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Standardization of "Gokshuradi Churna'': An ayurvedic polyherbal formulation

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ABSTRACT

In the few decades, there has been exponentially growth in the field of herbal remedies. Newer guidelines for standardization, manufacture, quality control and scientifically rigorous research will be necessary for traditional treatments. This traditional knowledge can serve as the powerful search engine that will greatly facilitate drug discovery. Standardization of herbal formulations is essential in order to assess the quality, purity, safety and efficacy of drugs based on the amounts of their active principles. The aim of the present work is to standardize Gokshuradi churna. The churna makes this traditional drug more stable for long term storage and hence, easier to prepare. The "Gokshuradi churna" an Ayurvedic polyherbal formulation used in the treatment of is a polyherbal Ayurvedic medicine used as a diuretic and cardiac tonic. One marketed and one in- house formulations were used for the study. All the formulations were standardized on the basis of organoleptic characters, physical characteristics and physico-chemical properties. 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 purposes. The analysis and quality control of herbal medicines are moving towards an integrative and Comprehensive direction, in order to better address the inherent holistic nature of herbal medicines. It was observed that all ingredients of commercial samples matched exactly with that of authentic standards after performing the standardization as per WHO guideline.

Keywords: Gokshuradi churna, Standardization, Quality tests, Physicochemical parameters, Pharmacopoeial standard, WHO.

INTRODUCTION

India having a rich heritage of traditional medicine constituting with its different components like *Ayurveda, Siddha* and *Unani*. Botanical constituent of major part of these traditional medicines. The development of these traditional systems of medicines with the perspectives of safety, efficacy, and quality will helps not only to preserve the traditional heritage but also to rationalize the use of natural products in healthcare

[1,2]. In India around 20,000 medicinal plant species have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases [3]. Standardization of herbal formulations is an essential factor in order to assess the quality, purity, safety and efficacy of drugs based on the concentration of their active principles [4]. It is very important to establish a system of standardization for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous. Plant material when used in bulk quantity may vary in its chemical content and therefore, in its therapeutic effect according to different batches of collection e.g. collection in different seasons and/or collection from sites with different environmental surroundings or geographical locations. The increasing demand of the population and the chronic shortage of authentic raw materials have made it incumbent, so there should be some sort of uniformity in the manufacture of herbal or Ayurvedic medicines so as to ensure quality control and quality assurance. With the growing need for a safer drug, attention has been drawn to the qualility, efficacy, and standard of Ayurvedic formulations [5]. Quality assurance of herbal products may be ensured by proper quality control of the herbal ingredients and by means of good manufacturing practice. Some of herbal products have many herbal ingredients with small amount of individual herb being present. Assuring the quality of Ayurvedic medicines was traditionally the responsibility [6] of the physician who prepared the medicine himself and maintained a fiduciary relationship with the patient. The absence of post-market surveillance and the paucity of test laboratory facilities also make the quality control of Ayurvedic medicines exceedingly difficult at this time [7].

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 [8].

In the present research work, an attempt was made to standardize " Gokshuradi churna" an ayurvedic polyherbal formulation made up of six herbs (Table 1) used in the treatment of is a polyherbal ayurvedic medicine used as a diuretic and cardiac tonic. It increases vitality and decrease body weakness. It is a excellent remedy for sexual performance as per WHO guidelines [9].

EXPERIMENTAL SECTION

Plant material

The crude drugs used in preparation of Gokshuradi churna were collected from local market in September 2010, Bhilai and identified in Department of Botany, Government Science College, Durg. One in house formulation was prepared, as per the procedure mentioned in Ayurvedic text “Ayurveda Sar Sangrah”. All plant parts were then dried in shade, powdered and passed through sieve no. 80 # and lastly packed in a well closed container to protect them from moisture. Each ingredients *Tribulus terrestris* (8.333gm), *Asparagus racemosus* (8.333gm), *Abutilon indicum* (8.333gm), *Grewia hirsuta* (8.333gm), *Hygrophila spinosa* (8.333gm), *Mucuna pruriens* (8.333gm) were weight separately, mixed together to obtain a homogeneous blend.

