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RESEARCH ARTICLE

Evaluation of Morphological, Phytochemical and Physicochemical Properties of Indian Polyherbal Formulation, Chyawanprash for Quality Evaluation

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Abstract:
The present study was designed to evaluate quality profile of Indian polyherbal formulation, chyawanprash and to find new additional parameters and methods which may be considered for its quality control. Different marketed formulations of Indian chyawanprash were assessed for their morphological, phytochemical and physicochemical properties. The data analysis revealed that physicochemical parameters were within the limits as per the Pharmacopoeial standards. However, there was a significant variation among chyawanprash preparations. Most of marketed brands of Indian chyawanprash were well within the limit but there was also significant difference in some parameters among chyawanprash preparations. Therefore the present investigation reveals that there is a need to make more stringent quality control parameters in order to reduce variation among different Indian chyawanprash preparations.

Keywords: Polyherbal formulation, morphological, phytochemical, physicochemical properties, marketed brands, quality control parameters.

1. INTRODUCTION

Chyawanprash is a traditional Indian polyherbal formulation. It is widely used as tonic, rejuvenator, anabolic, immunomodulator and memory enhancer [1]. Amla constitutes as the main ingredient 35% of chyawanprash. Amla is richest source of vitamin C, a powerful antioxidant [2]. Being a well-known Ayurvedic formulation, 'Chyawanprash' has been the subject of study among several researchers [3,4]. However, their main emphasis have been on correlating the ethnomedicinal uses of some of its ingredients with the medicinal properties attributed to it, and evaluation of its physicochemical properties for quality evaluation. The entire Indian market for c**hyawanprash** stands around 50- 60 tones [5], even though only very few quality control methods for its evaluation have been established [6,7]. Due to lack of adequate quality control standards of Ayurvedic/ traditional preparations, it is very difficult to ensure uniformity of their composition and consequently quality standards of final products. Even the reported official methods for quality assurance of chyawanprash do not include complete analysis parameters [8].

Therefore, the present study was undertaken firstly, to establish and validate the quality control parameters of Indian chyawanprash and secondly, to find new additional parameters and methods which may be considered for its quality control. Efforts have been made to compare the quality control parameters of different marketed chyawanprash preparations.

2. MATERIALS AND METHODS

2.1 Chyawanprash preparations

The following marketed chyawanprash preparations were used in the present study. Brand D (Batch No. PN0964), Brand B (Batch No. 86), Brand Z (Batch No. MCH-9020), Brand Dh (Batch No. BN.CPS.94) and, Brand A (Batch No. JVPF8085SJ). All brands of the Chyawanprash were procured from the local market.

2.2 Morphological Testing

All the organoleptic properties viz. color, odor, taste, and consistency of different chyawanprash preparations were noted down and assessed.

2.3 Phytochemical Evaluation

Aqueous and methanol extract of chyawanprash prepared by hot extraction, were used for the phytochemical investigations. All the phytochemical investigations were done as follows:

Tests for alkaloids *(Wagner's Test)* Small quantity of the extract was taken in a test tube and evaporated. To the residue 1 ml dilute HCl was added, shaken well and filtered. To the 1-2 ml of filtrate, Wagner's reagent was added. Brownish-red precipitate was formed showing the presence of alkaloid(s) (9).

Tests for carbohydrates *(Fehling's Test)* 1 ml Fehling's A solution and 1 ml of Fehling's B solution were mixed and boiled for one minute. Equal volume of extract was added to the above mixture. The solution was heated in boiling water bath for 5-10 minutes. Brick red precipitate was observed showing the presence of carbohydrates (10).

Test for fixed oils and fats *(Spot test)* A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oil (11).

Tests for flavonoids *(Shinoda Test)* 5 ml of 95% ethanol and few drops of concentrated HCl was added to few ml of the extract. To this solution, 0.5 g of magnesium turnings was added. Observance of pink tomato red coloration indicated the presence of flavonoid(s) (12).

