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Analytical standardization of *Shleshmantaka Agad*

Research Article

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Abstract

Background: To counter-act the action of *visa,(poison)* ancient *Acharyas* have mentioned the different types of *Agada* . Though Acharyas have mentioned various types of potent *Agadas* in various poisoning conditions, still these *Agadas* have neither undergone analytical study nor animal experiment. *Shleshmantaka Agad* is one of the drug remedy mentioned in *Shushrut Samhita, kalpa sthan* used for treatment of snake bite poisoning. Aim: In present work an attempt has been made to standardize *Shleshmantaka Agad.* Material & Methods: Physicochemical parameter was tested by Loss on drying, Acid insoluble Ash, Total ash value, Water & Alcohol soluble Extractive Value, HPTLC was used for phytochemical analysis. Result: In analytical study of *Shleshmantaka Agad* obtained value of Loss on drying, Total ash value, Acid insoluble Ash ,Water & Alcohol soluble Extractive Value are 12%, 10%, 2%, 13%, 1% respectively and in HPLC major constitute phytoamines was detected. Conclusion: Analytical findings of present study can be considered as reference standard for SA. As *Shleshmantaka Agada* is a combination of many phyto-constitutes but major constituent are phytoamines which may be possesses anti-snake venom property.

Key Words: *Shleshmantaka Agad*, Standardization, Physicochemical, Phyto-chemical.

Introduction

Sushrutsamhita is one of the famous compendiums of Ayurveda well focus on the aspect of toxicology in kalpasthana. To counter-act the action of *visa,* ancient *Acharyas* have mentioned *Agada*. These *Agada* are anti- poisonous remedy which is used in various types of animate and inanimate poisoning condition. In ancient text, some *Agada* have broad spectrum action which neutralize the effect of various animate and inanimate poisons. (1) Some *Agada* are target specific which act against particular poison or venom. '*Shleshmantaka Agad* (SA) is one of the formulation mentioned for treatment of snake bite poisoning.(2)It is also mentioned in AshtangHrudya in the name of *Tanduliyakadi Agad*. (3)Drugs or formulation are expected to exert a desired biological activity at particular concentration of their chemical constitution the overall aim of drug standardization is to ensure the quality and efficacy of product. (4) Hence standardization and development of reliable quality protocol is important. (5) Keeping in view this study has undertaken to develop the pharmaceutical standardization with three batches of formulation of SA. Organoleptic, physicochemical and phytochemical parameter were studied.

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Material and methods

Materials

Raw drugs were purchased from local market and Drugs identification and authentication done by Dravyaguna dept of M.G.A.C.H.RC, Wardha. Chemicals and solvents were procured E .Merck and S.D. fine chemicals, Mumbai for analysis of *Shleshmantaka Agad.*

Ingredients of SA depicted in (Table no 1).

Methodology

Preparation of *Shleshmantaka Agada*

Authenticated drugs were pulverized to powder and then sieved through 100 sieves. All the individual choornas were mixed together and Matoolung swarasa was added in it so that all the churnas get soaked and then it was dried for 24 hr and kept in air tight container. Three batches of SA was prepared.

Analysis of *Sheshmantaka Agada* : (6)

Total Ash

The 2gm sample was placed in a weighted dish and heated for 3 hours in a muffle furnace at 450°C. Continue heating was done until a constant weight was obtained. The dish was cooled in a desiccator and weighted. Then percentage of ash with reference to air dried sample was calculated as:

Total Ash = 100* (weight of Ash)/weight of sample obtained for test.

Table 1: Content of Sheshmantaka Agad

Sr no	Name of Drug	Botanical Name	Part Used	Quantity
1	Shleshmantak	*Cordia diacotoma* Forst	Bark	100gm
2	Tanduliyak	*Amaranthus Tricolor* Linn	Root	100gm
3	Katphal	*Myrica esculenta* Buch-Ham	Fruit	100gm
4	Shweta giriha	*Clitoria ternatea* Linn	Root	100gm
5	Kinihi	*Achyranthus aspera* Linn	Root	100gm
6	Matulung	*Citrus Medica* Linn	Fruit	200ml
7	Sita		Mishri	100gm

Acid Insoluble Ash

In a 100ml beaker, ash was mixed with 25ml weak hydrochloric acid and heated for a few minutes. After cooling it was then filtered through a 41-number Whattman filter paper and repeatedly rinsed with distilled water until it was chloride-free. The filter paper along with residue in a glass funnel was then placed in the oven for drying.

Later, the dry paper with residue were transferred to a pre-weighted crucible and heated to 600°C in a muffle furnace.

After cooling, it was weighed, and acid insoluble ash was computed using the weight of residue obtained.