Marketed samples

The marketed sample of Gokshuradi churna was procured of Vyas pharmaceuticals.

Botanical parameters

Organoleptic evaluation was carried out to assess the color, odor and taste of the marketed and in-house formulations [10].

Physicochemical investigation

Physico chemical investigations of the formulations carried out including determination of extractive values, ash values and loss on drying by I R moisture balance [11,12].

Loss on drying

Loss on dying is the loss of mass expressed as percent w/w. About 10g of dug samples of each formulation was accurately weighed in a dried and tared flat weighing bottle and dried at 105°C for 5hrs. Percentage was calculated with reference to initial weight.

Determination of total ash

Ashing involves an oxidation of the components of the products. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the formulation for marketing. Total ash determination constitutes detecting the physiological ash (ash derived from plant tissue) and non physiological ash (ash from extrageneous matter, especially sand and soil adhering to the surface of the drug). For its detection, 2g of powdered material was placed in a suitable tared crucible of silica previously ignited and weighed. The powdered drug was spread into an even layer and weighed accurately. The material was incinerated by gradually increasing the heat, not exceeding 450°C until free from carbon, cooled in a desiccators, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible & that of crucible with total ash.

Determination of Acid insoluble ash

The ash obtained as above was boiled for 5min with 25ml of dilute hydrochloric acid; the insoluble matter was hot water and collected on an ashless filter paper, washed with ignited to constant weight. The percentage of acid-insoluble ash with reference to the air-dried drug was calculated.

Determination of solvent Extractive values

Alcohol soluble extractive value

5g of coarsely powdered air-dried drug was macerated with 100ml of alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. It was then filtered rapidly; taking precautions against loss of solvent.25ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish at 105°C to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug and is represented as % value.

Water soluble extractive value

5g of coarsely powdered air-dried drug was macerated with 100ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish at 105°C to constant weight and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug and is represented as % value.

Determination of pH

The pH of different formulations in 1% w/v and 10% w/v of water soluble portions were determined using pH paper (Range 3.5–6) and (6.5–14) with standard glass electrode [13].

Estimation of crude fiber extract

2 gm of drug was taken in a beaker and 50ml of 10% nitric acid was added. It was heated to boil with stirring (30 sec.).This was strained through fine cloth on a buchner funnel. The residue was washed with boiling water and transferred to a beaker. 50ml of 2.5% v/v sodium hydroxide solution was added. It was strained and washed with hot water. The residue was transferred in a clean and dried crucible. The residue was weighed and the crude fiber content was determined [14].

Determination of physical characteristics of powder

Physical characteristics like bulk density, tap density, angle of repose, Hausner's ratio and Carr's index were determined for different formulations [15,16].

Bulk density and Tap density

The term bulk density refers to a measure used to describe a packing of particles or granules. The equation for determining bulk density (Db) is:

$$Db = M/Vb$$

Where M is the mass of the particles and Vb is the total volume of the packing. The volume of the packing can be determined in an apparatus consisting of a graduated cylinder mounted on a mechanical tapping device (Jolting Volumeter) that has a specially cut rotating can. 100gm of weighed formulation powder was taken and carefully added to the cylinder with the aid of a funnel. Typically the initial volume was noted and the sample was then tapped until no further reduction in volume was noted. The initial volume gave the Bulk density value and after tapping the volume reduced, giving the value of tapped density.

Angle of repose

Angle of Repose has been used as an indirect method of quantifying powder flowability; because of its relationship with interparticle cohesion. As a general guide, powders with angle of repose greater than 50 degree have unsatisfactory flow properties, whereas minimal angle close to 25 degrees correspond to very good flow properties. The fixed funnel and the free standing cone method employs a funnel that is secured with its tip at a given height, which was taken 2.5 cm (H), above the graph paper that is place on flat horizontal surface. Powder or granulation was carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel.

$$\text{Tan } \alpha = H/R \text{ or } \alpha = \text{arc tan } H/R$$

Where α is the angle of repose, R being the radius of the conical pile.

