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DEVELOPMENT AND EVALUATION OF POLY-HERBAL DIGESTIVE CHURNA

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Abstract

Ayurvedic medications are well known for their efficacy and safety in treating gastrointestinal issues. Using normal protocols, a churna prepared for its digestive qualities was created, and its safety and effectiveness were assessed using a variety of physical and analytical techniques. The churna formulation consists of a fine powder (sieve size 60) of *Carica papaya* leaves, *Terminalia chebula, Nigella sativa* seeds, and *Bunium persicum* in the proportions of 2:1:1:1. The formulation was subjected to various physical and chemical evaluations, including the determination of total ash, acid-insoluble ash, water-soluble extractive values, alcohol-soluble extractive values, and crude fiber content. Heavy metal analysis was done to make sure everyone was safe, especially for arsenic. Microbial load testing, specifically for the presence of Escherichia coli, was carried out to assess microbiological safety. The churna's digestive effectiveness was assessed by measuring its amylolytic and lipolytic activities and comparing them to Hajmola, a commercially available gastritis preparation. The churna's extractive and ash values were found to be within the allowed ranges. The arsenic level was determined to be 0.205 ppm, which is within safe limits. The formulation was found to be free of Escherichia coli and other bacteria, indicating its microbiological safety. The churna exhibited pronounced amylolytic activity and moderate lipolytic activity, which were found to be superior to Hajmola, indicating the churna's effective digestive properties.

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Introduction

Churna is a finely powdered form of one or more medicinal herbs used in the Ayurvedic system of medicine. The process of making a fine powder requires cleaning, drying, grinding, and sieving the herbs. When stored in an airtight container, churna retains its potency for up to one year. A few examples of frequently used churna formulas are Sudharsana churna, Drakeshadi churna, Trikatu churna, and Triphala churna. In contemporary medicine, churna formulations are occasionally transformed into tablet form for simpler and more accurate dosage [1].

Churna preparations are widely used in Ayurvedic medicine for various health conditions, particularly due to their small particle size, which enhances absorption rates in the gastrointestinal tract (GIT), leading to improved bioavailability. These preparations are often prescribed to address conditions such as diabetes, indigestion, and constipation. A large percentage of people suffer from the common condition known as indigestion. Antacids are commonly administered in allopathic treatment to address this problem. However, long-term usage of antacids that include aluminum has been associated with negative side effects, such as Alzheimer's disease. Consequently, there is a growing interest in finding alternative, safer remedies for indigestion [1].

This study investigated the formulation of a churna with natural substances that are frequently used in cooking as a less harmful treatment for dyspepsia. Papaya leaves (*Carica papaya*), kalonji (*Nigella sativa*), haritaki (*Terminalia chebula*), and shah jeera (*Bunium persicum*) were combined to create the churna. These components have long been utilized to treat digestive problems such as indigestion, bloating, and constipation. Papaya leaves, in particular, are known for their enzymatic activity, including the presence of papain [2], which aids in protein digestion. The churna made with these ingredients is said to have a variety of biological properties, such as carminative and

digestive effects [3-6]. These herbs have been shown in earlier research to improve digestion [7-9]. Thus, the aim of the present study was to evaluate the amylolytic, lipolytic, and proteolytic activities of the formulated churna and compare these activities with that of Hajmola, a widely marketed formulation used for treating gastritis and indigestion [10].

Materials and Methods

Preparation of Churna

The formulation was made using raw components like two parts *Carica papaya* leaves, one part *Terminalia chebula*, one part *Nigella sativa* seeds, and one part *Bunium persicum*. The ingredients utilized in this formulation were procured from the marketplace and verified by Sri Venkateswara College of Pharmacy's Pharmacognosy department. After being finely powdered, the raw materials of *Terminalia chebula, Nigella sativa, Carica papaya, and Bunium persicum* were passed through sieve number 60. Accordingly, churna was mixed in the appropriate ratios (2:1:1:1) accordingly. A glass jar was used to seal the churna [11].

Evaluation of Physical Parameters

1) Determination of pH

The pH of a 1% solution of the formulated churna was determined using a pH meter (Elico pH meter) according to standard procedures [12].

2) Determination of Moisture Content

The moisture content of the formulated churna was assessed using a halogen moisture analyzer (Mettler Toledo), in accordance with normal procedures

3) Determination of Ash Values

I. Total Ash Value

2g of formulated churna was accurately weighed and placed in a previously ignited and tarred silica crucible. The material was ignited by gradually increasing the heat to 500-600°C until it appeared white, indicating the absence of carbon. After cooling in a desiccator, the total ash was calculated in mg per gram of air-dried material.

