorbic acid loss and nonenzymatic browning in orange juice serum: Experimental rate constants. J. Food Sci. 1995; 60(3): 502–505.
21. Solomon O, Svanberg U, Sahlstrom A. Effect of oxygen and fluorescent light on the quality of orange juice during storage at 8°C. Food Chem. 1995; 53: 363–368.
22. Eskin N A M. Biochemistry of food processing: Browning reactions in foods. In Biochemistry of foods, London Academic Press, 2nd ed. 1990, p. 240-295.
23. Huelin, F E, Coggiola I M, Sidhu G S, Kennett B H. The anaerobic decomposition of ascorbic acid in the pH range of foods and in more acid solutions. Journal of Science of Food and Agricultural 1971; 22: 540–542.

24. Larisch B, Grob U, Pischetsrieder M. On the reaction of L-ascorbic acid with propylamine under various conditions: quantification of the main products by HPLC/DAD. Zeitschrift fur Lebensmittel Untersuchung Und-Forschung A 1998; 206: 333–337.
25. D'Archivio M, Filesi C, Benedetto R, Gargiulo R, Giovannini C, Masella R. Polyphenols, dietary sources and bioavailability. ANN IST SUPER SANITA 2007; 43 (4): 348-361.
26. Cheynier V. Polyphenols in foods are more complex than often thought. Am J Clin Nutr 2005; 81(suppl): 223S–229S.