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Preparation and Evaluation of Shatavaryadi Churna: An Ayurvedic Polyherbal Formulation

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ABSTRACT

In Present investigation Shatavaryadi Churna was prepared as per Ayurvedic Formulary of India. Shatavaryadi Churna Marketed Formulation (SCMF-1), house formulation and indivisual ingredients has been evaluated on the basis of organoleptic characters, preliminary Phytochemical analysis, Physico-chemical properties by Bulk density, Angle of Repose, Static angle of repose, Total and Acid insoluble ash, Extractive values, Moisture Content and in vitro antimicrobial activity by cup plate method. On the basis of the results obtained in this present investigation, that house formulation showed the presence of carbohydrates, amino acids, flavonoids and saponin glycosides but saponin glycoside were absent in marketed formulation. Flow properties of both the formulations showed good compared to individual ingredients, it may be due to additive effect of the poly herbs present in formulation. Both the formulations complies with standard ash values and moisture content.

Keywords: Shatavaryadi churna; Evaluation; Extractive; Antimicrobial; Phytochemical

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INTRODUCTION

India is rich heritage of traditional medicine and 80% populations of developing countries rely on traditional medicines, mostly on plant drugs for their primary health care needs [1].

Most of the traditional systems of medicines are effective but they need herbal drug evaluation for the purpose of research work on standardization of herbal formulations a profound knowledge of the important herbs found in India and widely used in Ayurvedic formulation is of outmost importance. India can emerge as the major country and play the lead role in production of standardized, therapeutically effective ayurvedic formulations. India needs to explore the medicinally important plants. This can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization.The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy [2].

Evaluation of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. Shatavaryadi Churna is an Ayurvedic formulation, in herbal powder form. It is used in the Ayurvedic treatment for rejuvenation and to relieve stress and tiredness. This medicine is most commonly used in North Indian Ayurvedic practice. Shatavaryadi churna was prepared as per Ayurvedic Formulary of India. In-house preparation and the marketed drug have been standardized on the basis of Organoleptic characters, Preliminary Phytochemical analysis, Physico-Chemical properties and *in vitro* antimicrobial activity. The set parameters were found to be sufficient to evaluate the Churna and can be used as reference standards for the quality control/quality assurance laboratory of a Pharmaceutical house [3].

Shatavaryadi churna

Shatavaryadi Churna is an Ayurvedic formulation, in herbal powder form. It is used in the Ayurvedic treatment for rejuvenation and to relieve stress and tiredness. This medicine is most commonly used in North Indian Ayurvedic practice.

Different powder ingredients of Shatavaryadi churna formulation

Studies reported on Shatavaryadi Churna, Determined the concentrations of twelve elements and *in vitro* antioxidant activity in two formulations of Shatavaryadi Churna and their ingredients [4]. Many activities were done on indivisual ingredients of Shatavaryadi Churna. Hepatotoxic, immunemodulatory, immune adjuvant and antilithiatic effects was reported for *Asparagus racemosus* [5]. is used for treating sexual and other nerve disorder due to it's great medicinal properties [6]. Ethanolic extract of Chlorophytum borivilianum was carried in rat model at dose of 125 mg/kg up to 250 mg/kg to treat sperm count. Sperm count increases up to 150% [7]. and also weight gain of body and reproductive organs were observed [8]. Increased libido, sexual vigor and sexual arousal were also reported [9]. Carried out the evaluation of *Withania somnifera* herbal formulation and reported standardization values [10]. For present study we have selected the Shatavaryadi Churna because after through literature survey till now there has been no documentation on pharmacognostic parameters, pharmaceutical parameters (angle of repose, bulk density tap density, carr index, hausner's ratio), *in vitro* antimicrobial activity to set standards for this poly herbal formulation and compare the classically prepared Shatavaryadi Churna with the market available sample using these parameters and understand whether there are any differences.

Aim

Aim of the present research work is to prepare and evaluate Shatavaryadi Churna herbal formulation.