II. Acid Insoluble Ash Value

After adding 25 ml of HCl, the crucible containing the complete ash was gently heated for five minutes. The crucible was filled with 5 ml of boiling water. After collecting the insoluble material on ashless filter paper and cleaning it with hot water until the filtrate was neutral, it was fired in a crucible to a constant weight. Once the residue had cooled, it was weighed.

4) Determination of Extractive Values

I. Water-Soluble Extractive Value

5g of formulated churna was accurately weighed and placed inside a glass stoppered conical flask. For 18 hours, the churna was macerated in 100 ml of chloroform water. Once the mixture was filtered, 25 ml of the filtrate were moved to a china dish and dried by evaporating it on a water bath. The residue was then cooled, weighed, and dried for six hours at 105°C.

II. Alcohol-Soluble Extractive Value

Instead of using chloroform water as a solvent, ethanol was utilized, and the process was the same as for the water-soluble extractive value.

5) Determination of Crude Fibre Content

2g of accurately weighed formulated churna was placed in a round-bottom flask, and 100ml of 0.128M sulphuric acid was added. The mixture was refluxed for 1 hour, then filtered through an ashless filter paper, and The filtrate was made neutral by washing the residue with water. The residue was weighed (a), ignited to ash, and the weight of ash (b) was determined. The difference between (a) and (b) represented the crude fibre content and was calculated on a dry weight basis [13].

6) Determination of Heavy Metal Contamination

I. Arsenic Content

Preparation of Standard Solution (10 ppm)

5 ml of 2M sodium hydroxide were used to dissolve 0.33 grams of arsenic trioxide, which was then diluted with 250 ml of water. This solution was further diluted to a volume of 100 ml.

Preparation of Sample

1g of formulated churna was dissolved in 100ml of distilled water to prepare the churna solution. 10ml of this solution was pipetted out into a flask, and 10ml of concentrated nitric acid was added, then evaporated to dryness on a water bath. The residue was dried at 130°C for 30 minutes. Afterward, hydrazine molybdate reagent was added and refluxed for 20 minutes. At 800 nm, the absorbance of the test and standard solutions was determined with a Perkin Elmer UV spectrophotometer [12].

II. Limit Test for Iron

Standard Solution (20 ppm)

A 0.1726% w/v solution of ferric ammonium sulfate was diluted with 0.05 M sulfuric acid to 10 volumes.

Limit test was performed in Nessler's cylinders. 2ml of both test and standard solutions were added, in addition to 0.1 ml of thioglycollic acid and 2 ml of a 20% citric acid solution. Iron-free ammonia was added to the mixture to make it alkaline, and it was then diluted to 50 ml. The standard solution and the color generated during the test were contrasted. The sample failed the test if the color of the test solution was more intense.

III. Limit Test for Lead

Standard Solution (20 ppm)

250ml of water containing 2ml of nitric acid was used to dissolve 0.4g of lead nitrate.

Similar to the iron test, a Nessler's cylinder was used. The test and standard solutions were treated with hydrogen sulfide solution and allowed to stand for 5 minutes. The test solution's color should not be more intense than the standard solution's. The sample does not pass the lead limit test if it does.

IV. Test for Mercury

To 10 drops of the test solution, 6M HCl was added to form a white precipitate. The precipitate was treated with 6M

ammonia. If the color of the precipitate changes to gray or black, it indicates the presence of mercury.

7) Determination of Microbial Content

1g of formulated churna was dissolved in lactose broth, and the volume was adjusted to 100ml. 10ml of the sample was transferred into Macconkey broth and incubated for 18-24 hours at 43-45°C. A subculture was prepared on Macconkey agar, incubated for the same amount of time, and checked for E. coli growth. Gram-negative rod colonies that are red and non-mucoid are a sign that E. coli is present [14].

Determination of Digestive Property

1) Preparation of Extract

100mg of accurately weighed formulated churna was extracted with 20% aqueous glycerol and phosphate buffer (pH 7.8) in a 1:4 ratio. The mixture was filtered, and the filtrate was used as the enzyme source. A similar preparation was made for the standard sample.

