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# DETECTION OF ADULTERANTS IN POLYHERBAL NUTRACEUTICAL "CHYAWANPRASH" BY PHYTOCHEMICAL ANALYSIS & QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS BY UV-SPECTROPHOTOMETRY

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**ABSTRACT:**

Qualitative and quantitative phytochemistry of polyherbal nutraceutical "Chyawanprash" is investigated for the first time in detail. Since, Chyawanprash is a part of dietary supplement of most Indians including children, young men, women and old aged group, but the dietary benefits and the possible adulterants have never been explored in details till date. Due to the growing demand and consumerism, lot of malpractices occur in such products that are made of multiple ingredients, having complex composition, difficult to detect not only by common men but by specialized laboratories also. The paper investigates the complete phytochemical profile of Chyawanprash of six different brands purchased from different stores of Gandhinagar, Gujarat, by different phytochemical tests along with the possible adulterants present. The quantitative estimation of Phenol by gallic acid calibration curve at 765 nm and Protein content by Bovine serum albumin (BSA) calibration curve at 660 nm by UV-Spectrophotometric analysis reveals the nutritional value of different brands of Chyawanprash. From the phytochemical analysis data, it can be deduced that alkaloids, amino acids, carbohydrates, coumarins, cardiac glycosides, diterpenes, emodins, flavonoids, proteins, phenols, tannins and caffeine were present in all the samples. Spectrophotometric data showed that the presence of phenol was minimum in sample 3 (257.6460 ppm) and maximum in sample 6 (402.7480 ppm) whereas the presence of protein was minimum in sample 1 (0.0440 µg/µL) and maximum in sample 6 (0.3170 µg/µL). Since the different brands had different protein content and it wasn't mentioned in the composition table, therefore there should be guidelines for various polyherbal nutraceuticals regarding their constituents. Caffeine was found to be present in all the samples which was not mentioned in the composition. Therefore it can be inferred that it was an adulterant. The paper represents a preliminary work and detailed analysis by various instrumental techniques is further required to confirm the adulterants in different forms.

**Keywords:** Chyawanprash, Nutraceutical, Phenol, Phytochemical, Polyherbal, Protein.

## INTRODUCTION:

India is a land of great traditional knowledge on herbal medicines comprising Ayurveda, Yoga, Naturopathy, Unani, Siddha and Homeopathy and has a vast floral biodiversity. Indian herbal market is one of the most promising markets because the herbal medicines are getting importance owing to its lot of health benefits, cost effectiveness and very less side effects. The demand in herbal product market is increasing worldwide as the herbal medicines do not cause overdose toxicity, are safer and more reliable than their synthetic counterparts [1]. The current Compound Annual Growth Rate (CAGR) in herbal medicines is projected around 7.6% with a market value of little more than US$ 130 billion as projected in 2017 in a report published by Future Market Insights on 13th September, 2017 [2].

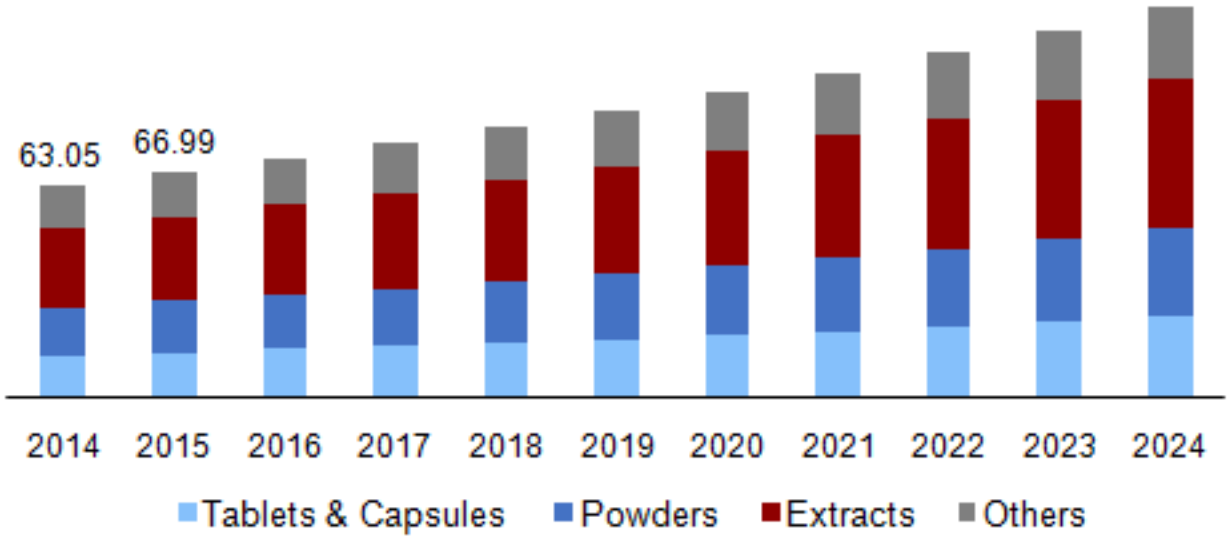
In western countries like America, a shift in trend towards the usage of ayurvedic medicines as dietary supplement or nutraceutical has been noticed. The population in European countries and other Asian nations is aging that has led to increase in demand for such nutraceuticals and thus fuelled the herbal medicinal market with lot of opportunities. Figure 1 shows the global herbal medicinal product market share projections in percentage during the year 2017 and 2027. The report has been published by Future Market Insights. The figure clearly shows the increasing trust in the market on ayurvedic medicines over other medicinal product categories [2].