Tests for glycosides *(Keller-Killiani Test)* To 2 ml of the extract, glacial acetic acid, one drop 5% $FeCl_3$ and conc. H_2SO_4 was added. Reddish brown color appeared at junction of two liquid layers and upper layer turned bluish green indicating the presence of glycoside(s) (9).

Test for proteins and free amino acids *(Millon's test)* A small quantity of both alcoholic and aqueous extracts was added in a few ml of distilled water, separately. To 2 ml of filtrate, 5-6 drops of Millon's reagent was added, red colored precipitate formation confirmed the presence of proteins and free amino acids (11).

Test for saponins *(Foam Test)* Drug extract was shaken vigorously with water. Formation of persistent foam indicates presence of saponins (9).

Tests for tannins and phenolic compounds

(a) $FeCl_3$ Solution Test*:* On addition of 5% $FeCl_3$ solution to the extract, appearance of deep blue black color indicates the presence of phenolic compound(s) (10).

(b) Dil. HNO_3 Test*:* On addition of few ml of dilute HNO_3 to the extract, appearance of reddish color indicated the presence of tannin(s) and phenolic compound(s) (10).

2.4 Physicochemical Evaluation

Water soluble extractive value

5 g of chyawanprash preparation was macerated with 100 ml of distilled water in a closed flask for 24 hours, frequently shaking for six hours and allowing standing for the next 18 hours. It was filtered rapidly, taking precautions against the loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105° C to constant weight and was weighed. The percentage of water soluble extractive was calculated with reference to the original amount of chyawanprash taken (13). It was done in triplicate for every chyawanprash preparation.

Alcohol soluble extractive value

5 g of chyawanprash preparation was macerated with 100 ml of alcohol of the specified strength in a closed flask for 24 hours, frequently shaking for six hours and allowing standing for the next 18 hours. It was then filtered rapidly, taking precautions against the loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105° C to constant weight and was weighed. The percentage of alcohol soluble extractive was calculated with reference to the original amount of chyawanprash taken (13). It was done in triplicate for every chyawanprash preparation.

Loss on Drying (LOD)

10 g of chyawanprash preparation (without any preliminary drying) after accurately weighing was placed in a tarred evaporating dish. It was then dried at 105° C for 5 hours, and weighed. Drying and weighing was continued at one hour interval until difference between two successive weighing (drying for 30 min and cooling for 30 min in a desiccator), show not more than 0.01 g difference. The percentage LOD was calculated with reference to the original amount of chyawanprash taken (13). It was done in triplicate for every chyawanprash preparation.

Total acidity in terms of anhydrous citric acid

2g chyawanprash was weighed accurately and suspended in 100 ml of water. It was titrated with 1 M sodium hydroxide using 0.5 ml of phenolphthalein solution as indicator (14). It was done in triplicate for every chyawanprash preparation.

Vitamin C content

25 ml of vitamin C standard solution was added to a 125 ml Erlenmeyer flask. 10 drops of 1% starch solution was added. Burette was rinsed with a small volume of the iodine solution and filled. The initial volume was recorded. Solution was titrated until the endpoint (Blue color) was reached. Final volume of iodine solution was recorded. Titration was done in triplicate.

Samples were titrated exactly the same way as standard. The initial and final volume of iodine solution required to produce the color change at the endpoint were recorded. And vitamin C content was calculated (15). It was done in triplicate for every chyawanprash preparation.

