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Determination Of Free Radical Scavenging Activity In Herbal Supplement: Chyawanprash

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Abstract

Earlier investigations have shown that there are a number of plants which shows an antioxidant activity due to the presence of flavonoids and other polyphenolic compounds. Since Chyawanprash is made with such type of plants or their parts so manufacturer has claimed their antiaging effects by , inhibiting the formation of free radicals in body. In the present work the free radical scavenging activity of ethyl acetate, methanolic and aqueous extracts of different brand of Chyawanprash were evaluated spectrophotometrically by in *vitro* DPPH (1, 1-diphenyl, 2-picryl hydrazyl) assay at 516nm.The absorbance decreases when the radical is reduced by antioxidants. As results indicates that ethyl acetate extract of all samples exhibited higher level of scavenging activity i.e. close to ascorbic acid (IC_{50} 20.693 µg/ml) as compared to its methanolic and aqueous extracts. Free radical scavenging activity of aqueous extracts of all brands is comparable and close to each other that is indicative that these brands are likely to exhibit similar free radical activity.

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Introduction:

About 5% or more of the inhaled oxygen (O_2) is converted to reactive oxygen species (ROS) such as O_2^{-}, H_2O_2, and OH by univalent reduction of O_2. [1, 2] Free radicals play a significant role in the causation of several diseases such as diabetes, obesity, cirrhosis, cancer and cardiovascular diseases. [3] The harmful effects of free radicals are neutralized by the enzymatic antioxidant defenses including the super

oxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). However, overproduction of the ROS arising from either mitochondrial electron transport chain, excessive stimulation of NAD (P) H, or exposure to environmental pollutants, cigarette smoke, ultraviolet rays, some parasitic infections, radiation and toxic chemicals results in oxidative stress- a phenomenal disturbance in the equilibrium status of pro-oxidant/antioxidants reactions in living systems, which mediates damage to cell structures, including lipids and membranes, proteins, and DNA.[4, 5] Thus, compounds or antioxidants that can scavenge free radicals have a vital role in the improvement of these diseased conditions.[6]Plants contain a wide variety of free radical scavenging molecules such as phenols, flavonoids, vitamins and terpenoids, which are rich in antioxidant activity.[7] Many dietary polyphenolic constituents derived from plants are more effective antioxidants in vitro than vitamins E or C and thus might contribute significantly to the protective effects in vivo.[8] Chyawanprash is one of the best example of such type of dietary food supplement containing more than 40 Ayurvedic herbs and spices with 'Amla berry' also known as Emblica officinalis or Indian gooseberry that forms the base. All these ingredients make Chyawanprash a rich source of phyto-nutrients and antioxidants. Fresh 'Amla berries' are the key ingredient in Chyawanprash. Amla berry has been studied for its anti-oxidant benefits; [9, 10] Immunomodulator and anti-cancer activity; [11] hypolipidemic activity; [12] Hepato-protective benefits.[13, 14] Hence, the aim of the present study is to compare and evaluate the authenticity with respect to free radical scavenging activity, of nine different marketed Chyawanprash with standard one (S1) by Using UV-Visible spectrophotometer at the wavelength of 516 nm.

Materials and Methods:

Materials:

All the ingredients used in preparation of Chyawanprash and nine other brands were purchased from the local market of Mandsaur (M.P.). The ingredients or herbs were authenticated by Dr. Devendra Puranik (Reg.No.3991), RMO, Dist.Ayurvedic Hospital, Mandsaur (M.P.). Chyawanprash was prepared according to text mentioned in Charka Samhita. Ethyl acetate, n-Hexane, Methanol and 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) (Sigma Aldrich Co.), Ascorbic acid and all other chemicals were of analytical grade were purchased from Qualigens.

Method:

Preparation of Fractional extracts of Chyawanprash:

Each brand of Chyawanprash (25 g) was macerated with 200 ml of n-hexane for 24 hr. to remove fats and waxes and then supernatant was decanted. Solid mass of different brands were macerated with ethyl acetate for 24 hr. and filtered under reduced pressure. The residue of different brands were further similarly extracted with 200 ml of alcohol and finally with 200 ml of distilled water. All the three fractions were dried under reduced pressure.

Evaluation of free radical scavenging activity:

A set of clean and dry test tubes prepared and then added 3 ml of methanol and 75µl of DPPH reagent solution in each test tube and mixed thoroughly. The initial absorbance (Ac) of each test tube was measured on UV-Visible spectrophotometer (model uv-1, Merck Thermo Spectronic) at 516 nm.