(Source: Future Market Insights, 2017)

**Figure 1: Global Herbal Medicinal Products Market Share (%)**

Western Europe is also a hub for herbal products, which is also contributing in the growth of the global herbal medicinal products market. In Eastern European countries, the herbal medicines have witnessed tremendous increase in the past few years. In Russia, majority of the population prefer traditional medicines as a primary option for the treatment. Many of the men and women suffering from infertility are adopting traditional treatment option despite the progressive development in contemporary medicines.

Herbal medicines are formulated in dosage forms such as tablets & capsules, powders, extracts, pastes, gels, and oils. The extracts segment generated revenue of USD 27.1 billion in 2016 and is expected to reach USD 44.6 billion by 2024. Higher absorption rates of extracts in comparison with other dosage forms are supposed to drive the market over the forecast period [3].




(Source: Market Research Report by Hexa Research, 2017)

**Figure 2: Global herbal medicine market revenue, by product, 2014-2024 (USD Billion)**

Polyherbal drug formulations comprise extracts from various plants in a fixed ratio. Chyawanprash is one such polyherbal formulation comprising around 50 herbal extracts in a bulk medium of Amla fruit base. Amla is a rich source of Vitamin C. Chyawanprash is a house hold remedy used in most of the parts of India and is gaining popularity worldwide due to its health benefits which aims at maintaining physique,

vigour, vitality, while delaying the ageing process [4]. Due to the growing demand, consumerism, and competition in market, lot of malpractices occur in such products that are made of multiple ingredients, having complex composition difficult to detect not only by common men but by specialized laboratories also. These products are very easy to be adulterated or substituted as the consumers have no knowledge of it and they blindly trust over ayurvedic medicines. The common adulterants can be antibiotics, caffeine, nicotine or the bulk volume of the formulation can be obtained from inferior quality of plant or exhausted plant part or residue, i.e. the adulteration can be done either to enhance the functional activity of nutraceutical or the similar physical appearance can be given to the product by adding look alike plant parts.

Chyawanprash as a polyherbal formulation has never been explored in detail earlier related to its chemical aspects. Few researchers had tried to review over its formulations, recipe, medicinal usage and market. In this paper, it has been tried to study the chemical constituents in various market samples of Chyawanprash qualitatively by phytochemical analysis and quantitatively by UV-Visible Spectrophotometry. As chyawanprash is consumed in the form of nutraceutical and its protein content is very much important, it was estimated by UV- Visible spectrophotometry along with total phenolic content which is important for its antioxidant effects in various market samples.