Hausner's ratio

It is related to interparticle friction and as such can be used to predict the powder flow properties. Powders with low interparticle friction such as coarse spheres, have a ratio of approximately 1.2, whereas more cohesive, less flowable powders such as flakes have a Hausner's ratio greater than 1.6. The equation for measuring the Hausner's ratio is:

Hausner's ratio = Df / Do, where Df = Tapped density and Do = Bulk density.

Carr's index

Another indirect method of measuring the powder flow from bulk density is Carr's index. The equation for measuring Carr's index is: % compressibility = (Df-Do/Do) × 100 where Df = Tapped density and Do = Bulk density.

Phytochemical screening

Active phytoconstituents like carbohydrates, glycosides, flavonoids, alkaloids were identified in aqueous extracts of all formulations [17].

Quantitative analysis of Flavonoids

1gm of powdered drug was boiled in 100 ml methanol for 1 hour followed by filtration. 1ml of filterate was placed in 10ml volumetric flask. 3ml methanol and 0.3 ml $NaNO_2$ were added in the flask. 3ml of $AlCI_3$ was added after 5min. 2ml of 1M NaOH was added and the net volume was made to 10 ml with methanol and absorbance was measured against a blank at 510nm. The total flavonoids content was calculated using following equation [18].

$$A = 0.01069c - 0.001163$$

A = absorbance, c = flavonoid content µg/g.

Heavy metal analysis

To 3 ml of the sample, 10 ml water, 2 ml Hydrochloric acid and 2 ml Nitric acid were added and boiled for 10 minutes. The mixture was cooled down and volume made up to 100 ml with water. 0.1N Nitric acid was used as blank. The samples were detected for presence of heavy metals like lead, copper, arsenic and mercury [19].

Statistical analysis

All the data obtained in the study has been expressed as Mean ±SEM.

RESULTS

All the formulations of Gokshuradi churna were evaluated as per WHO guidelines. In-house formulation was prepared as per procedure mentioned in Ayurveda Sar Sangrah. Botanical parameters revealed that the formulations were brown to dark brown in color, with pleasant odor and pungent taste (Table 2). The physicochemical comparisons of individual ingredients and in between in-house formulation and marketed formulations are given in Table 3 and 4 respectively. The results obtained with the market formulations and the in-house formulations were found to be comparable and variation was insignificant. The physical characteristics of the in-house formulation and market formulation (average values along with standard deviation) (n = 3) are shown in Table 5. The results of the market formulations and in-house formulation were found to be comparable. Total ash value for standard and in- house formulation are 18.5 ± 0.15 and 14.5± 0.16 respectively. The flowability of the formulation was found to be poor in both market formulations and in-house formulation, which was further confirmed by high values of Hausner's ratio(std.1.49±0.004 & In-house 1.51±0.003) and Carr's index (std.33.2±0.51 & In-house 32.1±0.67). The result obtained for phytochemical screening reveals that phytoconstituents like glycosides, carbohydrates, alkaloid and flavonoids were present in all samples (Table 6). Heavy metals may be present in crude drugs through atmospheric

pollution and through the soil. Moreover minerals and metals are also used in preparing Ayurvedic formulations.

Table 1 Ingredients of Gokshuradi churna

S.No.	Botanical name	Common name	Family	Part used
1	Tribulus terrestris	Gokhru	Zygophyllaceae	Fruit
2	Asparagus racemosus	Shatavari	Liliaceae	Root
3	Abutilon indicum	Atibala	Malvaceae	Root
4	Grewia hirsute	Nagbala	Tiliaceae	Root
5	Hygrophila spinosa	Talmkhana	Acanthaceae	Whole plant
6	Mucuna pruriens	Kaunch	Fabaceae	Seed

Table 2 Botanical parameters of various formulations of Gokshuradi churna

S. No.	Formulations	Appearance	Color	Taste	Odor
1.	In-house	Powder	Dark brown	Pungent	Pleasant
2.	Standard	Powder	Light brown	Pungent	Pleasant

Table 3 Physical and chemical evaluation of individual ingredients present in Gokshuradi churna

