Quality Assurance of Chyavanprash Through Determination of Free Radical Scavenging Activity

C.J. SHISHOO*, S.A. SHAH. I.S. RATHOD. AND S.G. PATEL.
Department of Quality Assurance, L.M. College of Pharmacy.
Navarangpura, Ahmedabad- 380 009.

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Free radical scavenging activity of ethyl acetate, alcohol and aqueous extracts of fresh amla fruits, freeze dried amla powder and Chyavanprash was determined by DPPH assay method. Freeze dried amla powder and fresh amla fruits exhibited very good scavenging activity in all the extracts comparable to that of vitamin C. The alcoholic and aqueous extracts of Chyavanprash exhibited a very low level of activity compared to its ethyl acetate extract. The scavenging activity of ethyl acetate extract of Chyavanprash is very high (EC_{50} 3.4 ug/ml) comparable to that of pure vitamin C (EC_{50} 2.99 ug/ml). All the three market samples of Chyavanprash showed comparable activity even though vitaminc C is absent. The results indicate that free radical scavenging activity by DPPH assay method may be used as one of the quality control parameters for Chyavanprash.

DURING hepatic detoxification of xenobiotics and toxic substances large amounts of reactive oxygen species are produced. Large acute doses of toxic agents or chronic exposure to such substances can overpower the antioxidant defense system of the body and cause cell damage. Free radicals may damage cell DNA in a way that eventually leads to cancer, may interact with cholesterol in the blood stream to produce clogged arteries, may help cause cataract and rheumatoid arthritis[1,2]. Aging process is associated with free radical damage of cells and tissues[3]. Any compound natural or synthetic that can quench free radicals would, therefore, contribute towards the partial or total alleviation of these damages.

Vitamin supplements of A,E, and C are effective and risk free forms of preventive medicine[1,4]. Combination of vitamin C, E and beta carotene, a popular nutrition supplement in U.S., is believed to help protect the somatic cell from free radicals that are thought to be responsible for wide ranging damage to the tissues.

In continuation of our work on quality assurance of Chyavanprash[5], it was thought of interest to determine the free radical scavenging activity of fresh amla fruits, freeze dried amla powder and market samples of Chyavanprash DPPH assay method[6] was employed to measure free radical scavenging activity.

The assay method[6] is based on the reduction of a methanolic solution of the colored free radical DPPH (1,1'-diphenyl-2-picryl hydrazyl) by free radical scavenger. The decrease in absorbance of DPPH at its absorbance maximum of 516 nm is proportional to the concentration of free radical scavenger added to the DPPH reagent solution. The activity is expressed as effective concentration as EC_{50} (i.e. the concentration of the test solution required to give 50% decrease in absorbance compared to that of blank solution).

Phyllanthus emblica fruits (amla), were collected from the local market in the month of January and stored in deep freezer(-10°). Three established brands of market formulation of Chyavanprash were procured from the market. DPPH (1,1- diphenyl picryl hydrazyl) was purchased from Sigma Aldrich Inc, from USA, and ascorbic acid, from BDH. Methanol, ethyl acetate, alcohol and n- hexane were of laboratory grade and procured from SD Fine Chemicals, Mumbai, spectrophotometer used was Shimadzu, UV 160A.

*For Correspondence

Table 1: Free Radical Scavenging Activity of Different Extracts of Chyavanprash and Amla Fruits

Sample	Extracts	Scavenging Activity EC_{50} (ug/ml)	r*
Chyavanprash I	Ethyl Acetate	03.46	0.9985
	Alcoholic	28.90	0.9968
	Aqueous	50.29	0.9943
Chyavanprash II	Ethyl Acetate	03.51	0.9983
	Alcoholic	30.97	0.9980
	Aqueous	50.51	0.9954
Chyavanprash III	Ethyl Acetate	03.48	0.9993
	Alcoholic	30.89	0.9980
	Aqueous	50.19	0.9967
Freez dried Amla fruits	Ethyl Acetate	04.53	0.9943
	Alcoholic	04.75	0.9972
	Aqueous	05.91	0.9968
Fresh amla Fruits	Ethyl Acetate	04.68	0.9951
	Alcoholic	04.98	0.9974
	Aqueous	06.00	0.9973
Abscorbic Acid	Ethanolic Solution	02.99	0.9947