## MATERIALS AND METHOD:

**Sample collection:** The samples of six brands of Chyawanprash were collected from various medical stores of Gujarat. Some samples were also bought from the online store. The samples were then used for further analysis**.**

**Preparation of extract**: Small quantity of sample was taken in 20mL of falcon tube & was filled with methanol. The mixture was vortexed for 5 minutes at 3000 rpm. The homogeneous solution was centrifuged at 3000 rpm for 5 minutes. The supernatant layer was collected & stored in falcon tubes. The extract was evaporated to dryness in a beaker & stored for further use for phytochemical test of each sample. Dried extract was reconstituted by adding few mL of methanol. The different phytochemical tests were then performed as follows [5]:

**Qualitative estimation of Phytoconstituents:**

**1) Alkaloids:**

**a)** ***Mayer's Test****:* Extracts were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

**b)** ***Wagner's Test:*** Extracts were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

**c)** ***Dragendroff's Test:*** Extracts were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

**d)** ***Hager's Test:*** Extracts were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

**2) Amino acids:**

***Ninhydrin test:*** To the 2 mL extract, 2 mL of ninhydrin reagent was added & boiled for few minutes; formation of blue colour indicates the presence of amino acid.

**3) Anthocyanin:**

2 mL of extract was added to 2 mL of 2N HCl and $NH_3$, the appearance of pink red turns blue violet indicates presence of Anthocyanin.

**4) Carbohydrates:**

***Benedict's test:*** Extracts were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

**5) Coumarin:**

3 mL of 10% NaOH was added to 2 mL of extract, formation of yellow colour indicates coumarins.

**6) Cardiac Glycosides:**

***Legal's Test:*** Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

**7) Diterpenes:**

***Copper Acetate Test:*** Extracts were dissolved in water and treated with 3-4 drops copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes [6-8].

**8) Emodins:**

2 mL of $NH_4OH$ and 3 mL of benzene were added to extract, appearance of red colour indicates presence of emodins.

**9) Flavonoids:**

***a) Alkaline Reagent Test:*** Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

***b) Lead acetate Test:*** Extracts were treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

**10) Fatty Acid:**

1g of Sudan III was mixed with 5mL of distilled water and then mixed with 1mL of extract. The appearance of dark red oil droplet in the upper layer indicates the presence of fatty acids.

**11) Leucoanthocyanin:**

5 mL of Isoamyl alcohol added to 5 ml of aqueous extract, upper layer appear red in colour indicates the presence of Leucoanthocyanin.

**12) Phlobatannins:**

Deposition of red precipitate when aqueous extract of each plant sample was boiled with 1% aqueous HCl was taken as evidence for presence of Phlobatannins.

**13) Phytosterols:**

***Salkowski's Test:*** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of Phytosterols.

**14) Proteins:**

***Xanthoproteic test:*** Extract was treated with few drops of concentrated $HNO_3$, formation of yellow indicates the presence of proteins.

**15) Phenols:**

***Ferric Chloride Test:*** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

**16) Saponin:**

5 mL of extract was mixed with 20 mL of distilled water then agitated in graduated cylinder for 15 min, formation of foam indicates Saponin.

**17) Steroids:**

1mL extract was dissolved in 10 mL of chloroform & equal volume of conc. Sulphuric acid was added from the side of the test tube. The upper layer turns red and Sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of Steroid.

**18) Tannin:**

4mL extract was treated with 4 mL $FeCl_3$, formation of green colour indicates that presence of condensed tannin.

**19) Terpenoids:**

2mL of the extract was mixed with 2mL of chloroform and 3mL Conc. Sulphuric acid was carefully added to form a layer. A reddish-brown colouration of the interface was formed to indicate the presence of Terpenoids.

**20) Caffeine:**

$KClO_3$+ extract+ a drop of HCl $\longrightarrow$ evaporated the sample to dryness & then exposed the resulting residue with ammonia vapour; development of pink colour will confirm for the presence of caffeine.

**21) Nicotine:**

**a)** 50mg $Cu(NO_3).3H_2O$ +10mL Methanol + nicotine extract ⟶ green (Cu-Ni complex).

**b)** 50mg $CoCl_2.6H_2O$+ 10mL Methanol ⟶ formation of pink coloured solution + nicotine extract ⟶green (Co-Ni complex).

The results of the above tests were then tabulated.

**Quantitative estimation of Phenols & Proteins:**

**Estimation of Total Phenolic Content:**

The aqueous extracts of samples were prepared by diluting the samples 100 times (0.1g sample in 10 mL of distilled water).