Saponification Value

2 g of chyawanprash preparation was weighed accurately, into a 200 ml flask of borosilicate glass fitted with reflux condenser. 25 ml of 0.5 M ethanolic potassium hydroxide and a little pumice powder was added and boiled under reflux on water bath for 30 minutes. 1 ml of phenolphthalein solution was added and titrated immediately with 0.5 M HCl (1 ml). Blank titration was carried out by omitting the substance under examination (b ml) (12). It was done in triplicate for every chyawanprash preparation. Saponification value was calculated from the expression:

$$\text{Saponification value} = \frac{28.05\ (b-a)}{W}$$

Acid Value

10 g of chyawanprash preparation was weighed accurately, and transferred in 50 ml of a mixture of equal volumes of ethanol (95%) and ether, previously neutralized with 0.1 M KOH to phenolphthalein solution. The flask was connected with a reflux condenser and warmed slowly, with frequent shaking, until the sample dissolved. 1 ml of phenolphthalein solution was added and titrated with 0.1 M KOH until the solution remained faintly pink after shaking for 30 seconds. It was done in triplicate for every chyawanprash preparation. Acid value was calculated from the expression:

$$\text{Acid value} = \frac{5.61\ n}{W}$$

Where, n = the number of ml of 0.1 M KOH required;

W= the weight, in g of the substance (14).

Ester Value

Ester value was calculated from the expression:

Ester Value = Saponification value - Acid value (14)

Total Ash

2g accurately weighed chyawanprash was incinerated, in a tarred silica dish at a temperature not exceeding 450^{o} C until free from carbon. It was then cooled and weighed. The percentage of ash was calculated with reference to the original amount of chyawanprash taken (13). It was done in triplicate for every chyawanprash preparation.

Acid Insoluble Ash

To the crucible containing total ash, 25 ml of dilute HCl was added. Insoluble matter was then collected on ashless filter paper and washed with hot water until the filtrate was neutral. Filter paper containing the insoluble matter was transferred to the original crucible, and ignited to constant weight. The residue was allowed to cool in desiccator for 30 minutes and then weighed. Acid insoluble ash was calculated with reference to the original amount of chyawanprash taken (13). It was done in triplicate for every chyawanprash preparation.

Total Fat

Accurately weighed chyawanprash (5g) was extracted using diethyl ether. 3 to 4 successive extractions were done. It was then decanted in a tared flat bottom dish. The solvent was evaporated by keeping on water bath. It was then cooled and weighed. The difference in weight gives the total fat content of the sample for the amount of sample taken. It was done in triplicate for every chyawanprash preparation.

pH

*p*H of 5 % solution of chyawanprash was determined using *p*H meter. It was done in triplicate for every chyawanprash preparation (14).

Weight ml^{-1}

Weight ml^{-1} of 1 % solution of chyawanprash was determined in triplicate for chyawanprash preparation (14).

3 RESULTS

3.1 Morphological Testing

For all the brands of chyawanprash following observations were recorded. Color, odor, taste and consistency of all the chyawanprash preparations were identical. No significant difference was observed (Table 1):

Table 1. Morphological properties of chyawanprash

S.No.	Organoleptic property	Observations				
		Brand D	**Brand B**	**Brand Z**	**Brand Dh**	**Brand A**
1.	Color	Brown colored	Brown Colored	Brown colored	Brown colored	Brown colored
2.	Odor	Distinct (spicy)	Distinct (spicy)	Distinct (spicy)	Distinct (spicy)	Distinct (spicy)
3.	Taste	sweet/ sour/ spicy	sweet/ sour/ spicy	sweet/ sour/ spicy	sweet/ sour/ spicy	sweet/ sour/ spicy
4.	Consistency	Jam like (Sticky) ++	Jam like (Sticky) ++	Jam like (Sticky) +++	Jam like (Sticky) +++	Jam like (Sticky) +

++ = Viscous, + = Relatively less viscous, +++ = Relatively more viscous

3.2 Phytochemical Evaluation

Both aqueous and alcoholic extract of all the chyawanprash preparations, showed the presence of various phytoconstituents viz. alkaloids, carbohydrates, flavonoids, glycosides, tannins and phenolic compounds, fixed oils and fats, and saponins (Table 2).