Methanolic solution of standard ascorbic acid (0.5mg/ml) was prepared and added in range of 5-35 µl in test tubes containing methanol and DPPH reagent solution, as control. All these tubes kept aside for 4 min at room temperature and measured the final absorbance (As) at 516 nm. The % reduction in absorbance was calculated from the initial and

final absorbance at each level by using the following formula (Table-1)

% Reduction = (Ac –As)/Ac *100

Ac = Control Absorbance

As = Sample Absorbance

Constructed a plot between concentration vs % reduction in absorbance of DPPH by adding the ascorbic acid (figure-1) and calculated the IC_{50} (Concentration of Ascorbic acid required for 50% reduction in absorbance) from the equation y=1.803x +12.69.

Similarly IC_{50} of Methanolic solution of all three residues i.e. ethyl acetate(0.5mg/ml), alcohol (2mg/ml) and water (2mg/ml) was determined by adding increased concentration i.e. ethyl acetate(5-35 µl), alcohol (25-150 µl) and water (25-150 µl) in above prepared test tubes containing methanol and DPPH reagent solution.

Table -1 Percentage reductions in absorbance of DPPH at 516 nm by Adding Ascorbic acid

Concentration (µg/ml)	Absorbance	% Reduction	IC_{50} (µg/ml)
5	0.779	22.333	
10	0.699	30.309	
15	0.608	39.382	
20	0.518	48.355	20.693
25	0.425	57.627	
30	0.321	67.996	
35	0.248	75.274	

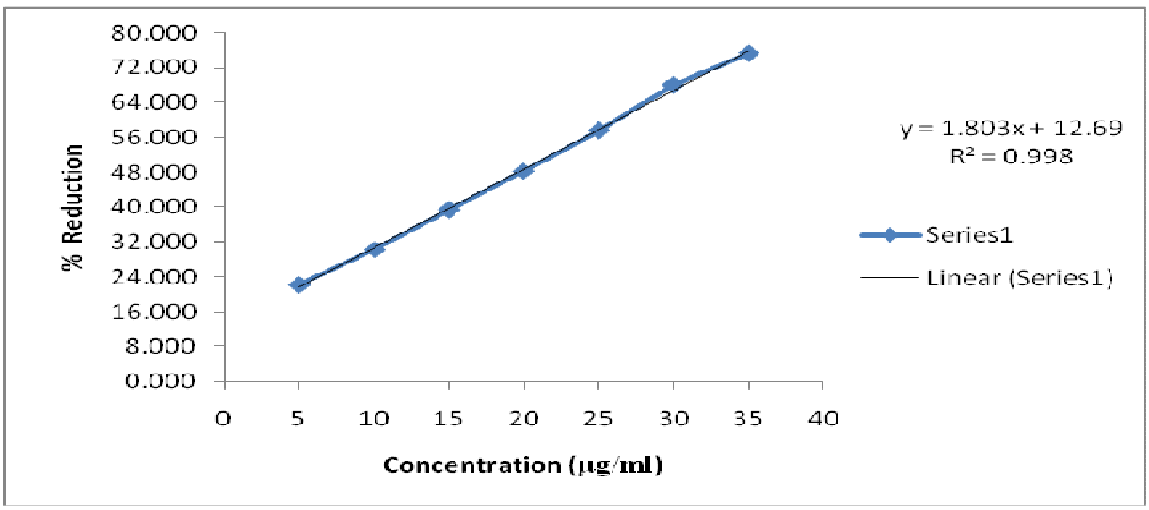

Figure – 1: Graphical presentation of % reduction in absorbance of DPPH at 516 nm by adding Ascorbic acid

Result and Discussion:

Unlike other free radicals such as the hydroxyl radical and super oxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. [15] A freshly prepared DPPH solution exhibits a deep purple colour with absorption maximum at 516nm. The purple colour generally fades or disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them to a colorless (i.e., 2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 516nm. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract. This test is a

commonly employed assay in antioxidant studies of specific compounds or extracts across a short time scale. The principle advantage of DPPH is that its reduction can be measured directly in the reaction medium by a continuous spectrophotometric assay. DPPH assay is known to give reliable information concerning the antioxidant ability of the tested compounds. [16, 17]

Free radical scavenging activity of ethyl acetate, methanol & aqueous extracts of various brands of Chyawanprash were determined by DPPH assay method. Table–2 & figure-2 shows the IC_{50} (Concentration of the test solution required to give 50% decrease in absorbance compared to that of blank solution) of various brand of Chyawanprash in different extracts. As results indicates that ethyl acetate extract of all samples exhibited higher level of scavenging activity i.e. close to ascorbic acid (IC_{50} 20.693 µg/ml) as compared to its methanolic and aqueous extracts. Free radical scavenging activity of aqueous extracts of all brands is comparable and close to each other that is indicative that these brands are likely to exhibit similar free radical activity. Thus the results of free radical scavenging activity by DPPH assay method helps to design the quality control protocol and might be useful to rate the product of various manufacturers hence help consumer chose the right brand and the manufacturers improve upon the quality of their product.