The total phenolic contents of the aqueous extracts were estimated using the Folin Ciocalteu reagent as described by Singleton and Rossi [9]. The calibration curve was plotted by mixing 1 mL aliquots of 50, 100, 150, 200, 250, 300, 350, 400 and 450 mg/mL Gallic acid solutions with 5.0 mL of Folin Ciocalteu reagent (diluted tenfold) and 4.0 mL of sodium carbonate solution (75 g/L concentration). The mixture solutions were stored for 30 minutes and then absorbance was measured at 765 nm. For both of the aqueous extracts (1 g/100 mL or, 0.1 g/10 mL), 1 mL was mixed separately with the same reagents, as was done for constructing the calibration curve. After 1 h, the absorbance was measured to determine the total phenolic contents in each extract separately [10, 11].

**Estimation of Total Protein Content:**

The aqueous extracts of samples were prepared by diluting the samples 1000 times (0.01g sample in 10 mL of distilled water).

The phenolic group of tyrosine and tryptophan residues (amino acid) in a protein will produce a blue purple colour complex, with maximum absorption in the region of 660 nm wavelength, with Folin- Ciocalteu reagent which consists of sodium tungstate molybdate and phosphate. Thus the intensity of colour depends on the amount of these aromatic amino acids present and will thus vary for different proteins. Most proteins estimation techniques use Bovine Serum Albumin (BSA) universally as a standard protein, because of its low cost, high purity and ready availability. The method is

sensitive upto 10µg/mL and is probably the most widely used protein assay despite its being only a relative method, subject to interference from Trisbuffer, EDTA, non-ionic and cationic detergents, carbohydrate, lipids and some salts. The incubation time is very critical for a reproducible assay. The reaction is also dependent on pH and a working range of pH 9 to10.5 is essential [12-14].

**RESULTS & DISCUSSION:**

The results of phytochemical test are tabulated below in **Table 1**. From the table, it can be deduced that alkaloids, amino acids, carbohydrates, coumarins, cardiac glycosides, diterpenes, emodins, flavonoids, proteins, phenols, tannins and caffeine are present in all the samples while anthocyanin, fatty acids, phlobatannins, leucoanthocyanin and nicotine are absent in all the samples. As caffeine was not mentioned in the composition list of the products, it can be considered as an adulterant because the possibility of presence of caffeine from any of the herbal product used is zero. Beside caffeine, amino acids were present in high concentration in all the samples. The results of phytochemical analysis were based on the simple colour change upon the addition of a particular reagent [9].

**Table 1: Results of Phytochemical tests**

| S.No. | TESTS | Control (Methanol) | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
|---|---|---|---|---|---|---|---|---|
| **1.** | **Alkaloids** | | | | | | | |
| **a.** | **Mayer's Test** | - | - | + + | + + | - | + | + + |
| **b.** | **Wagner's Test** | - | + + | + + | + + | + + | + + | + + |
| **c.** | **Dragendroff's Test** | - | + + | + + | + + | + + | + + | + |
| **d.** | **Hager's Test** | - | + + | + + | + + | + + | + + | + |
| **2.** | **Amino acids** | - | + + + | + + + | + + + | + + + | + + + | + + + |
| **3.** | **Anthocyanin** | - | - | - | - | - | - | - |
| **4.** | **Carbohydrate Benedict's test** | - | + + | + + | + | + | + | + |
| **5.** | **Coumarins** | - | + + + | + + + | + + + | + | + | + |