S. No.	Ingredients	Alcohol soluble extractive (%)	Water soluble extractive (%)	Total ash value (%)	Acid insoluble ash (%)	Water insoluble ash (%)
1.	*Asparagus racemosus*	30.63± 0.16	17.26± 0.11	9± 0.17	5± 0.02	8± 0.30
2.	*Tribulus terrestris*	12.37± 0.17	13.72± 0.17	6± 0.15	6± 0.01	4± 0.35
3.	*Hygrophila spinosa*	19.48± 0.18	20.78± 0.14	8± 0.17	8± 0.07	7± 0.28
4.	*Abutilon indicum*	7.36± 0.14	9.67± 0.15	7± 0.19	3± 0.03	7± 0.45
5.	*Grewia hirsute*	4.89± 0.16	8.39± 0.10	8± 0.14	2± 0.02	6± 0.32
6.	*Mucuna pruriens*	10.78± 0.17	6.84± 0.17	8± 0.18	6± 0.05	5± 0.28

Value are expressed in Mean ±SEM

Table 4 Physical and chemical evaluation of samples of Gokshuradi churna

S. No.	Parameters	Standard	In house
1	Total ash value (%w/w)	18.5± 0.15	14.5± 0.16
2	Acid insoluble ash value (%w/w)	1.3± 0.01	2.2± 0.04
3	Water soluble ash value (%w/w)	14± 0.26	12± 0.19
4	Alcohol soluble extractive value (%w/w)	30.4± 0.17	33.1± 0.15
5	Water soluble extractive value (%w/w)	33.5± 0.10	36.7± 0.15
6	Loss on drying (%w/w)	3.6± 0.06	4.8± 0.09
7	pH 1 % solution (%w/v)	6.9± 0.06	5.87± 0.08
8	pH 10% Solution (%w/v)	6.3± 0.08	5.6± 0.04
9	Crude fiber (gm)	0.19± 0.06	0.33± 0.09
10	Total flavonoid content	6.33± 0.016	8.43± 0.019

Values are expressed as Mean ±SEM (n=3)

However, heavy metals have been associated with various adverse effects [20] including status epilepticus, fatal infant encephalopathy, hepatotoxicity, congenital paralysis and deafness, and developmental delay. Many case studies have reported serious adverse conditions due to heavy metals in Ayurvedic and other herbal drugs [21]. Hence, heavy metals need to be detected in such preparations. In this study, all the samples tested negative for the presence of heavy metals lead, copper, arsenic and mercury, thereby further confirming the non toxic nature of the preparation.

Table 5 Physical characteristics of samples of Gokshuradi churna

S.No.	Parameters	Standard	In house
1	True density gm/cm^3	0.833± 0.014	0.625± 0.02
2	Bulk density gm/cm^3	0.555± 0.016	0.416± 0.015
3	Porosity %	86± 0.018	89± 0.016
4	Angle of Repose	50± 0.21	48± 0.29
5	Tapped density	0.845± 0.008	0.65± 0.003
6	Hausner's ratio	1.49± 0.004	1.51± 0.003
7	Carr's Index	33.2± 0.51	32.1± 0.67

Values are expressed as Mean ±SEM

Table 6 Phytochemical screening of Gokshuradi churna

S.No.	Phytoconstituents	In-house formulation	Standard
1	Carbohydrate	+	+
2	Flavonoids	+	+
3	Alkaloid	+	+
4	Glycoside	+	+
5	Steroid	-	-
6	Tannin	-	-
7	Amino acids	+	+
8	Mucilage	+	+
9	Gums	+	+

+ indicates presence ; - indicates absence

DISCUSSION

Gokshuradi churna is a safe polyherbal formulation containing many phytoconstituents. The churna shows poor flowability. The churna was evaluated based on different physical and chemical evaluation parameters for ingredients as well as both formulations. The formulations are free from any toxic material. The results obtained in this study may be considered as tools for assistance to the regulatory authorities, scientific organization and manufacturers for developing standards.

CONCLUSION

Ayurvedic medicine Gokshuradi Churna has been standardized by intervention of modern scientific quality control measures in the traditional preparation described in classical texts. Pharmacognostic characters established for the raw materials could be employed as Q.C. standards for evaluating its identity and can be used for routine analysis. Purity and potency of the materials and formulations following the procedure given could be performed in QC/QA laboratory of pharmaceutical house.

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