



Digestive Activity of Pancha Harithakadi Churna

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ABSTRACT

The traditional systems of medicine are really effective but the problem with them is they lack in quality assurance. Standardization is the need of the hour in ayurvedic system of medicine. PanchaHarithakadi Churna (PHC) is a traditional polyherbal formulation which consists of five household ingredients used for indigestion. It is mainly used for Constipation and Bloating. Churna's will play a major role in gastro intestinal problems and they have greater bioavailability because of smaller particle size. It consists of fine powder (sieve 100 size) of ginger rhizomes, fennel fruits, myrobalan fruits, senna leaflets and pink rock salt in equal proportions (1:1:1:1:1) are mixed well. PHC was formulated by standard procedures and evaluated by microscopic characterization, inorganic analysis and digestive studies. Microscopical characters indicate the presence of genuine crude drugs used in the formulation. Inorganic analysis shows the presence of calcium, magnesium, sodium, chloride and phosphate. The PHC showed pronounced amylolytic activity and trypsin activity whereas moderate lipolytic activity, proteolytic activity and pepsin activity, mild chymotrypsin activity in treating indigestion. In future we will carry out in vivo digestive studies.

Keywords: PHC, Digestion, Inorganic analysis, Enzymes, Ayurveda.

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INTRODUCTION

Ayurvedic formulations have numerous uses in Ayurveda. They help to rectify the three doshas in the body.¹In the last few decades, there has been an exponential growth in the field of ayurvedic medicine. There are great need of standardization and quality control of ayurvedic formulations. The CCRAS and WHO has introduced certain standards and guidelines to maintain uniformity between the production batches. Good manufacturing practices and quality control of the ingredients and products can result in ensuring quality assurance of the formulation.²

The present study is to formulate and standardize PHC, which is used to treat gastrointestinal problems. The churna is evaluated by microscopic evaluation, inorganic analysis and digestive studies.

MATERIALS AND METHODS

Preparation of Churna

The raw materials such as Ginger rhizomes (1part), Fennel fruits (1part), Myrobalan fruits (1part), Senna leaflets

(1part) and Pink Rock salt (1part) were used for the preparation of PHC. The raw materials of PHC were purchased from the market and authenticated by the Botany Department of Hindu College based on their microscopical characters of powdered drugs. All the ingredients were powdered separately, passed through sieve number 100 and mixed together in specified proportions. The churna was packed in an air tight glass container.³

Standardization Parameters

The PHC was standardized by microscopic evaluation, inorganic analysis and digestive studies.

Determination of Microscopic Evaluation

All the ingredients were powdered and their microscopical characters were observed as shown below.^{4,5}

Determination of Inorganic Analysis

Take the ash of PHC, add 50%v/v HCl or 50%v/v HNO₃ then kept for 1 hour or longer and filtered. By using filtrate we can perform the following tests.⁶

1. Tests for Calcium:

(a) To 10mL of filtrate, 1 drop of dil. NH₄OH and saturated ammonium oxalate solutions were added, white precipitate of calcium oxalate was formed which was soluble in HCl but insoluble in acetic acid.

(b) Ammonium carbonate solution gives white precipitate which was insoluble in NH₄Cl solution.



2. Tests for Magnesium:

- Heat and cool the filtrate with the solution of sodium phosphate in dilute ammonia solution which gives white crystalline precipitate.
- It gives white ppt. with ammonium carbonate solution but not with NH_4Cl solution.

3. Test for Sodium:

Flame test: Thick paste of ash was prepared with conc. HCl, paste was taken on platinum wire loop and introduced to bunsen flame then yellow flame was observed.

4. Tests for Potassium:

- Few drops of sodium cobalt nitrite solution were added to the filtrate, yellow ppt. of potassium cobalt nitrite was observed.
- Flame test: It gives violet flame.

5. Tests for Iron:

- Few drops of 2% potassium ferrocyanide were added to the filtrate, dark blue coloration was observed.
- Few drops of 5% ammonium or potassium thiocyanate were added to the filtrate, blood red color was formed.