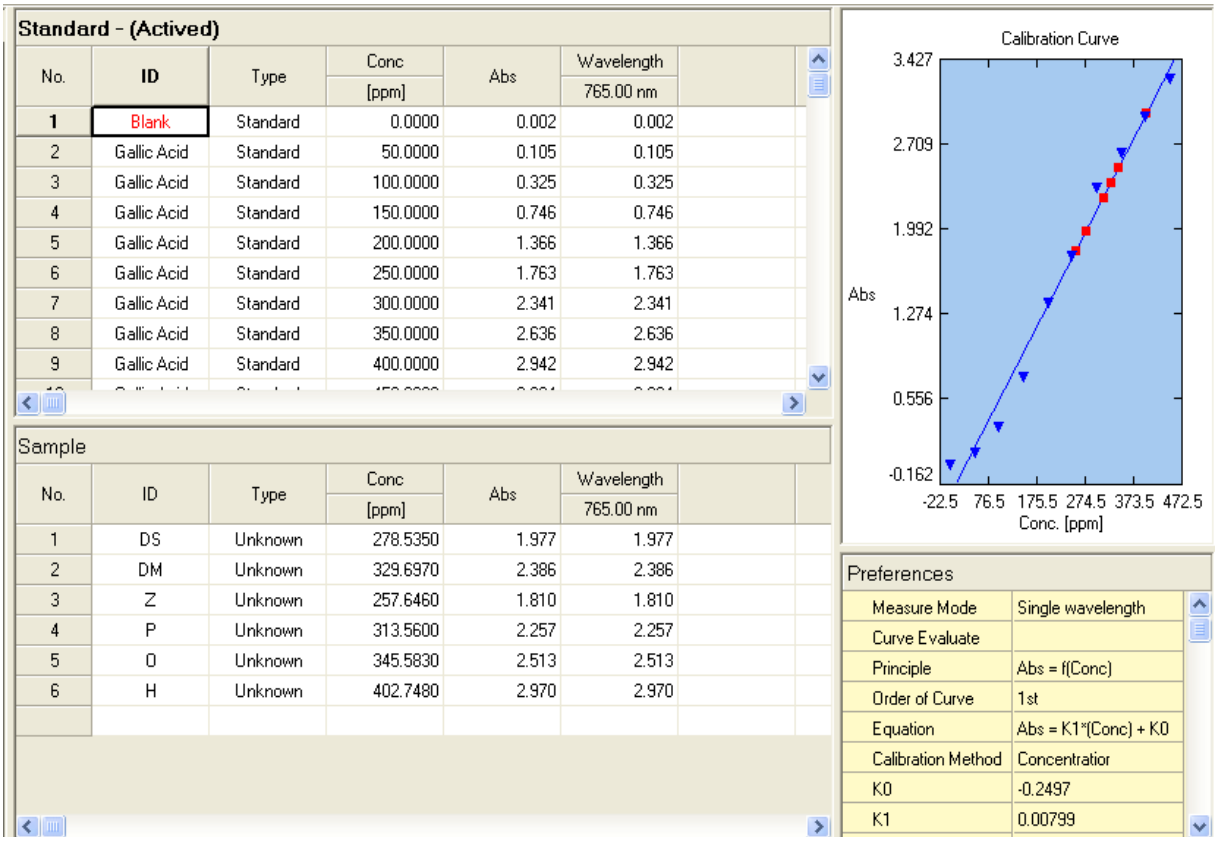
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **6.** | **Cardial Glycosides** | - | + + + | + + + | + + + | + | + | + + |
| **7.** | **Diterpenes** | - | + + + | + + | + + | + + + | + + | + + |
| **8.** | **Emodins** | - | + + + | + + + | + + | + + + | + + | + + + |
| **9.** | **Flavonoids** | | | | | | | |
| **a.** | **Alkaline reagent test** | - | + + | + + + | + + + | + + + | + + + | + + + |
| **b.** | **Lead acetate test** | - | + + + | + + | + + | + | + | + |
| **10.** | **Fatty acids** | - | - | - | - | - | - | - |
| **11.** | **Leucoantho-cyanin** | - | - | - | - | - | - | - |
| **12.** | **Phlobatannins** | - | - | - | - | - | - | - |
| **13.** | **Phytosterols** | - | - | + | + | + | - | - |
| **14.** | **Proteins** | - | + + + | + + + | + + | + + | + + | + + |
| **15.** | **Phenols** | - | + + + | + + + | + + | + + | + + | + + |
| **16.** | **Saponins** | - | - | + + | + | | - | - |
| **17.** | **Steroids** | - | + + + | + + + | + + | + + + | + + + | - |
| **18.** | **Tannins** | - | + + + | + + + | + | + + + | + + + | + + |
| **19.** | **Terpenoids** | - | + | + | + | - | - | + |
| **20.** | **Caffeine** | - | + | + | + | + | + | + |
| **21.** | **Nicotine** | - | - | - | - | - | - | - |

(Note: '+' present, but in low concentration; '+ +' present in moderate concentration; '+ + +' present in high concentration; '-' absent)

**Quantitative Estimation of Phenol and Protein Content:**

The results of UV are shown below in **Figure 3** and **Figure 4** for phenol and protein content respectively. The concentration of phenol is given in ppm for every 0.1 g of sample in 10 mL water and concentration of protein is given in µg/µL for every 0.01g sample in 10 mL water.

