FORMULATION AND EVALUATION OF CHURNA FOR DIGESTIVE PROPERTY

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ABSTRACT

Back ground:

Ayurvedic medicines play an important role in gastro intestinal problems due to safety and efficacy in it. Hence churna meant for digestive property has been formulated by standard procedures and evaluated by physical and analytical methods.

Method:

The formulation consists of fine powder (sieve 60 size) of dried rhizomes of Zingiber officinale, fruits of Foeniculum vulgarae, barks of Cinnamomum zeylanicum and fruits of Trachyspermum ammi in appropriate proportions (2:2:1:1) and mixed well. Physical parameters viz, total ash, acid insoluble ash, water extractive values, alcohol soluble extractive values and crude fibre content besides heavy metal analysis were carried out. The microbial load of formulation for Escherichia coli was also determined. The efficiency of churna for digestive property is determined by finding the amylolytic activity and lipolytic activity and compared with GASTRAP a marketed formulation for gastritis.

Results:

Ash values and extractive values were found to be within prescribed limits. The arsenic level was found to be 0.205ppm. Churna did not show the presence of any Escherichia coli and other microorganisms. The churna showed pronounced amylolytic and moderate lipolytic activity when compared to GASTRAP proving its efficiency for digestive problem.

Key words: *Ayurvedic Medicines, Digestion, Complementary Therapies*

INTRODUCTION

Churna is defined as a fine powder of drug or drugs in Ayurvedic system of medicine. Drugs mentioned in patha, are cleaned properly, dried thoroughly, pulverised and then sieved. The churna is free flowing and retains its potency for one year, if preserved in an airtight containers. Triphala churna, Trikatu churna, Drakeshadi churna and Sudharsana churna are some of examples. Churna formulation are similar to powder formulations in Allopathic system of medicine. In recent days churna is formulated into tablets in order to fix the dose easily. These forms of medicament are prescribed generally because of their particle size. Smaller the particle size greater is the absorption rate from g.i.t and hence the greater is bioavailability. It is prescribed by the Ayurvedic physician for treating conditions such as diabetes, indigestion, constipation etc. Indigestion is a common ailment affecting the general population and in allopathy system antacids are commonly prescribed. Since the usage of such aluminium containing antacids cause deleterious effects like Alzheimer's disease upon long term usage, we explored an alternative and safe remedy for indigestion. Hence we prepared a churna with natural ingredients commonly used by mankind for culinary purposes. Thus the present study examined the favourable influence of four spices formulated into churna said to have digestive property. The common ingredients of these churna were Ginger (*Zingiber officinale*), Ajowan (*Trachyspermum ammi*), Cinnamon (*Cinnamomum zeylanicum*) and Fennel (*Foeniculum vulgare*). The formulated churna derived from above said drugs is reported to have a wide range of biological activity. Ginger contains aromatic principle like Zingiberine and bisaboline while pungent principles are gingerols and shogaols. Other components are nerol, geraniol, d-camphor, β-Phellandrene, linalool, α-farnesene, [1] Shagoal, [2] and also diarylheptanoids such as gingerone A&B. This is used in the treatment of flatulence, colic, indigestion, vomiting, constipation. It also maintains the tonicity of intestine muscle [3,4]. Ajowan was found to contain essential oil that contains 50% thymol. This is used in traditional medicine for the treatment of indigestion and also as antispasmodic [5]. Cinnamon contains cinnamaldehyde, which is a phenylpropene derivative [6]. It was found to possess antibacterial property and is mostly used as carminative. Fennel contains anethole and fenchone. This is mainly used as a carminative [7,8,9,10].

An earlier report on the digestive and carminative property of the mentioned ingredients prompted us to formulate and evaluate the digestive enzyme activity namely amylolytic, lipolytic and proteolytic activity in comparison with GASTRAP (marketed formulation) used as a digestive agent.