6. Test for Sulphate:

- Few drops of 5% BaCl_2 solution were added to the filtrate, white crystalline BaSO_4 ppt. was observed which was insoluble in HCl.
- It gives white ppt. with lead acetate reagent, which was soluble in NaOH.

7. Test for Phosphate:

To 5mL of filtrate in HNO_3 , few drops of ammonium molybdate solution were added and heated for 10 min. then yellow crystalline ppt. of ammonium phosphomolybdate was observed.

8. Tests for Chloride:

- To 3mL of filtrate in HNO_3 , few drops of 10% AgNO_3 solution were added then white ppt. of AgCl was observed which was soluble in dil. ammonia solution.
- It gives white ppt. with lead acetate solution, which was soluble in hot water.

9. Tests for Carbonate:

- It forms CO_2 with dilute acid.
- It gives brownish red ppt. with HgCl_2 solution.
- It produces white ppt. with MgSO_4 solution.

10. Tests for Nitrate:

- It liberates red fumes when warmed with H_2SO_4 and copper.

- It does not form brown color with FeSO_4 solution but with H_2SO_4 it forms brown color at the junction of two liquids.

Determination of Digestive Activity**Preparation of Extract:**

About 100mg of accurately weighed quantity of churna was extracted with 20% aqueous glycerol and phosphate buffer (pH 7.8) in 1:4 ratio and filtered. The filtrate was used as an enzyme source. The standard sample was prepared similar to the test sample.⁷

Amylolytic activity:

Extract (1ml) of churna and standard were incubated separately for 15minutes at 27°C and added to 1ml of the substrate (soluble starch 1% in phosphate buffer). The enzyme reaction was interrupted by the addition of 2ml of DNS reagent and heated for 5minutes. The absorbance was measured at 520nm.^{8,9}

Lipolytic Activity**Preparation of Substrate Solution:**

2ml of castor oil was, neutralized to pH7 and stirred well with the 25ml of water in the presence of 100mg of bile salt (sodium taurocholate) till an emulsion was formed.^{8,9}

Procedure:

Take 20ml substrate and added 5ml phosphate buffer at pH 7. The contents were stirred slowly in magnetic stirrer and the temperature was maintained at 35°C . The electrodes of the pH meter were dipped in reaction mixture and the pH was adjusted to 7. The enzymes extract (0.5ml) was added immediately and pH recorded. The timer was set such that at zero time the pH was observed as 7. Then pH dropped by 0.2 units with addition of N/10 NaOH was noted. The pH was brought to initial value and was continued for 30 to 60 minutes. The volume of alkali consumed at each time was noted.

$$\text{Lipolytic activity} = \frac{\text{Volume of alkali} \times \text{Strength of alkali}}{\text{Weight of sample} \times \text{Time in minutes}}$$

Proteolytic activity**Preparation of Substrate Solution:**

200ml of boiled milk was treated with acetic acid till casein precipitates out. The precipitate was then removed, dried and powdered. One gram of prepared casein was diluted to 100ml using distilled water.^{8,9}

Procedure:

1ml of substrate solution added to 1ml of 0.1M phosphate buffer (pH 7.6) and 1ml calcium chloride. To this 1ml crude enzyme extract was added and digestion stopped after 1hour of incubation with 3ml of 5% trichloro acetic acid solution. After 10minutes precipitate was removed by centrifugation and one portion of supernatant was mixed with 5ml Lowry's reagent. The mixture was then stained with dilute folin-ciocalteu reagent (1:2) and optical density



measured at a wavelength of 650nm. The proteolytic activity was then calculated from standard curve in milligrams of tyrosine. Protein estimated by standard method and results were given in milligrams of liberated tyrosine per milligrams of dissolved protein per hour at 37°C as specific activity.

Pepsin Activity

Preparation of Sample:

0.1g of PHC was homogenized with 1mL Assay buffer, after 2 hours centrifuged at 7000 rpm (8000g) at 4°C for 10 minutes and the supernatant was kept on ice for detection.¹⁰

Assay Procedure:

Warm the substrate to room temperature before use, added the following reagents in the micro centrifugal tubes.