2) Amylolytic Activity

1ml of the formulated churna and Hajmola extract were incubated separately for 15 minutes at 27°C, after which it was added to 1 ml of the substrate (1% soluble starch in phosphate base). Adding DNS reagent stopped the reaction, heated for 5 minutes, and the absorbance was measured at 520nm [11].

3) Lipolytic Activity

Substrate Solution Preparation:

100 mg of sodium taurocholate (bile salts) and 25 ml of water were used to emulsify 2 ml of castor oil after it had been neutralized to pH 7.

To assess the lipolytic activity, a substrate solution was first prepared by taking 20 ml of the substrate and adding 5 ml of phosphate buffer at pH 7A magnetic stirrer was then used to slowly agitate the liquid while the temperature was carefully kept at 35°C for the duration of the experiment. The pH was first set to 7 by dipping the electrodes of a pH meter into the reaction liquid. After adjusting the pH, 0.5 ml of the enzyme extract was added immediately to the mixture. At this point, the pH was recorded as the zero time measurement. Over time, as the reaction progressed, a decrease in pH was observed, which was noted to drop by 0.2 units. To counter this pH drop, N/10 NaOH was added to bring the pH back to its initial value of 7. The reaction was then allowed to continue for a period ranging from 30 to 60 minutes, and the volume of alkali consumed to maintain the pH at 7 was recorded at different time intervals. This data was used to calculate the lipolytic activity of the enzyme extract [13].

$$\text{LIPOLYTIC ACTIVITY} = \frac{\text{Volume of alkali x Strength of alkali}}{\text{Weight of sample x Time in minutes}}$$

4) Proteolytic Activity

Substrate Solution Preparation

200 ml of boiled milk was treated with acetic acid until casein precipitated. The precipitate was dried and powdered, and 1g of casein was diluted to 100ml with distilled water.

To assess the proteolytic activity of the formulated churna, a reaction mixture was prepared by combining 1 ml of substrate solution (casein), 1 ml of 0.1M phosphate buffer (pH 7.6), and 1 ml of calcium chloride. Following this, 1 ml of crude enzyme extract from the churna was added, and the mixture was incubated at 37°C for 1 hour. After incubation, the reaction was stopped by adding 3 ml of 5% trichloroacetic acid solution, and the mixture was left to stand for 10 minutes to allow the precipitate to form. The precipitate was then removed by centrifugation, and the supernatant was collected for further analysis. To the supernatant, 5 ml of Lowry's reagent was added, followed by staining with dilute Folin-Ciocalteau reagent (1:2). The optical density of the resulting mixture was measured at 650 nm using a spectrophotometer. The proteolytic activity was calculated based on a standard curve of known tyrosine concentrations, with the amount of liberated tyrosine determined from the optical density. The specific proteolytic activity was expressed in milligrams of liberated tyrosine per milligram of dissolved protein per hour at 37°C. This method allowed for the quantification of the proteolytic activity of the formulated churna, contributing to the evaluation of its digestive properties [11,13].

Results & Discussion

The results of the physical parameter evaluation such as heavy metals, moisture content, ash values including total ash value, acid insoluble ash value, extractive values such as water soluble & alcohol soluble extractive values and crude fiber content were given in table 1 and detection of heavy metals such as arsenic, iron, lead and mercury in table 2. The result of microbial detection had shown no growth of microorganism.

Table 1. Evaluation of physical parameters of formulated churna

S. No	Physical Parameters	Result
1	pH	Slightly acidic
2	Moisture content	10%
3	Ash Values	
	I. Total ash 10% w/w II. Acid insoluble ash 5% w/w	8.5% 4%
4	Extractive values	
	I. Water soluble extractive value II. Alcohol soluble extractive value	4g 1.42g
5	Crude fiber content	1.3g

Table 2. Detection of heavy metals in formulated churna

S. No	Heavy metal	Values
1	Arsenic (Spectrophotometry)	0.2 ppm
2	Iron (Limit test)	Within the limit
3	Lead (Limit test)	Within the limit
4	Mercury (Qualitative analysis)	absent

Detection of Digestive Activity

Amylolytic activity

The amylolytic activity of the formulated churna was found to be 1.36 mg/ml while that of Hajmola was found to be 1.24 mg/ml. The results are represented in figure 1.