MATERIALS AND METHODS

PREPARATION OF CHURNA:

The raw materials such as rhizomes of *Zingiber officinale* (2 parts), fruits of *Foeniculum vulgarae* (2 parts), barks of *Cinnamomum zeylanicum* (1 part) and fruits of *Trachyspermum ammi* (1 part) were used for the preparation of the formulation. The raw materials used for this formulation were purchased from the market and authenticated in the Pharmacognosy department of Sri Ramachandra College of Pharmacy. The authentication is

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carried out based on the microscopic characteristics of powdered drug. The finely powdered raw materials were passed through sieve number 60 and mixed in appropriate ratios (16.7g of *Zingiber officinale* and *Foeniculum vulgarae*, 8.7g of *Cinnamomum zeylanicum* and 8.7g of *Trachyspermum ammi*). The churna was packed in an air tight glass container, [11]

EVALUATION OF PHYSICAL PARAMETERS:

1) Determination of pH [13]

The pH of 1% solution of formulated churna was determined using pH meter (Elico pH meter).

2) Determination of Moisture content [13]

The moisture content of churna was found using halogen moisture determining apparatus (Mettler Toledo).

3) Determination of Ash Values [13]

I. Total Ash Value

2gms of churna was weighed accurately in a previously ignited and tarred silica crucible. The material was then ignited by gradually increasing the heat to 500-600° C until, it appeared white indicating absence of carbon. It is then cooled in a dessicator and total ash in mg per gm of air dried material is calculated.

II. Acid Insoluble Ash Value

To the crucible containing total ash, 25ml of Hcl was added and boiled gently for 5minutes, then about 5ml of hot water was added and transferred into crucible. The insoluble matter was collected on an ashless filter paper. This was then washed with hot water until filterate is neutral and the filter paper along with the insoluble matter was transferred into crucible and ignited to constant weight. The residue was then allowed to cool and then weighed.

4) Determination of Extractive Values [13]

I. Water Soluble Extractive Value

5gms of churna was accurately weighed and placed inside a glass stoppered conical flask. It is then macerated with 100ml of chloroform water for 18hours. It was then filtered and about 25ml of filterate was transferred into a chinadish and was evaporated to dryness on a waterbath. It was then dried to 105° C for 6hours, cooled and finally weighed.

II. Alcohol Soluble Extractive Values

Ethanol was used as solvent in place of chloroform water and remaining procedure was the same as that of water-soluble extractive value.

5) Determination Of Crude Fibre Content [14]

2gms of accurately weighed churna was placed in a round bottom flask and then 100ml of 0.128 M sulphuric acid was added and refluxed for 1 hour then filtered through ashless filter paper and the residue was washed with water until filterate becomes neutral. The residue was then weighed (a), ignited to ash and finally the weight of ash (b) was determined. The difference between a and b represented the crude fibre content and was calculated on dry weight basis.

6) Determination of Heavy Metal Contamination

I. Arsenic Content [14]

Preparation of Standard Solution (10PPM)

0.33gms of arsenic trioxide was dissolved in 5ml of 2M Sodium hydroxide solution and then diluted to 250ml with water. One volume of this was then diluted to 100 volume with water.

PREPARATION OF SAMPLE

Preparation of Churna solution

The churna solution was prepared by means of diluting 1gm of churna to 100ml using distilled water. This is used to carryout limit test for iron and lead and also to perform qualitative test for mercury.

10ml of churna solution was pipetted out into a flask and about 10ml of concentrated nitric acid was added and evaporated to dryness on a waterbath. The residue was then dried at 130° C for 30minutes then about 10ml of hydrazine molybdate reagent was added and refluxed for 20minutes. The solution was then cooled and absorbance of both standard and test solution was measured at 800nm using Perkin Elmer UV spectrophotometer.

Ii. Limit test for Iron [14]

Preparation of Standard Solution (20 PPM)

One volume of 0.1726% w/v solution of ferric ammonium sulphate solution was diluted in 0.05 M sulphuric acid to ten volume using distilled water.

PROCEDURE

Limit test was performed in Nessler's cylinder. 2ml of test and standard solutions were taken in separate cylinders and then 2ml of 20% solution of citric acid and 0.1 ml thioglycollic acid were added. The solution was then mixed and made alkaline with iron free ammonia, diluted to 50ml with distilled water. It was then allowed to stand for 5minutes and colour obtained in sample was compared with that of standard colour. If the colour produced in test is more when compared to that of standard solution then the sample was said to fail the limit test and said to pass the test if vice versa occurs.

III. Limit Test For Lead [14]

Preparation of Standard (20 PPM)

0.4 gm of lead nitrate was dissolved in water containing 2ml of nitric acid and sufficient water to produce 250ml. About 1volume of above solution was diluted to 10 volume using distilled water.