Table 2. Phytochemical investigations of chyawanprash preparations

S.No.	Class of phytoconstituents	Brand D		Brand B		Brand Z		Brand Dh		Brand A	
		Aq. Extract	Alc. Extract	Aq. Extract	Alc. Extract	Aq. Extract	Alc. Extract	Aq. Extract	Alc. Extract	Aq. Extract	Alc. Extract
1.	**Alkaloids** (Wagner's reagent)	+	+	+	+	-	+	-	+	+	+
2.	**Carbohydrates** (Fehling's solution)	+	+	+	+	+	+	+	+	+	+
3.	**Fixed oils and fats** (Spot Test)	-	+	-	+	+	+	+	-	-	+
4.	**Flavonoids** (Shinoda test)	-	+	+	+	+	+	+	+	+	+
5.	**Glycosides** (Keller-Killani test)	+	+	+	+	-	-	+	+	+	+

	Saponins (Foam Test)	+	+	+	+	+	+	+	+	+	+
6.	**Tannins and Phenolic compounds**										
7.	**Ferric chloride test**	+	+	+	+	+	+	+	+	+	+
8.	**Dilute HNO_3 test**	+	-	+	+	+	+	+	-	+	+
9.	**Proteins and free Amino Acids** (Millon's test)	+	+	+	+	-	-	+	+	+	+

3.3 Physicochemical Evaluation

3.3.1 Water Soluble Extractive Value

According to API 2007, water extractive value of Chyawanprash should not be less than 50 %. As seen in the table below, all brands of chyawanprash were within the limits, expect for Brand Dh. However, Brand B was found to have highest water soluble extractive value as compared to other chyawanprash preparations (Table 3).

Table 3. Water Soluble Extractive Value of different marketed formulations of chyawanprash

S.No.	chyawanprash preparations	Mean ± SEM value (% w/w)
1.	Brand D	63.400± 2.1
2.	Brand B	69.134±2.76
3.	Brand Z	65.296±3.84
4.	Brand Dh	43.288±1.02
5.	Brand A	55.729±2.39

3.3.2 Alcohol Soluble Extractive Value

According to API 2007 alcoholic soluble extractive value should not be less than 50 %. As per the results obtained, it was seen that all the marketed preparations of chyawanprash, included in the study complied with the limits mentioned. Brand D was found to have maximum alcohol soluble extractive value whereas, brand Dh lowest alcohol soluble extractive value (Table 4).

Table 4. Alcoholic Soluble Extractive Value of different marketed formulations of chyawanprash

S.No.	chyawanprash preparations	Mean value±SEM (% w/w)
1.	Brand D	83.141±3.87
2.	Brand B	78.377±1.59
3.	Brand Z	80.758±1.97
4.	Brand Dh	51.554±2.95
5.	Brand A	74.011±4.722

3.3.3 Loss on Drying (LOD)

LOD as per API 2001 should not be more than 9 % for chyawanprash. All the brands of chyawanprash were well within the limit; except for brand J. Brand D chyawanprash was found to have lowest value of LOD (Table 5).

Table 5. LOD of different marketed preparations of chyawanprash

S.No.	chyawanprash preparations	Mean value ± SEM (% w/w)
1.	Brand D	6.026±0.37
2.	Brand B	9.797±0.63
3.	Brand Z	8.35±0.24
4.	Brand Dh	8.90±0.31
5.	Brand A	11.054±0.29

3.3.4 Total acidity in terms of anhydrous citric acid

Total acidity in terms of anhydrous citric acid for different marketed formulations of chyawanprash obtained is as shown in Table 1.6. Brand D was found to have lowest total acidity (in terms of anhydrous citric acid) whereas, brand Dh showed maximum total acidity (in terms of anhydrous citric acid) in comparison to other chyawanprash preparations.

Table 6. Total acidity (in terms of anhydrous citric acid) of different marketed preparations of chyawanprash

S.No.	chyawanprash preparations	Mean value±SEM (% w/w)
1.	Brand D	0.027±0.37
2.	Brand B	0.046±0.63
3.	Brand Z	0.03±0.24
4.	Brand Dh	0.051±0.31
5.	Brand A	0.033±0.29

3.3.5 Vitamin C content

Brand D (0.334), brand Z (0.267) and brand J (0.187) showed comparatively higher values of vitamin C than that of brand B (0.167) and brand Dh (0.133). Brand D was found to have maximum vitamin C content whereas, brand Dh showed lowest vitamin C content in comparison to other chyawanprash preparations (Fig 1).