Spectrophotometric data showed that the presence of phenol was minimum in sample 3 (257.6460 ppm) and maximum in sample 6 (402.7480 ppm) whereas the presence of protein was minimum in sample 1 (0.0440 µg/µL) and maximum in sample 6 (0.3170 µg/µL).



Standard - (Actived)

| No. | ID | Type | Conc [ppm] | Abs | Wavelength 765.00 nm |
|---|---|---|---|---|---|
| 1 | Blank | Standard | 0.0000 | 0.002 | 0.002 |
| 2 | Gallic Acid | Standard | 50.0000 | 0.105 | 0.105 |
| 3 | Gallic Acid | Standard | 100.0000 | 0.325 | 0.325 |
| 4 | Gallic Acid | Standard | 150.0000 | 0.746 | 0.746 |
| 5 | Gallic Acid | Standard | 200.0000 | 1.366 | 1.366 |
| 6 | Gallic Acid | Standard | 250.0000 | 1.763 | 1.763 |
| 7 | Gallic Acid | Standard | 300.0000 | 2.341 | 2.341 |
| 8 | Gallic Acid | Standard | 350.0000 | 2.636 | 2.636 |
| 9 | Gallic Acid | Standard | 400.0000 | 2.942 | 2.942 |

Sample

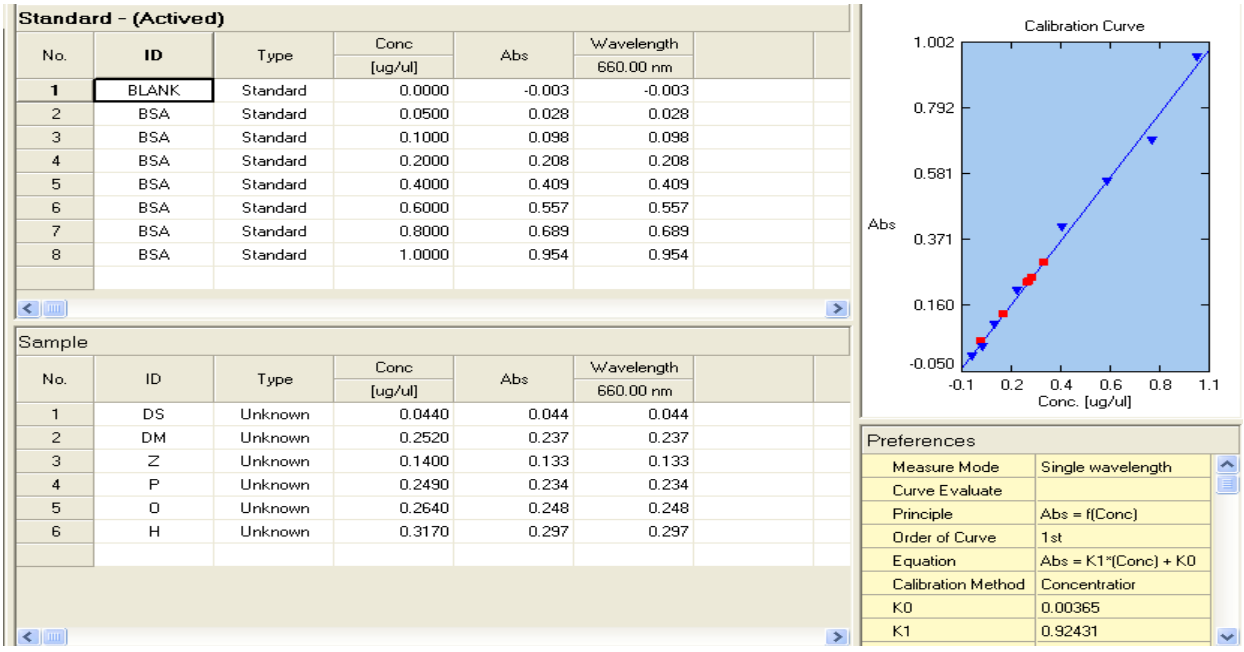
| No. | ID | Type | Conc [ppm] | Abs | Wavelength 765.00 nm |
|---|---|---|---|---|---|
| 1 | DS | Unknown | 278.5350 | 1.977 | 1.977 |
| 2 | DM | Unknown | 329.6970 | 2.386 | 2.386 |
| 3 | Z | Unknown | 257.6460 | 1.810 | 1.810 |
| 4 | P | Unknown | 313.5600 | 2.257 | 2.257 |
| 5 | O | Unknown | 345.5830 | 2.513 | 2.513 |
| 6 | H | Unknown | 402.7480 | 2.970 | 2.970 |