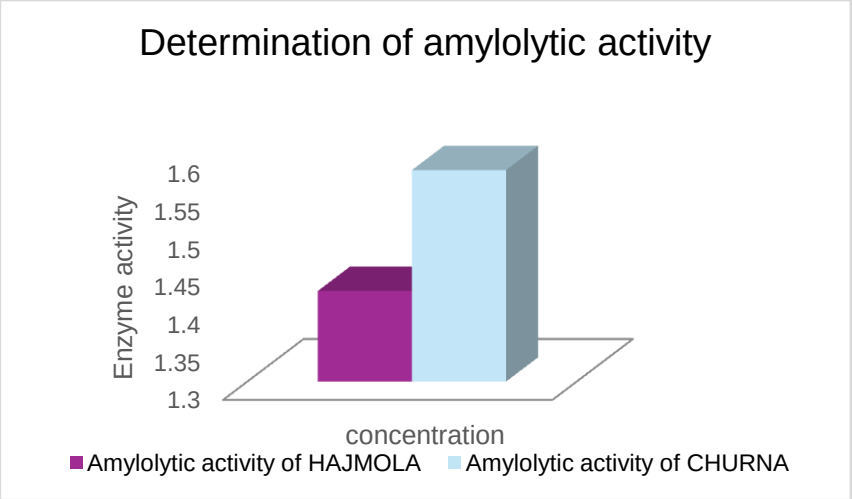

Figure 1. Amylolytic activity of the formulated churna

Lipolytic activity

The lipolytic activity of churna was found to be 1.58 while that of hajmola was found to be 0. 1.42. The results are represented in figure 2.

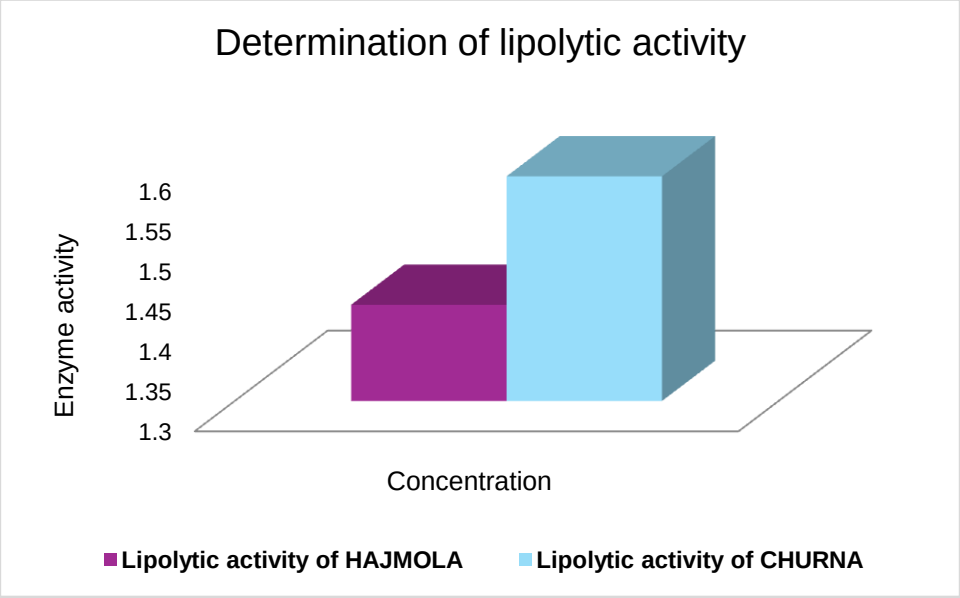

Figure 2. Lipolytic activity of the formulated churna

Proteolytic activity

The proteolytic activity of churna was found to be 54.15 mg/ml while that of Hajmola was found to be 31.052 mg/ml. The results are represented in figure 3.