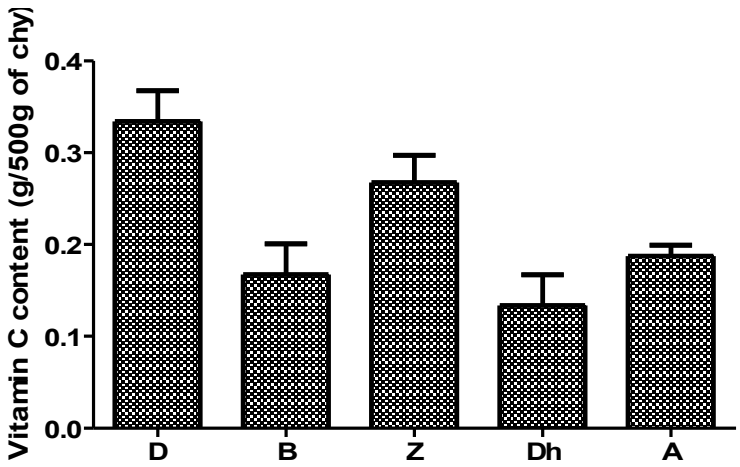

Fig. 1. Comparison of Vitamin C content of different marketed preparations of chyawanprash

3.3.6 Saponification Value

Brand D (3.222), brand Z (3.161) and brand J (3.413) showed similar saponification value, while brand B (4.493) and brand Dh (4.898) showed comparatively higher saponification values. Brand D was found to have lowest saponification value and brand Dh showed maximum saponification value in comparison to other chyawanprash preparations (Fig. 2).

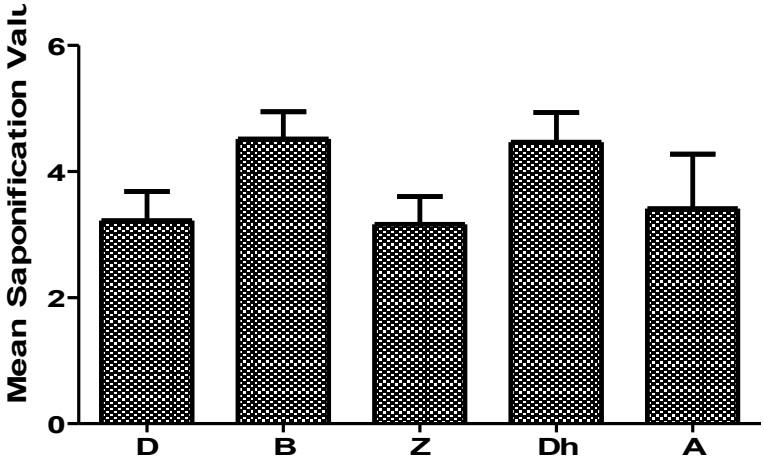

Fig. 2. Comparison of saponification value of different marketed preparations of chyawanprash

3.3.7 Acid Value

Brand D (1.788), brand Z (1.641) and brand J (1.535) showed similar acid value, while brand B (2.432) and brand Dh (2.443) showed comparatively higher acid values. Brand J had lowest acid value whereas, Brand Dh showed maximum acid value in comparison to other chyawanprash preparations (Fig. 3).