Objectives

Preparation of the ethanolic extract of each individual ingredients of Shatavaryadi Churna herbal formulation, marketed and house formulation. Shatavaryadi Churna was evaluated by: Organoleptic, Preliminary Phytochemical analysis, Determination of Extractive values, Ash values, moisture content,Bulk density,Tap density, Carr index, Hausner's ratio, Angle of repose and *In vitro* antimicrobial activity.

EXPERIMENTAL SECTION

Materials and methods

Collection of plant material:

Shatavaryadi Churna consists of 5 ingredients. *Asparagus racemosus* tubers powder, *Withania somnifera* root powder, *Tribulus terrestris* fruit powder, *Mucuna pruriens* seed powder *Chlorophytum borivilianum* root powder were collected from Tissue Vidhyalay at Renigunta, Tirupati. *Chlorophytum borivilianum* root powder was purchased from Yahiya sons at Gandhi road, Tirupati and Satavaryadi Churna marketed formulation (SCMF-1) procured from Om Ayurveda medical shop, Ramulavari South Mada Street, Tirupati.

Method of preparation of shatavaryadi churna house formulation:

All the above plant ingredients are powdered separately and mixed together in specified quantity, stored in air tight container in dry place.

Preparation of ethanolic extract:

Ethanolic extract of Shatavaryadi Churna herbal formulation was prepared by cold maceration method. Fifty grams of each plant material in powder form was weighed in an Erlenmeyer of 500 ml to which 200 ml of ethanol (96%) and distilled water (20:80 v:v) is added for pre-extraction. The Erlenmeyer is placed in dark for three days in room temperature. The mixture was filtered using What man No.1 filter paper. The filtrates were exposed to 60^{o} C in water bath for 30 min for ethanol evaporation. The filtrates were kept at 4^{o} C until use.

Chemicals:
All the Solvents and Reagents were used as analytical grade.

Phytochemical analysis:
Preliminary Phytochemical tests were conducted on test extracts to detect the presence of Phyto constituents and carried out according to standard procedures [11].

Methods
Flow properties of powders:
Bulk density: The density of a powder is often determined using a jolting volumeter. A known weight of sample is placed into a measuring cylinder and 'tapped' until a consistent volume is reached which corresponds to the maximum packing density of the material. By measuring both the untapped volume and the tapped volume the following can be determined [12]. Pour (or Bulk) density = mass / untapped volume, Tapped density = mass / tapped volume
Hausner ratio = tapped density / pour density, Carr's Index = (tapped density – bulk density) x 100 / tapped density.

Angle of repose: The angle of repose provides a reliable, quick and simple method to measure the flowability of different powders. Lower angles of repose correspond to freely flowing powders, whereas higher angles indicate a cohesive or poor flowing material.Classified powders according to their flowability using the angle of repose [12].

Measurement of static angle of repose: The device used to measure the static angle of repose in the current study consisted of a glass conical funnel, with an outlet diameter of 0.9 cm, fixed on a metal stand; the funnel outlet was kept at a height of 6 cm above the base. Then powder is poured up to the tip of the pile touches the funnel. Then take the diameter of the pile is measured and substituted in a formula [12].

$$Ø = \tan^{-1}(h/r), \text{ here } h = \text{height of the pile.}$$
$$r = \text{radius of the circle}$$

Ash values:
Total ash: Take about 2 gm accurately weighed of the ground drug in tarred platinum or silica dish previously ignited and weighed Scatter the ground drug in a fine even layer on the bottom of the dish. Incarnated by gradually increasing the heat-not exceeding dull red heat- until free from carbon, cool and weigh [13].

Acid- insoluble ash: Boil the total ash for five minutes with 25 ml of dilute hydrochloric acid, collect the insoluble matter in a Gooch crucible or on an ash less filter paper, wash with hot water, ignite and weigh. Calculate the percentage of acid- insoluble ash with reference to the air dried drug [13].