PROCEDURE

Limit test was performed in Nessler's cylinder. 1ml of standard lead solution and test solution were taken in separate cylinders and were diluted to 25ml using distilled water

and then pH was adjusted to value 3-4 by adding dilute acetic acid or dilute ammonia solution and then diluted to 35ml using distilled water. To both the solutions 10ml freshly prepared hydrogen sulphide solution was added, mixed and diluted with water to 50ml. It was then allowed to stand for 5minutes and viewed downwards over white surface. The colour produced in test solution should not be more intense than that of standard solution, if so then the sample is said to pass the limit test for lead.

IV. Test for Mercury

To 10 drops of test solution 6M Hcl was added to get a white precipitate. The precipitate was then treated with 6M ammonia solution. If the colour of precipitate changes to grey or black colour then it indicates the presence of mercury.

7) DETERMINATION OF MICROBIAL CONTENT

1gm of churna was dissolved in lactose broth and volume adjusted to 100ml with the same medium. About 10ml of sample was transferred into 100ml of Macconkey broth and incubated for 18-24 hours at 43-45°C. A subculture was prepared on a plate with Macconkey agar and incubated at 43-45°C for 18-24 hours. The growth of red, generally non-mucoid colonies of gram negative rods appearing as reddish zones indicates the presence of *E.coli* if not then it indicates the absence of *E.coli*.

Determination of Digestive Property

Preparation of Extract

About 100mg of accurately weighed quantity of churna was extracted with 20% aqueous glycerol and phosphate buffer (pH7.8) in 1:4 ratio and filtered and the filtrate was used as enzyme source. [15,16]. The standard sample was prepared similar to the test sample.

i. Amylolytic activity

Extract (1ml) of churna and GASTRAP were incubated separately for 15minutes at 27°C and added to 1ml of the substrate (soluble starch1% 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. [17, 18].

ii. Lipolytic activity [19,20]

Preparation of Substrate Solution

2ml of castor oil was, neutralized to pH 7 and stirred well with 25ml of water in the presence of 100mg of bile salts (sodium taurocholate) till an emulsion was formed.

PROCEDURE:

Taken 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 enzyme 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 unit 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.

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

i. Proteolytic activity [22]

Preparation of Substrate Solution

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

PROCEDURE

Taken 1ml of substrate solution added 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% trichloroacetic acid solution. After 10 minutes 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- Ciocalteau reagent (1:2) and optical density measured at a wavelength of 650 nm. The proteolytic activity was then calculated from standard curve in milligrams of tyrosine. Protien estimated by standard method and results were given in milligrams of liberated tyrosine per milligram of dissolved protien per hour at 37° C as specific activity.

RESULTS:

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 fibre content were given in table 1 and detection of heavy metals such as arsenic, iron, lead and mercury in table 2. Finally the result of microbial detection was given in table 3.

TABLE 1

EVALUATION OF PHYSICAL PARAMETERS OF CHURNA

S.No	Physical Parameters	Values
1	pH	5.357
2	Moisture content	10.8 % w/w
3	Ash Values	
	I. Total ash II. Acid insoluble ash	10% w/w 5% w/w
4	Extractive values	
	I. Water soluble extractive value II. Alcohol soluble extractive value	0.12% w/w 2% w/w
5	Crude fibre content	9.75% w/w

TABLE 2
DETECTION OF HEAVY METALS IN CHURNA

S.No	HEAVY METAL	Values
1	Arsenic (Spectrophotometry)	0.205 ppm
2	Iron (Limit test)	Within the limit
3	Lead (Limit test)	Within the limit
4	Mercury (Qualitative analysis)	Absent

TABLE 3
DETECTION OF MICROBES IN CHURNA

S.No	MICROORGANISM	Present / Absent
1	*Escherichia coli*	Absent

The amylolytic activity of the churna was found to be 0.294mg/ml while that of GASTRAP was found to be 0.28 mg/ml. The values are represented in fig 1.

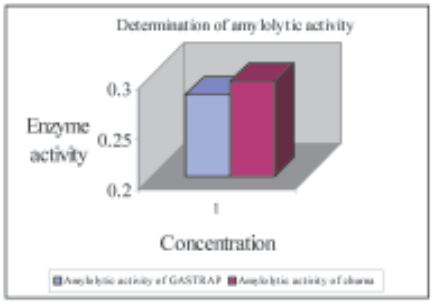

Fig 1.