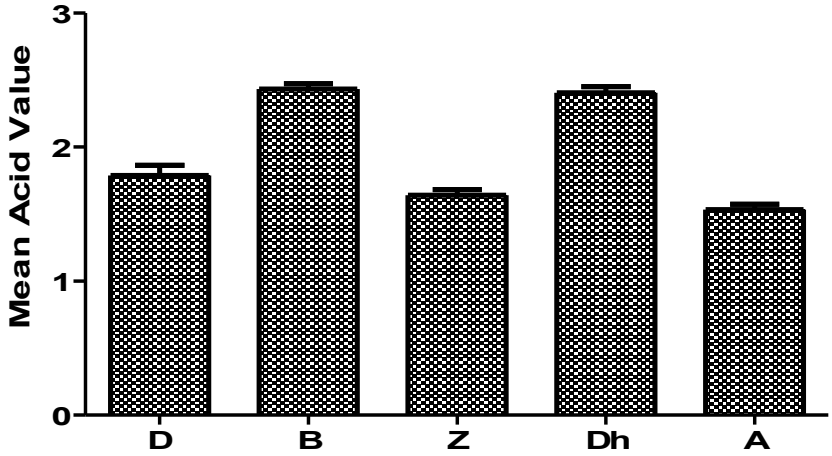

Fig. 1.3. Comparison of acid value of different marketed formulations of chyawanprash

3.3.8 Ester Value

Brand D (1.434), brand Z (1.52) and brand J (1.878) showed similar ester value, while brand B (2.061) and brand Dh (2.455) showed comparatively higher ester values. Brand D was found to have lowest ester value whereas, brand Dh showed maximum ester value in comparison to other chyawanprash preparations (Fig. 4).

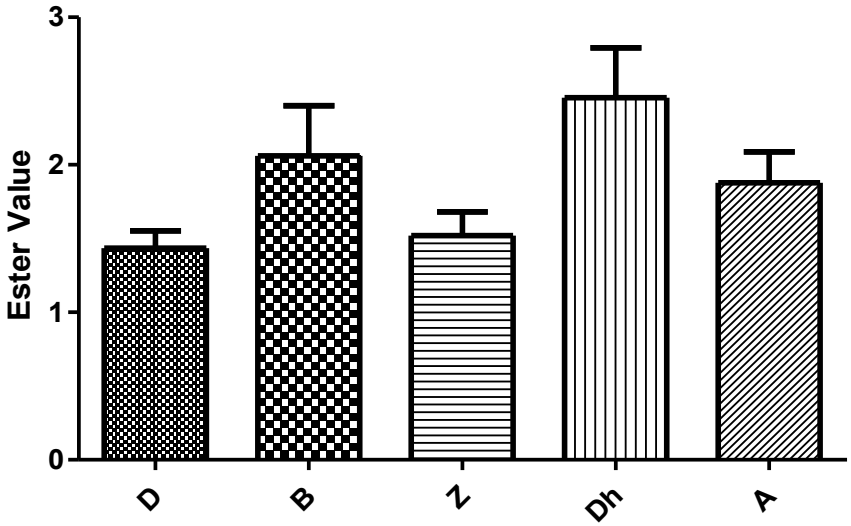

Fig. 4. Comparison of ester value of different marketed formulations of chyawanprash

3.3.9 Total Ash

According to API 2001, total ash of chyawanprash should not be more than 2%. All the marketed formulations of chyawanprash except brand Dh were below the limit. Brand D was found to have lowest ash value as compared to other chyawanprash preparations (Table 7).

Table 7. Total Ash value of different marketed formulations of chyawanprash

S.No.	chyawanprash preparations	Mean value±SEM (% w/w)
1.	Brand D	1.238±0.33
2.	Brand B	1.896±0.21
3.	Brand Z	1.469±0.45
4.	Brand Dh	2.171±0.26
5.	Brand A	1.582±0.24

3.3.10 Acid Insoluble Ash

Acid insoluble ash as per API 2001 should not be more than 1%. All the brands of chyawanprash included in the study were below 1%, except for brand Dh. Brand D was found to have lowest value for acid insoluble ash in comparison to other chyawanprash preparations (Table 8).