Determination of extractive values:
5g of air dried Powder of ingredients of Shatavaryadi Churna and Shatavaryadi Churna formulations were taken and macerated with 100 ml of solvent (i.e. ethanol, water, chloroform, ethyl acetate and benzene) in a closed flask for 24 hours, shaking frequently for the first 6 hrs and allowed to stand for 18 hrs, then filtered with taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and finally dried at 105° C and weighed. The percentage of the water soluble extractive values were calculated with reference to air dried powder [11].

Determination of moisture content:
5 g of sample (without preliminary drying) were placed after accurately weighing it in a tarred evaporating dish. After placing the above said amount of the sample in the tarred evaporating dish dry at 105^{o}C and continue the drying and weighing at 10 minutes interval until difference between two successive weighing corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccators, shows not more than 0.01g difference. Finally moisture content was measured directly in percentage [11].

Determination of antimicrobial activity by cup plate method:
Antimicrobial activity was carried out by Cup plate method [14].

Preparation of nutrient media:

Suspend 28 grams in 1000 ml distilled water, heat to boiling to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before pouring [15].

Preparation of plates:
To Petri dishes (Pyrex dishes, 100 mm in diameter, selected for uniform flat bottoms 2) 40ml of sterile assay agar was added preferably with dispensing pipette. The agar is allowed to harden with the Petri dish tops tilted or removed, while the plates are still warm. The amount to be used is best determined by actual test in the assay procedure, that quantity being selected which gives large sharp zones. The organism Escherichia coli are added to the liquefied agar at 45^{o}C and the agar medium is maintained at that temperature until all the plates are poured. The plates are stored at 2^{o}C to 4^{o}C as soon as they have hard and may be kept for the several days with no effect on the assay. It is essential that the plates be prepared on an absolutely level table top.

Preparation of sample:
The Shatavaryadi Churna marketed formulation, house formulation and each individual ingredient of house formulation and dilutions done with ethanol (rectified spirit). The dilutions are 20 µg/ml, 40 µg/ml, 80µg/ml, 160µg/ml and 240µg/ml are prepared.

Incubation of plates:
The plates are incubated at 30^{o}C for 24 hour. After this time no significant change in the size of the zone occurs up to 24 hours of incubation. At 37^{o}C the zone size reduced, and no long incubation the organism trends to overgrowth the cleared area. However , the zones develop more rapidly at 37^{o}C, and the readings may be made after 4 to 6hours of incubation, thus affording a shorter assay time.

Estimation of potency:
The diameters of the zones of inhibition are measured, to the closet one –quarter millimeter and replicates are averaged. A daily curve is prepared from dilutions of the standard by plotting the zone diameters in mm against the concentration in units per ml. The potency of the unknown samples is determined from the standard curve [16].

RESULTS

Phytochemical analysis
Ethanolic extract of the Shatavaryadi Churna showed the presence of phytochemical constituents like Carbohydrates, Amino acids, Flavonoids, Saponin glycosides and Alkaloids and were shown in Table 1.

Table 1: Phytochemical constituents in ethanolic extract of the shatavaryadi churna

Tests	AR	WS	TT	CB	MP	HF	MF
Carbohydrates							
Molish test	+	+	+	+	+	+	+
Fehling's test	+	-	-	-	+	+	-
Benedict's test	+	+	+	+	-	+	+
Amino acids							
Ninhydrin test	+	+	+	+	+	+	+
Flavonoids							
Reaction with alkali and dilute acids	+	+	+	+	-	+	+
Saponins							
Foam test	+	-	+	+	-	+	-
Glycosides							
Baljet test	+	-	+	+	+	+	+
Lejal's test	+	-	+	+	+	+	+
Borntrager's test	-	-	-	-	-	+	-
Alkaloids							
Dragondroff's test	-	+	+	-	+	+	+
Mayer's test	-	+	-	-	-	+	+
Hager's test	-	+	-	-	-	+	+
Wagner's test	-	+	-	-	-	+	+

AR: *Asparagus racemosus*; WS: *Withania somnifera;* TT: *Tribulus terrestris;* CB*: Chlorophytum borivillianum;* MP*: Mucuna pruriense*; HF: House formulation; MF: Marketed formulation; Here, + = present; - = absent

Organoleptic evaluation

The following ingredients of Shatavaryadi Churna, Marketed and House preparations were evaluated for their Colour, Odour, Taste and Texture and shown in Table 2.