The lipolytic activity of churna was found to be 0.01633 while that of GASTRAP was found to be 0.02294.The values are represented in fig 2.

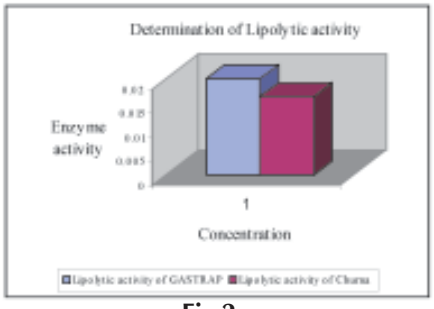

Fig 2.

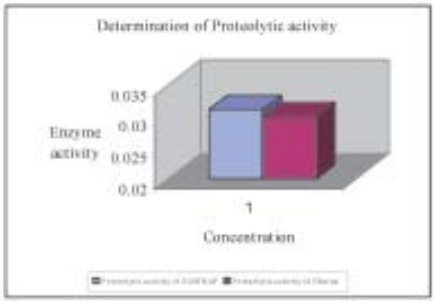

Fig 3.

The proteolytic activity of churna was found to be 0.030 mg/ml while that of GASTRAP was found to be 0.031 mg/ml.

DISCUSSION:

The churna consisting of fine powder of herbs in appropriate ratio was subjected to standardisation by means of various physical, chemical and microbiological methods. The physical parameters such as pH was determined to avoid gastric irritation and the moisture content was determined to find out any increase in weight caused by moisture absorption. The value obtained was found to be within the standards. Since ashing process involves oxidation of components of product, an increase in ash value indicates contamination, substitution and adulteration. The total ash value is an indicative of total amount of inorganic material after complete incineration and the acid insoluble ash value obtained is an indicative of silicate impurities, which might have arised due to improper washing of crude drugs. Both the ash values obtained were found to be within the standard limits. The extractive values namely water-soluble and alcohol soluble indicates the amount of active constituent in given amount of plant material when extracted with respective solvents, a lower value compared to standard value indicates presence of exhausted material. In the present study both the extractive values were found to be more than the standard values. The determination of crude fibre content is an indicative of fibre content in formulation and was found to comply with the standard value. Heavy metals if present in formulations will have a deleterious effect on different organs of body in particular kidneys and leads to renal toxicity. Hence evaluation of heavy metals is an important role. Heavy metals include arsenic, iron, lead and mercury. In the present study arsenic was evaluated by means of spectrophotometry, iron & lead by means of limit test where the allowed maximum limit were 20ppm respectively and were found to be within the limits. The presence of mercury was determined qualitatively and found to be absent. The formulated churna was finally subjected to microbiological evaluation namely for *E.coli* and was found to be absent hence the formulated churna complied with the WHO requirements.

The biological activity of churna was evaluated by means of evaluating amylolytic, lipolytic and proteolytic activity in comparison with the standard marketed formulation GASTRAP. The amylolytic activity involves the break down of starch into maltose by the action of amylase enzyme. Determination of amylolytic activity brings out the ability of churna to digest starch. In the present study the amylolytic activity of formulated churna was found to be 1.4% greater than that of marketed formulation GASTRAP. Hence the formulated churna was considered to possess the activity of digesting starch. Lipolytic activity is another enzymatic activity that involves the break down of lipids into fatty acids by the action of lipase enzyme. Determination of lipolytic activity brings out the ability of digesting lipids by particular substance. In the present study the lipolytic activity of formulated churna was found to be slightly lesser than that of

GASTRAP. Proteolytic activity is an enzymatic activity that involves break down of proteins into aminoacids by the action of protease enzyme. Determination of proteolytic activity brings out the ability of digesting proteins by a particular substance. In the present study it was determined by means of using folin-ciocalteau method where the phenolic group present in the liberated aminoacid namely tyrosine forms a complex with the reagents added and found to absorb in a wavelength of 660nm. The intensity of colour depends on the amount of aromatic aminoacids present and hence gives the proteolytic activity of churna. In the present study the proteolytic activity of formulated churna was found to be almost equal to that of marketed formulation GASTRAP.

CONCLUSION:

The physical parameters evaluated confirm the standard of the formulated churna. The invitro 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 GASTRAP.

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