* r = Correlation co-efficient

All the samples of chyavanprash (25 g), were macerated for 24 h with 200 ml of n-hexane to remove fats and waxes. The samples were then macerated for 24 h with 200 ml of ethyl acetate and filtered. The ethyl acetate filtrate was dried under reduced pressure. The residue was further extracted similarly with 200 ml alcohol and finally with 200 ml distilled water. All the three fractions were dried under reduced pressure.

Freeze dried amla powder (10 g) or fresh amla fruits (25 g) were macerated for 24 h. with 200 ml of ethyl acetate followed by 200 ml of alcohol, and finally with 200 ml of water as described above. The collected fractions were dried under reduced pressure.

To a set of clean and dry test tubes containing 3 ml of methanol, 75 ul of DPPH reagent solution was added with micro pipette and mixed thoroughly. The initial absorbance of each test tube was measured at 516 nm. To these tubes the methanolic solution of extractive residues were added in increasing concentration in the following manner. In case of Chyavanprash, 5-30 µl of methanolic solution of ethylacetate extractive residue (0.5 mg/ml), 10-70 ul alcoholic residue solution (2mg/ml) and 10-110 µl of aqueous residue solution (2 mg/ml) and in case of fresh amla fruit and freeze dried amla powder, 5-25 ul of ethylacetate and alchoholic extractive residue solution (1 mg/ml) and 10-60 µl of aqueous residue solution (0.5 mg/ml) were added. The ethanolic solution of ascorbic acid (0.5 mg/ml) was added in range of 5-25 µl as control. The solution was mixed, allowed to stand for 4 min at room temperature and final absorbance was measured at 516 nm.

The % reduction in absorbance was calculated from the initial and final absorbance at each level. Concentration of extract required for 50% reduction in absorbance (EC_{50}) was calculated from the calibration curve of concentration of extract (ug) vs. % reduction in absorbance. The result was subjected to linear regression between 10 and 80%.

The aqueous, alcoholic, and ethyl acetate extracts of

freeze-dried amla powder and fresh amla fruits exhibited very good free radical scavenging activity (Table 1). The scavenging activity of alcoholic and ethyl acetate extract was higher than that of the aqueous extract. The results indicate that freeze-dried amla powder and fresh amla fruits are identical in their free radical scavenging activity.

Similarly, when Chyavanprash extracts were analysed for free radical scavenging activity by DPPH assay, the alcoholic and aqueous extract exhibited a very low level of activity compared to its ethyl acetate extract (Table 1). The scavenging activity of ethyl acetate extract is very high and is comparable to that of vitamin C activity (Table 1). All the three samples of Chyavanprash showed comparable activity even though vitamin C is absent[5].

The aqueous extract of fresh amla and its freeze dried powder exhibited better free radical scavenging activity compared to its preparation, Chyavanprash. It is known that both amla and its dehydrated powder contain vitamin C, which is extracted in water, therefore, the activity of aqueous extract is mainly due to the presence of vitamin C in it. Low activity of aqueous extract of Chyavanprash again confirms the absence of vitamin C in it[5].

Interestingly, the ethyl acetate extract of Chyavanprash and amla fruits exhibited very high scavenging activity comparable to Vitamin C. This may be due to the presence of flavonoids, tannins, polyphenols and other antioxidant constituents.

Aqueous, alcoholic and ethyl acetate extracts of three Chyavanprash market samples exhibited identical activity in their respective extracts (Table 1). Thus, free radical scavenging activity by DPPH assay method could be exploited as quality control parameter of Chyavanprash.

Free radical scavenging activity data indicates that Chyavanprash and amla fruit possess very good antioxidant property, eventhough Chyavanprash does not contain vitamin C. Free radical scavenging activity can thus be used as a routine quality control parameter for Chyavanprash.

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