Table 2: Evaluation of shatavaryadi churna ingredients

Samples	Colour	Odour	Taste	Texture
Asparagus racemosus	Silver white	None	Slightly bitter followed by sweet	Rough
Chlorophytum borivillianum	Pale white	Milk like odour	Mucilaginous	Smooth and Sticky
Mucuna pruriens	Grey to dark brown	Characteristic	Bitter	Smooth
Tribulus terrestris	Green	Slightly aromatic	Bitter	Smooth
Withania somnifera	Light brown	Characteristic	Bitter and acrid	Smooth
House formulation	Whitish to green	Characteristic	Slightly bitter	Smooth
Marketed formulation	Light whitish	Characteristic	Slightly bitter	Smooth

Extractive values

Shatavaryadi Churna herbal formulations were subjected with different Solvents. The solubility of Constituent are extracted in particular solvents used and shown in Table 3.

Table 3: Solubility of constituents

Sample	Water	Ethanol	Chloroform	Ethyl acetate	Benzene
Asparagus racemosus	70%	25%	35%	25%	85%
Withania somnifera	89%	23%	36%	2%	2%
Tribulus terrestris	33%	10%	30%	3%	38%
Chlorophytumborivilianum	2%	3%	25%	2%	30%
Mucuna pruriense	33%	3%	32%	87%	2%
House formulation	88%	37%	33%	30%	2%
Marketed formulation	87%	36%	33%	30%	2%

Flow properties of shatavaryadi churna

Shatavaryadi Churna formulation was observed for following flow properties like bulk density, tap density, cars index, Hausners ratio and Angle of repose were showed in Table 4 and 5.

Table 4: Flow properties of shatavaryadi churna

Sample	Bulk density	Tap density	Hausner's ratio	Carr's index
Asparagus racemosus	0.27	0.37	1.37	27.02
Withania somnifera	0.43	0.67	0.24	35.82
Tribulus terrestris	0.3	0.45	1.5	33.33
Chlorophytum borivilianum	0.45	0.62	1.24	19.35
Mucuna prurience	0.43	0.59	1.37	27.11
House formulation	0.43	0.59	1.37	27.11
Marketed preparation	0.31	0.45	1.45	31.11

Table 4: Angle of repose of shatavaryadi churna

Sample	Angle of repose
Asparagus racemosus	37º
Withania somnifera	41º
Tribulus terrestris	47º
Chlorophytum borivilianum	39º
Mucuna pruriense	47º
House formulation	40º
Marketed preparation	40º

Ash values

Shatavaryadi Churna was evaluated for the presence of any earthy matter, siliceous substances, and water exhaustive material contamination by doing ash values and was shown in Table 6.

Moisture content

Moisture content is responsible for the bacterial growth hence Shatavaryadi Churna was evaluated for any moisture content and results were shown in Table 7.

Table 6: Evaluation for the presence of any earthy matter

Sample	Total ash value	Water soluble ash	Acid insoluble ash
Asparagus racemosus	7.67	5.14	1.02
Withania somnifera	6	3	1.5
Tribulus terrestris	12.57	10.52	4
Chlorophytum borivilianum	3	2.68	0.356
Mucuna pruriense	4.26	2.51	0.289
House formulation	5.55	2.99	0.622
Marketed formulations	5.34	2.96	0.578

Table 7: Evaluated for any moisture content

Sample	Moisture content (%w/w)	Reference value (%w/w)
Asparagus racemosus	0.4	Not more than 15%
Withania somnifera	0.1	Not more than 12%
Tribulus terrestris	0.04	Not more than 5%
Chlorophytum borivilianum	0.11	-
Mucuna pruriense	0.6	-
House formulation	0.01	-
Marketed preparation	0.001	-

Anti-microbial activity

Ethanol extract of each individual ingredient of Shatavaryadi Churna house formulation and marketed formulation were evaluated for their *in vitro* antimicrobial activity and results were shown in Table 8.