Table 8. Acid insoluble ash value of different marketed formulations of chyawanprash

S.No.	chyawanprash preparations	Mean value±SEM (% w/w)
1.	Brand D	0.365±0.0043
2.	Brand B	0.814±0.0076
3.	Brand Z	0.470±0.0057
4.	Brand Dh	1.112±0.009
5.	Brand A	0.509±0.005

3.3.11 Total Fat

Brand B (0.851) showed maximum total fat, of all the brands included in the study. It is then followed by Brand Z (0.741), brand Dh (0.697), and brand J (0.627). Brand D (0.56) showed least total fat content. Brand D was found to have lowest total fat whereas, brand B showed maximum value of total fat in comparison to other chyawanprash preparations (Fig. 5).

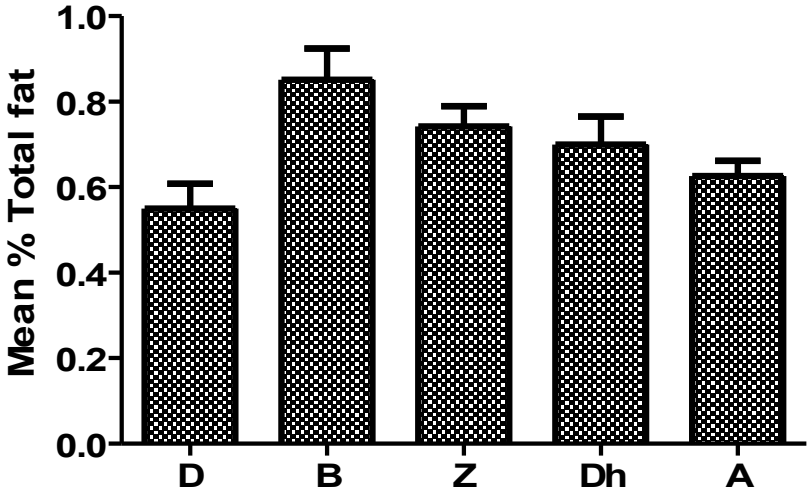

Fig. 5. Comparison of % total fat value of different marketed formulations of chyawanprash

3.3.12 pH

As per API 2001, *p*H of chyawanprash should be from 3.82 to 4.23. *p*H values of all the brands of chyawanprash were well within the limit. Brand Dh was found to be more on acidic side whereas, brand B chyawanprash was more on the basic side (Table 9).

Table 9. *p*H value of different marketed formulations of chyawanprash

S.No.	chyawanprash preparations	Mean value ± SEM (% w/w)
1.	Brand D	3.94±0.061
2.	Brand B	4.20±0.07
3.	Brand Z	4.11±0.115
4.	Brand Dh	3.90±0.084
5.	Brand A	4.19±0.113

3.3.13 Weight ml^{-1}

Observed weight ml^{-1} values of different marketed formulations of chyawanprash included in the study are as shown in Table 1.10. Brand D was found to have highest weight ml^{-1} value and brand J chyawanprash had lowest weight ml^{-1} value (Table 10).

Table 10. Weight ml^{-1} values of different marketed formulations of chyawanprash

S.No.	Chyawanprash preparations	Mean value±SEM (% w/w)
1.	Brand D	1.0086±0.0023
2.	Brand B	1.0004± 0.0028
3.	Brand Z	1.0051±0.0012
4.	Brand Dh	1.0038±0.0022
5.	Brand A	0.9971±0.0030

4.

DISCUSSION

Comparative physicochemical evaluation of various preparations of chyawanprash was done using various parameters, viz. extractive value, loss on drying, ash value, total acidity in terms of anhydrous citric acid, vitamin C content, saponification value, acid value, ester value, total fat, *p*H, etc. Both official standards as well as some other tests were included for the comparative

evaluation of the different chyawanprash preparations. The data analysis revealed that physicochemical parameters were within the limits as per the Pharmacopoeial standards. However, there was a significant variation among chyawanprash preparations.