Reagent	Sample	Control	Standard	Blank
Sample	20 µL	-	-	-
Substrate	100 µL	100 µL	-	-
Mixed and kept in water bath at 37°C for 10 min.				
Stop Solution	100 µL	100 µL	-	-
Sample	-	20 µL	-	-
Mixed and centrifuged at 1000g, 4°C for 10 min, the supernatant was taken into the microplate.				
Supernatant	60 µL	60 µL	-	-
Standard	-	-	60 µL	-
Substrate diluent	-	-		60 µL
Reaction Buffer	120 µL	120 µL	120 µL	120 µL
Dye Reagent	20 µL	20 µL	20 µL	20 µL
Mixed and after 20 min, absorbance was measured at 580nm.				

Calculation:

Unit Definition: One unit of Pepsin activity is the enzyme that generates 1 µmol of Tyrosine per minute.

According to the weight of sample,

$$\text{Pepsin (U/g)} = 22 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W$$

Trypsin Activity

Preparation of Sample:

0.1g of PHC was homogenized with 1mL Assay buffer on ice, after 2 hours centrifuged at 7000 rpm (8000g) at 4°C for 10 minutes and the supernatant was kept on ice for detection.¹⁰

Assay Procedure:

Warm the reagents to room temperature before use, added following reagents into the microplates.

Reagent	Sample	Standard	Blank
Reaction Buffer	180 µL	-	200 µL
Substrate	10 µL	-	-
Standard	-	200 µL	-
Distilled water	-	-	-
Sample	10 µL	-	-
Mixed and measured at 253nm, recorded the sample absorbance of 10 th second and 130 th second.			

Calculation:

Unit Definition: One unit of Trypsin activity is defined as the enzyme produce 1 µmol Benzoyl-Arginine per minute.

According to the weight of sample,

$$\text{Trypsin (U/g)} = 10 \times (\text{OD}_{\text{sample}(130\text{S})} - \text{OD}_{\text{Sample}(10\text{S})}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W$$

Chymotrypsin Activity

Preparation of Sample:

0.1g of PHC was homogenized with 1mL Assay buffer on ice for 1 hour, centrifuged at 7000 rpm (8000g) at 4°C for 10 minutes and the supernatant was kept on ice for detection.¹⁰

Assay Procedure:

Warm the substrate to room temperature before use, added following reagents into the microplate.

Reagent	Sample	Blank
Sample	10 µL	-
Assay Buffer	-	10 µL
Substrate	190 µL	190 µL
Mixed and measured at 237nm, recorded the sample absorbance of 30 th second and 330 th second.		

Calculation:

Unit Definition: One unit of Chymotrypsin activity is defined as the OD value changed 0.01 in the reaction system per minute.

$$\text{Chymotrypsin (U/g)} = 4 \times [(\text{OD}_{\text{Sample}(330\text{S})} - \text{OD}_{\text{Sample}(30\text{S})}) - (\text{OD}_{\text{Blank}(330\text{S})} - \text{OD}_{\text{Blank}(30\text{S})})] / W$$

RESULTS

PHC was standardized by microscopical evaluation, inorganic analysis and digestive studies. Microscopical characters show the presence of genuine drugs rather than adulterated drugs. Inorganic analysis reveals the presence of various inorganic constituents like calcium, magnesium, sodium, chloride and phosphate. Digestive activity was evaluated by amyolytic, lipolytic, proteolytic, pepsin,



trypsin and chymotrypsin studies in comparison with the standard. The amylolytic activity of a churna was found to be 0.38mg/mL at 1mg/mL and that of standard was 0.31mg/mL, lipolytic activity was found to be 0.44mg/mL and the standard was 0.58mg/mL then proteolytic activity was found to be 0.69mg/mL and that of standard was found to be 0.78mg/mL, pepsin activity was found to be 25.32µmol/mL, trypsin activity was found to be 30.67µmol/mL and chymotrypsin activity was found to be 4.55µmol/mL.