Table 8: Evaluated for antimicrobial activity

Zone of inhibition(cm)					
Sample	**240µg/ml**	**160µg/ml**	**80µg/ml**	**40µg/ml**	**20µg/ml**
Asparagus racemosus	2.1	2	1.8	1.7	1.5
Withaniasomnifera	2.6	2.5	2.3	2.2	2
Tribulus terrestris	2.4	2.3	2.1	2	1.9
Chlorophytum borivilianum	2.2	2	1.8	1.6	1.4
Mucuna pruriense	2.7	2.5	2.3	2	1.8
House formulation	2.8	2.4	2.3	2.1	2
Marketed preparation	2.7	2.4	2.1	1.6	1.4
Streptomycin	2.9	2.6	2.5	2.3	2

DISCUSSION

Organoleptic evaluation of house formulation showed the light greenish colour and marketed formulation showed light whitish colour this may be due to low concentration of *Tribulus terrestris* powder in marketed formulation**.** Both the formulation showed bitter in taste, characterstic odour and having smooth in texture.

Preliminary Phytochemical analysis of Shatavaryadi Churna carried out in marketed formulation, house formulation and each individual ingredients, revealed the presence of carbohydrates, amino acids, flavonoids and saponin glycosides. Marketed formulation of Shatavaryadi Churna showed the absence of saponin glycosides it indicates the marketed formulation may not contain *Asparagus racemosus* because Phytochemical analysis of *Asparagus racemosus* showed the presence of saponin moiety. Extractive values of individual ingredients of Shatavaryadi Churna showed lower extractive values in all the solvents except in water but in both house and marketed formulation showed more water soluble extractives. Among all the ingredients as well as the formulations, house formulation exhibit more water soluble extractive, it indicates the formulation having more polar constituents such as steroidal glycosides. Flow properties were evaluated based on angle of repose, hausner's ratio and carr's index. House formulation showed better flow properties compared to marketed formulation. Individual ingredients of Shatavaryadi Churna showed poor flow property except *Withania somnifera* but the house formulation showed better flow properties, this may be due to additive effect of the polyherbs.

Tribulus terrestris showed high amount of total ash values, this may be due to presence of siliceous contamination (or) other earthy matter whereas house and marketed formulation showed almost similar ash values and complies with the standard values (< 5 shows normal range). Individual ingredients except *Tribulus terrestris* showed within the normal range .

Tribulus terrestris also showed high amount of water soluble ash and acid insoluble ash this may be due presence of a water exhausted material, presence of varying amount of calcium oxalate crystals. All other formulation and ingredients of Shatavaryadi Churna showed within the normal. The antimicrobial activity of marketed formulation, house formulation and each individual ingredient was studied by cup plate method against Escherichia coli. This assay are based on the use of cups as reservoirs containing solutions as substances to be examined. House formulations showed good activity against Escherichia coli, when compared with marketed formulation. Streptomycin was taken as positive control. Anti microbial activity may be due to presence of amino acids, flavonoids in poly herbs of house formulation, the marketed formulation showed moderate activity, it may be due to absence of *Asparagus racemosus* plant in marketed formulation.

CONCLUSION

On the basis of the results obtained in this present investigation, we concluded that ethanolic extract of house formulation showed the presence of Carbohydrates, Amino acids, Flavonoids and Saponin glycosides but Saponin glycosides were absent in marketed formulation .Flow properties of house formulation and marketed formulation showed good flow properties compared to individual ingredients, this may be due to additive effect of the poly herbs present in the formulation. Marketed and house formulation complies with standard ash values and moisture content. The result of this study may be used as the reference standard in further research under taking of its kind.

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