Water soluble extractive

A 5gm coarsely powdered, air dried sample was transferred into a glass-stoppered, 250ml reflux conical flask, followed by 50ml of boiled water. The flask was shaken vigorously before being put aside for 10 minutes. It was filtered and cooled. The filtrate was transferred to a 7.5cm diameter evaporating dish, where the solvent was evaporated on a water bath, then allowed to dry for 30 minutes before being dried in an oven and the residue weighed.

Alcohol soluble extractive

In a closed flask, 5 gm. of dried samples was macerated with 100 ml of alcohol, shaking frequently for the first 6 hours, and then left to stand for 18 hours separately. Following that, it was quickly filtered in order to minimize methanol loss. In a tared flat bottom shallow dish dried at 105 0 C and weighted, evaporate 25ml of filtrate to dryness. With the air dried samples as a reference, the percentage of alcohol soluble extractive was calculated.

HPLC analysis:

a) Instrumentation

Chromatographic analysis was performed with Shimadzu Prominence HPLC instrument. It is equipped with quaternary pump, (LC20 -80) degasser (DGU-20As) column oven (CTO-10As) Autosample (SIL-20 AC) Diode-Array-Detector (UVSPD- M20). Phytochemicals were analysed with PrimeSIL C18 column (250*4.6mm. ID) and was purchased from local purchaser.

b) Chemicals

HPLC grade methanol and water were used for HPLC analysis. They were purchased from Merck (Mumbai MH.India), HPLC grade and ammonium acetate was purchased from Sigma-Aldrich (Mumbai MH,India). 0.2µ sample filters and 0.45µ nylone solvent filters were purchased from Milipore, India. Sample sonication was performed in Labman sonicator, purchased from Multilab, Ltd, Chennai, India.

Methodology

20 ul of freshly prepared stock solution of *Shelshmantak Agad* was injected to the C18 column (200* 46 mM) and eluted at the flow rate of 1.5 ml. min-1. Binary composition of methanol and water including ammonium acetate (50 Mm) were employed throughout the HPLC analysis. 254nm UV wavelength and ambient temperature were considered for achieving better peak shape and peak area.

Microbial Test

Sample was dissolved in Soybean casein digest medium prepared as per SOP of media preparation. Incubate at 30 to 35^0 for 18 to 24hours.after incubation sample was tested for Staphylococcus aureus, Pseudomonas aueruginosa, Salmonella, Ecoli.

Observation and Result

Table no 2: Quantity of ingredients and yield obtained in preparation of SA

Batch no.	Name of the drug	Quantity (gm)	Liquid media	Obtained quantity	% weight gain
1	*Shleshmantaka Agad*	100gm	Matoolunga Swaras	113gm	13%
2	*Shleshmantaka Agad*	100gm	Matoolunga Swaras	118gm	18%
3	*Shleshmantaka Agad*	100gm	Matoolunga Swaras	115gm	15%
Average		**100gm**		**115gm**	**15%**

Table no.3: Organo-leptic character of SA

Sr. no.	Organo-leptic character	Batch 1	Batch 2	Batch 3
1	Colour	Buff	Buff	Buff
2	Odour	Non-Characteristics	Non-Characteristics	Non-Characteristics
3	Taste	Sweet	Sweet	Sweet
4	Consistency	Hard	Hard	Hard

Table no. 4: Physicochemical property of SA

Sr no.	Test parameter	Batch 1	Batch 2	Batch 3
1	Loss on drying at 105^0C	12.5%	12%	12.2%
2	Total Ash Value	10%	10.2%	9.6%
3	Acid insoluble ash	2%	2.2%	2.4%
4	Water soluble extractive	13%	12.8%	13.4%
5	Alcohol soluble extractive	1%	1.5%	1.2%
6	pH	5.45	5	5.25