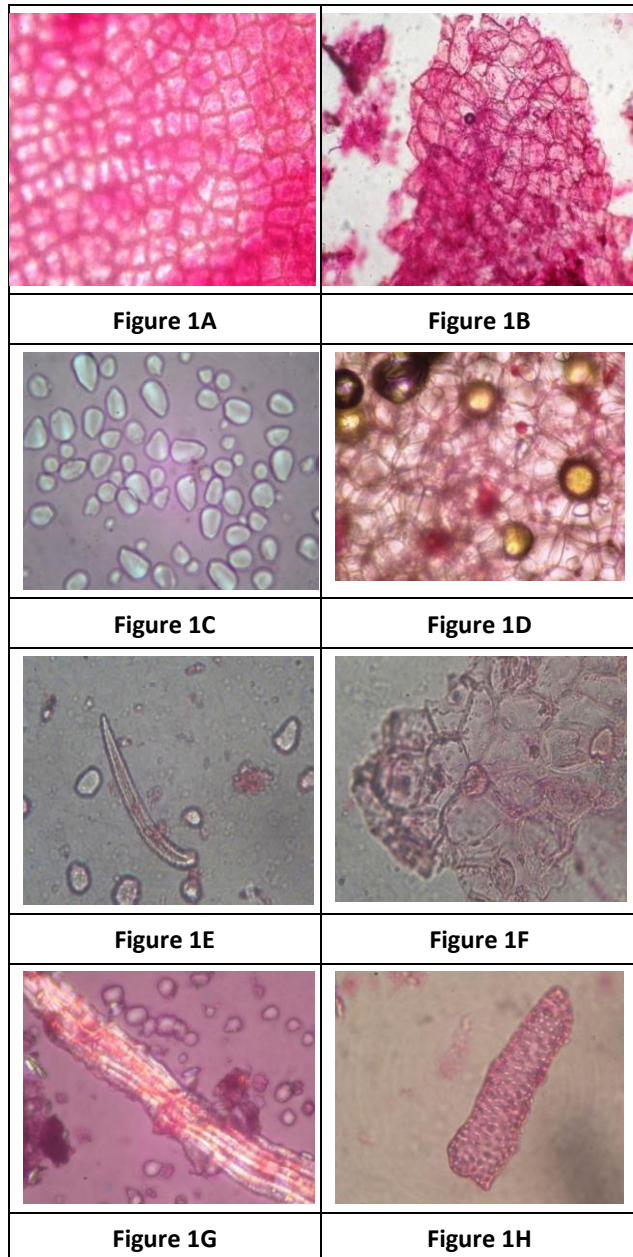


Figure 1: 1A- Epicarp of Fennel, 1B- Mesocarp of Fennel, 1C- Starch of Ginger, 1D- Oleo resin of Ginger, 1E- Covering trichome of Senna, 1F- Paracytic stomata of Senna, 1G- Fiber bundle of Myrobalan, 1H- Sclereids of Myrobalan.

Table 1: Inorganic Analysis of PHC

S. No.	Test	PHC	Marketed Churna
1.	Calcium	+	+
2.	Magnesium	+	+
3.	Sodium	+	+
4.	Potassium	-	-
5.	Iron	-	-
6.	Sulphate	-	-
7.	Phosphate	+	+
8.	Chloride	+	+
9.	Carbonate	-	-
10.	Nitrate	-	-