Table -2: Free radical scavenging activity of different extracts of various Brand of Chyawanprash

Sample	Extracts	Scavenging Activity IC_{50} (µg/ml)	R^2
S1	Ethyl Acetate Methanol Water	24.936 132.769 155.377	0.988 0.982 0.982
S2	Ethyl Acetate Methanol Water	26.513 205.991 263.967	0.993 0.991 0.989
S3	Ethyl Acetate Methanol Water	32.429 192.325 254.104	0.993 0.983 0.984
S4	Ethyl Acetate Methanol Water	23.003 171.699 107.845	0.985 0.998 0.991
S5	Ethyl Acetate Methanol Water	21.950 129.508 138.495	0.994 0.990 0.996
S6	Ethyl Acetate Methanol Water	27.405 166.919 201.156	0.983 0.986 0.994
S7	Ethyl Acetate Methanol Water	29.685 160.812 209.418	0.984 0.987 0.987
S8	Ethyl Acetate Methanol Water	22.901 156.988 244.443	0.989 0.989 0.990
S9	Ethyl Acetate Methanol Water	24.307 153.302 156.989	0.985 0.991 0.989
S10	Ethyl Acetate Methanol Water	30.987 143.170 200.485	0.991 0.994 0.992
Ascorbic Acid	**Methanol**	**20.693**	**0.998**

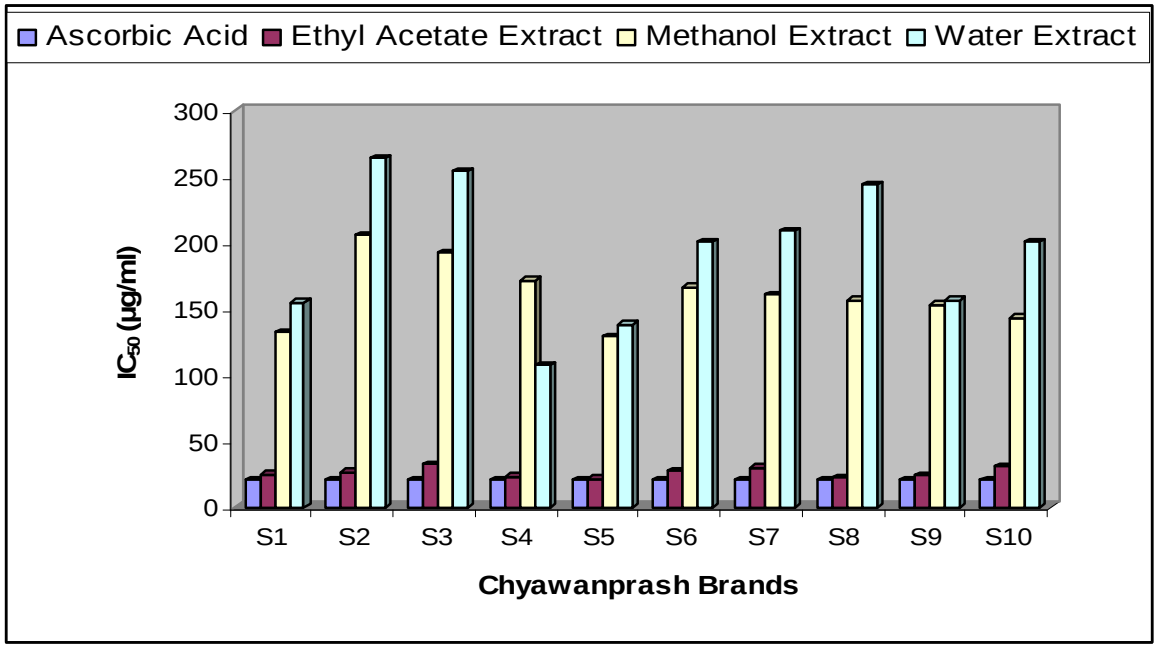

Figure-2: Graphical Comparison of Free radical scavenging activity of different extracts of various Brand of Chyawanprash with standard Ascorbic Acid.

Conclusion:

The free radical scavenging activity of different extracts was evaluated based on the ability to scavenge the synthetic DPPH. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. The results obtained in the present study indicate that the ethyl acetate extract of different brands exhibit potent free radical scavenging and antioxidant activity as close to the standard ascorbic acid (IC_{50} 20.693 µg/ml).

The overall antioxidant activity might be attributed to its polyphenolic content and other phytochemical constituents. The findings of the present study suggest that free radical scavenging activity of aqueous extracts of all brands is comparable and close to each other that is indicative that these brands are likely to exhibit similar free radical activity and preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases but comparatively less than standard ascorbic acid.

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