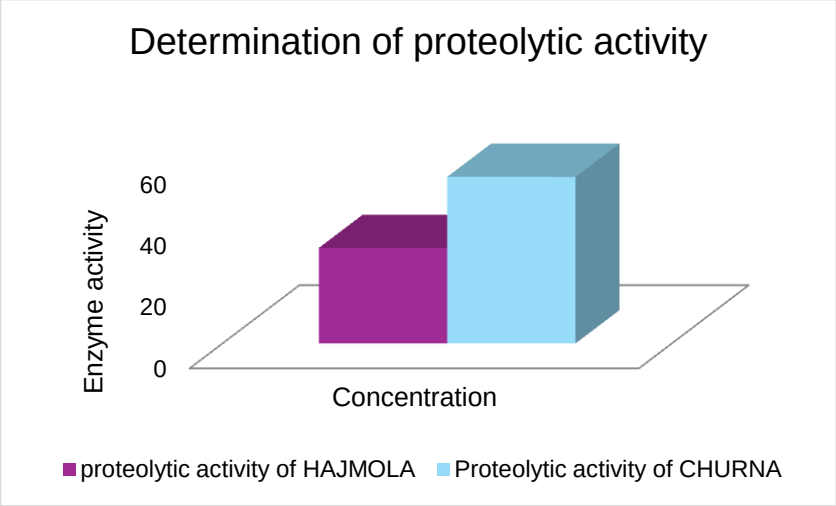

Figure 3. Proteolytic activity of the formulated churna

The formulated churna was subjected to a thorough standardization process to ensure its quality, safety, and compliance with relevant regulatory standards. Several physical, chemical, and microbiological tests were performed to assess various parameters. The pH of the churna was measured to prevent any potential gastric irritation upon consumption. The value obtained was found to be within the acceptable range, indicating that the product is safe for oral use without adverse effects on the digestive system. The moisture content was determined to assess if the churna had absorbed excessive moisture, which could lead to weight increase and potential degradation. The moisture content was found to be within the standard limits, ensuring the product's stability and preventing issues such as microbial growth or spoilage.

The ash values were assessed, including both the total ash value and the acid-insoluble ash value. The total ash value indicates the amount of inorganic material present after the complete incineration of the churna, with an increase in this value possibly suggesting contamination, substitution, or adulteration. The obtained total ash value was within the acceptable limits, indicating no such issues. The acid-insoluble ash value reflects the presence of silicate impurities, which may arise from improper washing of crude plant materials. This value was also found to be within standard limits, suggesting that the raw materials were adequately cleaned before processing.

The extractive values, both water-soluble and alcohol-soluble, were determined to measure the amount of active constituents present in the churna. The results showed that both the water-soluble and alcohol-soluble extractive values exceeded the standard, indicating a higher concentration of active compounds in the formulation and confirming that the plant material used was not exhausted or of poor quality.

The crude fiber content was also measured, as it is an important component of the formulation. This value was found to be within the standard. Heavy metal testing was performed to detect any toxic metals that could pose a health risk. The metals tested included arsenic, iron, lead, and mercury. Arsenic content was determined using spectrophotometry, while iron and lead were tested through a limit test with the permissible maximum limit being 20 ppm. All of these metals were found to be within the acceptable limits, confirming the safety of the formulation. Mercury was tested qualitatively and was found to be absent, further ensuring that the churna was free from harmful heavy metals. Finally, microbiological testing was carried out to evaluate the presence of harmful microorganisms. The churna was tested for Escherichia coli, and the result showed that it was absent, confirming that the formulation meets the microbiological safety standards set by the World Health Organization (WHO).

The biological activity of the formulated churna was evaluated by assessing its amylolytic, lipolytic, and proteolytic activities, which were compared with the standard marketed formulation, Hajmola, to determine its effectiveness in aiding digestion. In the study, the formulated churna demonstrated an amylolytic activity that was 1.4% greater than that of Hajmola, suggesting that the churna has a slightly superior capacity for starch digestion. The formulated churna exhibited slightly lower lipolytic activ-

ity compared to Hajmola, indicating that while the churna is still capable of digesting lipids. The formulated churna showed comparable proteolytic activity to Hajmola, indicating that both formulations have similar abilities to digest proteins.

Conclusion

The physical parameters evaluated confirm the standard of the formulated churna. The in vitro study of enzymatic activity carried out by above methods brings out the fact that the formulated churna possess the property of digesting starch, lipids and proteins similar to that of marketed formulation Hajmola. In conclusion, the formulated churna demonstrated superior amylolytic activity, slightly lower lipolytic activity, and comparable proteolytic activity when compared to Hajmola, suggesting that it has the potential to aid in the digestion of starch and proteins, though it may require further optimization in terms of lipid digestion.

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Conflict of Interest

The authors declare no conflict of interest.

Inform Consent and Ethical Statement

Not Applicable

Author Contribution

Concept: M.S.Riyazullah, design: M.S.Riyazullah, data collection S.Snehalatha, Md.Kadir Ansari, M.Vamsi Naik, P.Prudhvi, S.Ganesh, analysis: D. Jothieswari, writing: S.Snehalatha, Md.Kadir Ansari, M.Vamsi Naik, P.Prudhvi, S.Ganesh

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