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Short Communication

FORMULATION AND EVALUATION OF NARASIMHA *CHURNA* FOR AMYLOLYTIC ACTIVITY

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ABSTRACT

Ayurvedic medicines play an important role in immune problems due to safety and efficacy in it. Hence Narsimha Churna meant for amylolytic activity has been formulated by standard procedures and evaluated by physical and analytical methods. The formulation consists of fine powder (sieve 60 size) of dried roots of *Asparagus racemosus*, fruits of *Tribulus terrestris*, rhizome of *Dioscorea bulbifera,* steam of *Tinospora cordifolia* and fruit of *Semecarpus anacardium* 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 finds the amylolytic activity.

Ash values and extractive values were found to be within prescribed limits. The arsenic level was found to be 0.205 ppm. *Churna* did not show the presence of any *Escherichia coli* and other microorganisms. The *churna* showed pronounced amylolytic activity.

Keywords: Ayurvedic Medicines, Digestion, Complementary Therapies, Amylolytic Activity.

1. INTRODUCTION

Churna is defined as a fine powder of drug or drugs in Ayurvedic system of medicine [1-3]. Drugs mentioned in *patha*, are cleaned properly, dried thoroughly, pulverized and then sieved. The *churna* is free flowing and retains its potency for one year, if preserved in airtight containers. Triphala *churna*, Trikatu *churna*, Drakeshadi *churna* and Sudharsana *churna* are some of examples. *Churna* formulation is similar to powder formulations in Allopathic system of medicine. In recent days *churna* is formulated into tablets in order to fix the dose easily [4]. These forms of medicament are prescribed generally because of their particle size. Smaller the particlesize greater is the absorption rate from GIT and hence the greater is bioavailability [5-6]. 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 [7]. Hence we prepared a *churna* with natural ingredients commonly used by mankind for culinary purposes. Thus the present study examined the favorable influence of four spices formulated into *churna* said to have immunomodulation property [8]. The common ingredients of these *churna* were Shatavari (*Asparagus racemosus)*, Gokshura (*Tribulus terrestris*), Varahi (*Dioscorea bulbifera*), Guduchi (*Tinospora cordifolia*), Bhallataka (*Semecarpus anacardium*) [9-12].

2. MATERIAL AND METHODS

2.1. Preparation of *churna*

The raw materials used for this formulation were purchased from the market and authenticated in the Pharmacognosy department of Guru Nanak Dev University. The authentication is carried out based on the microscopic characteristics of powdered drug.

2.2. Evaluation of physical parameters

2.2.1. Determination of pH [13]

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

2.2.2. Determination of Moisture content [13]

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

2.2.3. Determination of Ash Values [13]

2.2.3.1. Total Ash Value

Two gms 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 was then cooled in a desiccator and total ash in mg per gm of air dried material was calculated.

Table 1: Formulation Profiling of Narasimha *Churna*

Name of Plant	Botanical Source	Part used	Quantity	Uses
Shatavari	*Asparagus racemosus*	Root	768 gm	Galactogogue
Gokshura	*Tribulus terrestris*	Fruit	768 gm	Aphrodisiac, Diuretic and Nervine
Varahi	*Dioscorea bulbifera*	Rhizome	960 gm	Diarrhea and Dysentery
Guduchi	*Tinospor acordifolia*	steam	1.20 kg	Immunomodulator
Bhallataka	*Semecarpus anacardium*	Fruit	1.53 kg	Leprosy and Nervous debility
Chitraka	*Plumbago zeylanica*	Root	480 gm	Headache, Antidiarrheal
Tila	*Sesamum indicum*	Seed	768 gm	Antioxidant, Anticancer
Ardhraka	*Zingiber officinale*	Rhizome	128 gm	Colitis, Nausea, Piles
Maricha	*Piper nigrum*	Fruit	128 gm	Bacteriostatic and Fungistatic
Pippali	*Piper longum*	Fruit	128 gm	Tonic, Useful in respiratory discomfort,
Sugar	------	----	3.36 kg	Sweetening agent
Honey	----	------	1.68 kg	Sweetening agent
Clarified butter	----------		840 gm	Moistening agent
Vidarikanta	*Pueraria tuberose*	Root	768 gm	Boosts immunity

2.2.3.2. Acid Insoluble Ash Value

To the crucible containing total ash, 25 ml of HCl was added and boiled gently for 5 minutes, 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 was 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.

2.2.4. Determination of Extractive Values [13]

2.2.4.1. Water Soluble Extractive Value

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

2.2.4.2. 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.

2.2.5. Determination of Crude Fibre Content [14]

Two gms of accurately weighed *churna* was placed in a round bottom flask and then 100 ml of 0.128 M sulphuric acid was added and refluxed for 1 hour, filtered through ash less filter paper and the residue was washed with water until filtrate became neutral. The residue was then weighed (a), ignited to ash and finally the weight of ash (b) was determined. Finely powdered raw materials were passed through sieve number 60 and mixed in appropriate ratios. The *churna* was packed in an air tight glass container [11].

2.3. Tests 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.

2.4. Determination of microbial content

A 01gm of *churna* was dissolved in lactose broth and volume was 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*.

2.5. Determination of Digestive Property

2.5.1. 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 ratios and filtered and the filtrate was used as enzyme source [15, 16]. The standard sample was prepared similar to the test sample.

2.5.2. 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 520 nm [17, 18].

20 ml substrate was taken and 5ml phosphate buffer was added 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 (0.5 ml) was added immediately and pH was 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 [19].

3. RESULTS AND DISCUSSION

The results of the physical parameters 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 2 and detection of heavy metals such as arsenic, iron, lead and mercury in table 3. Finally the result of microbial detection was given in table 4.

The amylolytic activity of the *churna* was found to be 0.294 mg/ml while that of GASTRAP was found to be 0.28 mg/ml.

Table 2: 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	 3 % w/w 5% w/w
4	Extractive values I. Water soluble extractive value II. II. Alcohol soluble extractive value	 0.12% w/w 2% w/w
5	Crude fibre content	9.75% w/w

Table 3: 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 4: Detection of microbes in *churna*

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

The *Churna* consisting of fine powder of herbs in appropriate ratio was subjected to standardization 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 [20-21]. 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 activity in comparison with the standard marketed formulation. The amylolytic activity involves the breakdown 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. Hence the formulated *churna* was considered to possess the activity of digesting starch.

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. In the present study the proteolytic activity of formulated *churna* was found to be almost equal to that of marketed formulation.

4. 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.

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