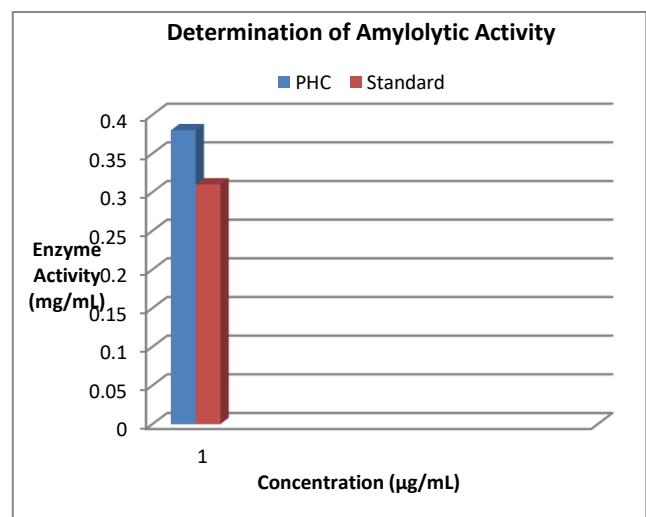


Figure 2: Amylolytic Activity of PHC

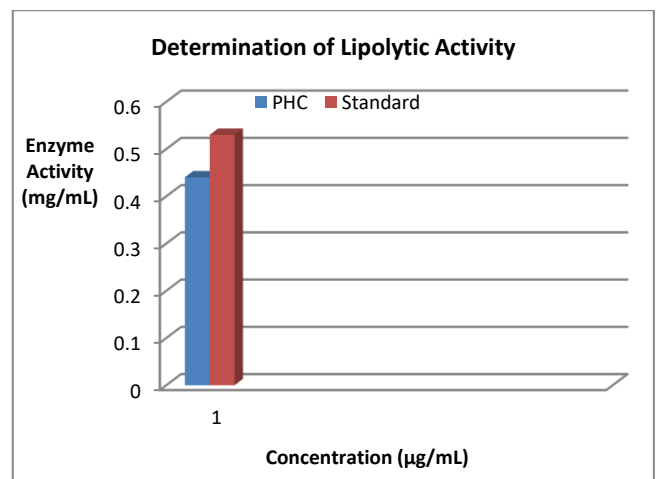


Figure 3: Lipolytic activity of PHC

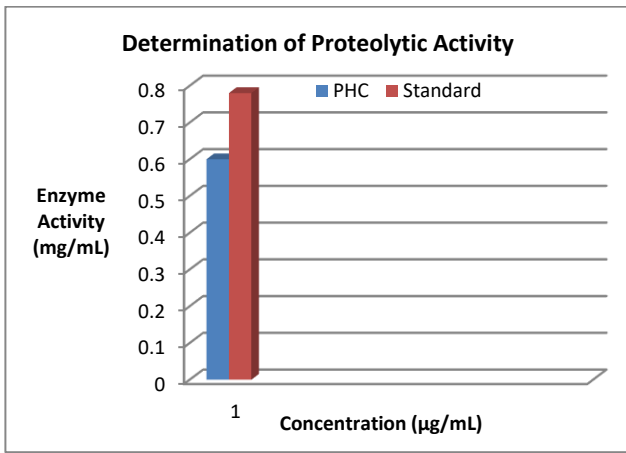


Figure 4: Proteolytic Activity of PHC

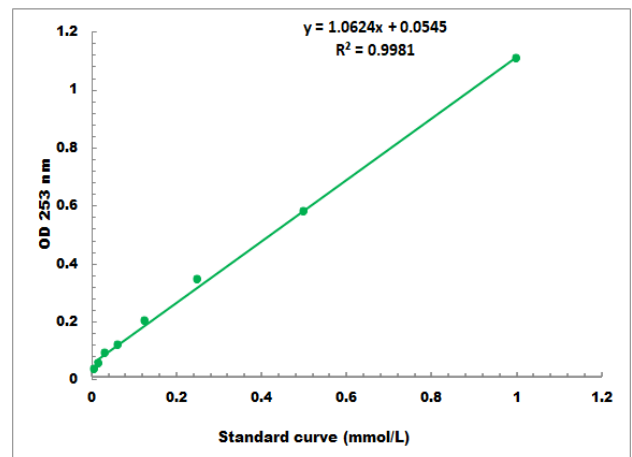


Figure 6A: Standard Curve of Trypsin

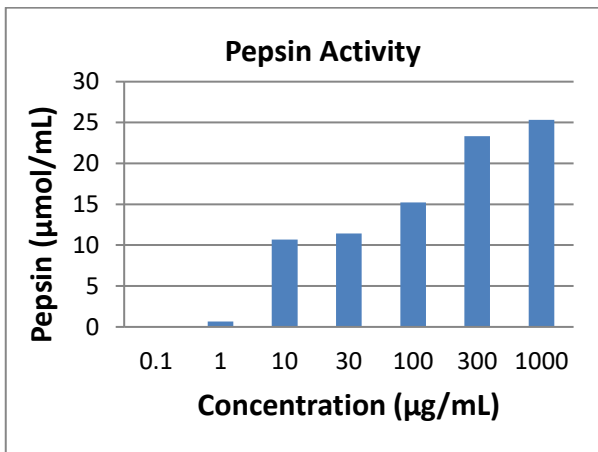


Figure 5: Pepsin Activity of PHC

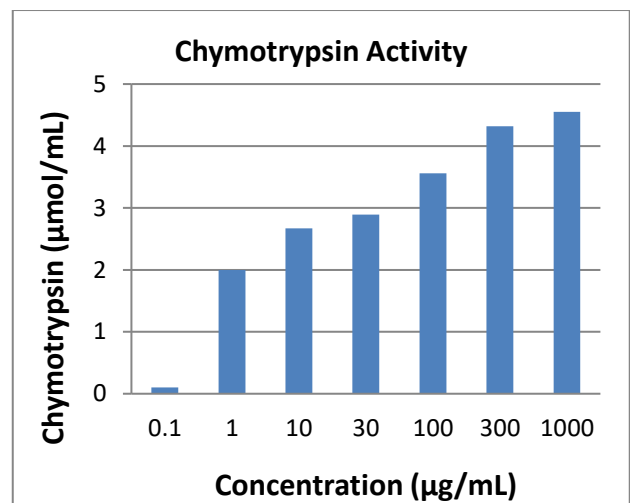


Figure 7: Chymotrypsin Activity of PHC

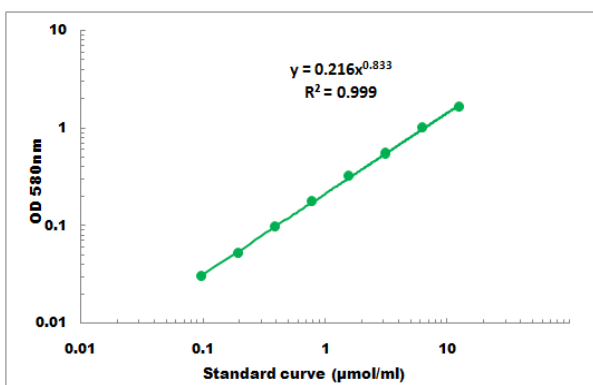


Figure 5A: Standard Curve of Pepsin

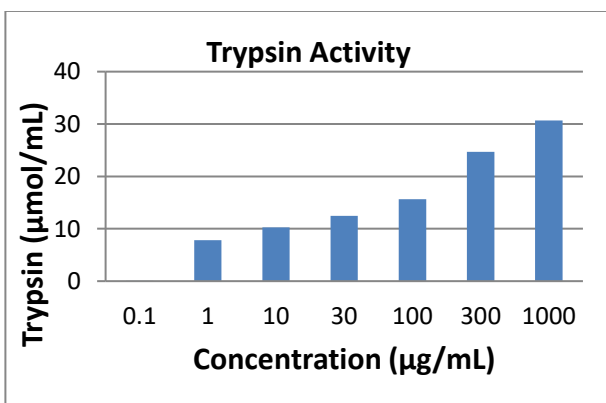


Figure 6: Trypsin Activity of PHC

DISCUSSION

Churna was subjected to standardization by microscopical characters, inorganic analysis and digestive studies. Microscopical characters suggests that the PHC was comprises of genuine crude drugs having digestive property. Inorganic analysis shows the presence of essential inorganic elements. Digestive studies reveal that churna was having strong amyolytic & trypsin activity, moderate lipolytic, proteolytic and pepsin activity whereas mild chymotrypsin activity.

CONCLUSION

Churna has been standardized by modern scientific quality control measures. The microscopical characters, inorganic analysis and digestive studies comply with the WHO standards. The *in vitro* digestive activity of PHC reveals the property of digesting carbohydrates, proteins and fats. Further investigation and isolation of compounds is necessary to establish the exact chemical constituents responsible for digestive activity. PHC as rich source of phytoconstituents and interaction of all these chemicals might be revealed in synergistically enhanced the digestive activity.

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