Preferences

| | |
|---|---|
| Measure Mode | Single wavelength |
| Curve Evaluate | |
| Principle | Abs = f(Conc) |
| Order of Curve | 1st |
| Equation | Abs = K1*(Conc) + K0 |
| Calibration Method | Concentratior |
| K0 | -0.2497 |
| K1 | 0.00799 |

**Figure 3: Estimation of Total Phenol Content**



Standard - (Actived)

| No. | ID | Type | Conc [ug/ul] | Abs | Wavelength 660.00 nm |
|---|---|---|---|---|---|
| 1 | BLANK | Standard | 0.0000 | -0.003 | -0.003 |
| 2 | BSA | Standard | 0.0500 | 0.028 | 0.028 |
| 3 | BSA | Standard | 0.1000 | 0.098 | 0.098 |
| 4 | BSA | Standard | 0.2000 | 0.208 | 0.208 |
| 5 | BSA | Standard | 0.4000 | 0.409 | 0.409 |
| 6 | BSA | Standard | 0.6000 | 0.557 | 0.557 |
| 7 | BSA | Standard | 0.8000 | 0.689 | 0.689 |
| 8 | BSA | Standard | 1.0000 | 0.954 | 0.954 |

Sample

| No. | ID | Type | Conc [ug/ul] | Abs | Wavelength 660.00 nm |
|---|---|---|---|---|---|
| 1 | DS | Unknown | 0.0440 | 0.044 | 0.044 |
| 2 | DM | Unknown | 0.2520 | 0.237 | 0.237 |
| 3 | Z | Unknown | 0.1400 | 0.133 | 0.133 |
| 4 | P | Unknown | 0.2490 | 0.234 | 0.234 |
| 5 | O | Unknown | 0.2640 | 0.248 | 0.248 |
| 6 | H | Unknown | 0.3170 | 0.297 | 0.297 |



Preferences

| | |
|---|---|
| Measure Mode | Single wavelength |
| Curve Evaluate | |
| Principle | Abs = f(Conc) |
| Order of Curve | 1st |
| Equation | Abs = K1*(Conc) + K0 |
| Calibration Method | Concentratior |
| K0 | 0.00365 |
| K1 | 0.92431 |

**Figure 4: Estimation of Total Protein Content**

## CONCLUSION:

The present work was taken up in the view to standardize the polyherbal formulation 'Chyawanprash' in accordance to WHO norms and standard laboratory procedures. Formulation was investigated for the first time for their phytochemical parameters and phenolic and protein content. The research outcomes of the standardization can be used for evaluating the quality and purity of the formulations [15]. Phenolic compounds in plants are very important because their hydroxyl group possess scavenging abilities [16]. Proteins pose a wide range of other functions in the body, such as enzymatic activity and transport of nutrients and other biochemical compounds across cellular membranes [17]. In order to maintain these important functions, it is essential to provide the body with good quality proteins through diet. Inadequate intake of dietary proteins containing essential amino acids results in increased turnover of muscular proteins, leading to reduced growth and loss of muscle mass. Impaired immunity, as well as reduced hormonal and enzymatic activity may subsequently follow [18]. Being such important constituents of human diet it is crucial to know the protein and phenolic content in foods and thus it is important to have reliable analytical methods [19]. Thus, strict norms are needed to be given related to such nutraceuticals which are getting deep rooted in peoples' daily routine and are becoming a necessary part of their diet.

## ACKNOWLEDGEMENT:

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## Conflict of Interest:

The authors declare no conflict of interest.

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