A systematic study of a crude drug embraces thorough consideration of both primary and secondary metabolites derived as a result of plant metabolism. Hence, the plant material is subjected to phytochemical screening for the detection of various plant constituents (11) (Table 1.2). Extractive value indicates the amount of active constituents extracted with solvents from a given amount of material. Water soluble polar constituents of crude drugs containing tannins, sugar, plant acids, mucilage, glycosides etc. are determined by water extractive value. Results obtained were within the limits (Table 1.3 and 1.4). But lower values of extractive values in case of brand Dh, suggests the presence of less amount of active constituents (tannins, sugar, plant acids, mucilage, glycosides etc.). Loss on drying is the loss of weight expressed as percentage w/ w resulting from water and volatile matter of any kind that can be driven off under specified conditions (14). Excess of water may lead to growth of microorganism or fungi and may also deteriorate plant material following hydrolysis. So this necessitates the determination of water content. All the preparations of chyawanprash included in the study were well within the limits (Table 1.5). But higher values of LOD in case of Jeevanprash chyawanprash suggests, that it may be more susceptible to microbial growth as compared to other chyawanprash preparations.

Even though the official methods for quality assurance of chyawanprash (16) do not include Vitamin C content, there are conflicting reports on the presence of Vitamin C in Chyawanprash (16) probably due to adoption of less sensitive and nonspecific methods for its determination. As per the results obtained vitamin C was found to be present in all the chyawanprash preparations, although its content differed among them. Presence of Vitamin C directly points towards the antioxidant potential of chyawanprash. Antioxidant activity of chyawanprash is further supported by the presence of tannins and phenolic compounds and flavonoids, which are well known for their antioxidant potential (17). Lesser values of vitamin C in brand D and brand B, accounts for its lower antioxidant potential (Fig. 1).

Saponification value is the number of milligrams of potassium hydroxide necessary to neutralize the free acids and to saponify the esters present in 1 g of the substance (14). Saponification occurs in an inverse proportion to the average molecular weight of fatty acids (FA) present. So it allows for comparison of average FA chain length. The results obtained suggest presence of long chain FA (Fig. 2). The acid content and ester value of oils and fats is given by the quantity of free fatty acids deriving from the hydrolytic rancidity of triglycerides. This alteration happens

when the edible fats are not stored or treated correctly; hence, this test gives a fundamental index of the genuineness of the product (18). Results obtained points towards greater rancidity of brand D and brand B (Fig. 3 and 4).

All vegetable drugs on incineration leave an inorganic ash. Presence of ash in medicinal plants is determined as total ash, acid insoluble ash, sulfated ash, water soluble ash. Ash is defined as the amount of material remaining after ignition/ incineration of medicinal plant. It represents inorganic salt naturally occurring in drug/preparation or deliberately added to it. Amount of ash is one of the important indicator of the quality and purity of the plant material. Acid insoluble ash is often more valuable than total ash. With the help of it, evidence of the presence of excessive earthy matter can be obtained. Results obtained in the study were within the specified limits, but maximum value was obtained with brand D indicating the presence of maximum inorganic material in it. Minimum value was obtained with that brand D suggesting its good quality (Table 7).

Total fat determination gives idea about the amount of the crude fat present in the preparation. According to the results obtained brand B showed the maximum value for total fat and brand D showed minimum value (Fig. 5). Weight ml^{-1} is the weight in g of 1 ml of a liquid when weighed in air at 20°C, unless otherwise specified in the monograph. Its purpose is to weigh accurately and the weight per ml determined in order to calculate the content as weight in volume (g per ml), thereby indicating about the viscosity of the preparation (14). As per the results brand D showed the maximum value and brand J showed minimum value (Table 10). Total acidity and pH indicates about the stability of the preparation. All the values obtained were well within the specified limits (Table 9).

5. CONCLUSION

All the parameters studied above, together can be used successfully for quality control of chyawanprash preparation. Although most of chyawanprash preparations were well within the limit but there is significant difference among chyawanprash preparations. Hence there is a need to make more stringent quality control parameters in order to reduce variation among chyawanprash preparations.

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