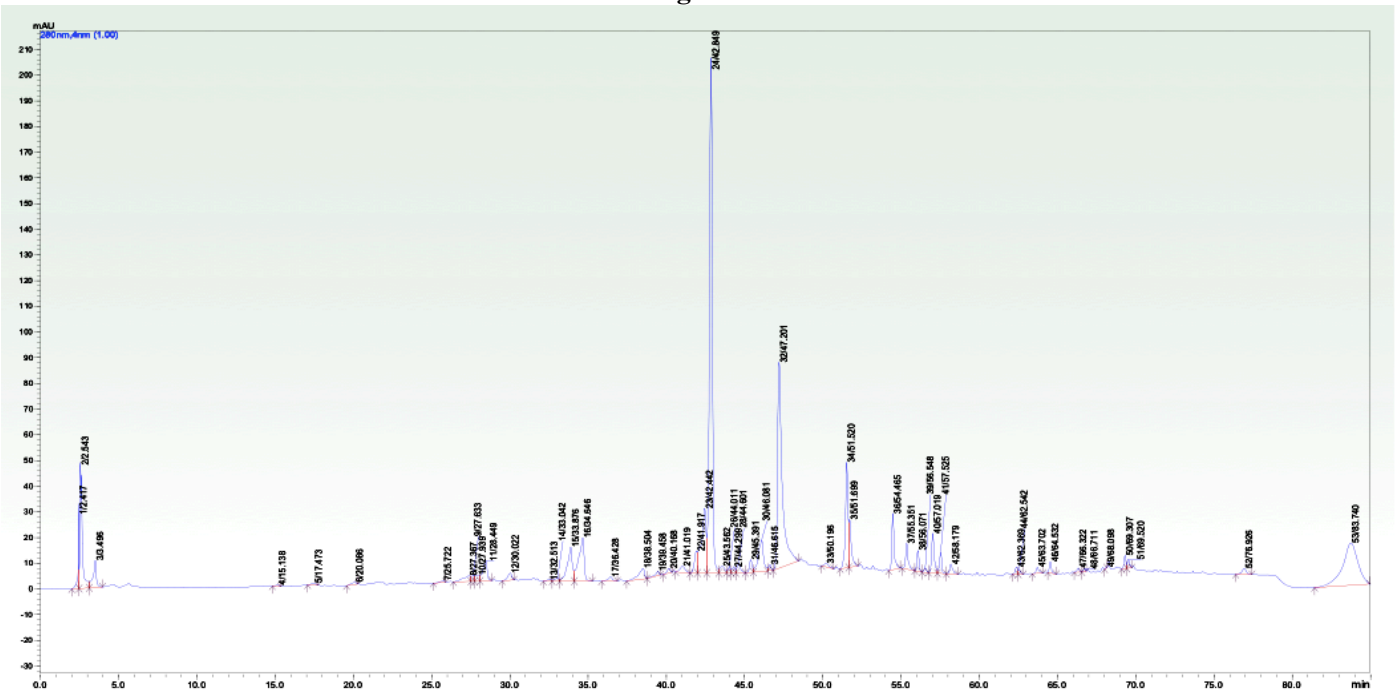
Table no 5: Microbial specification

Specification	Pharmacopeia standard	Batch 1	Batch 2	Batch 3
Total Viable count	Maximum 10^5/gm	No growth	No growth	No growth
Enterobacteriaceae	10^3/gm	Absent	Absent	Absent
Total fungus count	Maximum 10^3/gm	Absent	Absent	Absent
E- coli	Maximum 10^3/gm	Absent	Absent	Absent
Salmonella	None	Absent	Absent	Absent
Staphylococcus .aureus	Absent	Absent	Absent	Absent
Pseudomonas aueruginosa	Absent	Absent	Absent	Absent

Phyto-chemical analysis

After HPLC (High performance liquid chromatography) of SA phytoamine were detected at 27 minutes run time. Few polar flavonoids were detected. Among the pk-24 (42.84 min.); pk-32 (47.20) exhibits the maximum proportion of all separated compounds. Followed by them, few moderately polar/ organic polyphenols were identified between the run times of 50 to 60 minutes possibly which contains the sugar conjugated sterols like glycosides, interpreted between 51.52 – 58.17 min. run times.

Image no. 1



Discussion

SA is a poly-herbal formulation indicated for cobra and krait snake poisoning. After the preparation of formulation there was increase in weight 115 gm, it may be due to the addition of Matoolung swaras. Principle of SA preparation is a mixing of all the

ingredients with Matoolung Swarasa . While preparation of SA Bhavana is not mentioned, concept behind it may be the loss of constitute due to the friction. After studying physicochemical property it was buff in colour having Madhur rasa (sweet taste). Loss on drying of SA was Avg12% which is suggestive that compound is not likely to get contaminated by fungal growth. Avg Total Ash value of SA was 10% which was within prescribed limit (7, 8) Solubility test will indicate about the bioavailability and it was seen that water soluble extract 13% which was more than alcoholic extract 1%. As all the batches were prepared by taking required hygiene care and utilizing sterilized instruments, thus result of microbial content study showed absence of Enterobacteriaceae, fungus count, E-coli, Salmonella, Pseudomonas aueruginosa.

High performance liquid chromatography (HPLC) analysis of SA has been performed to identify the various class of components present in this formulation.HPLC of SA shows combination of phytoamines, moderately Antioxidents ,flavnoids/ polyphenols . Flavnoids are therapeutically proved as anti-inflammatory, antioxidant, anti-allergic and vaso-protective effects against snake venomm. (9). after the analytical study it was anticipated that the Phytoamines and flavanoids present in SA may of use in the management of snake bite.

Conclusion

Analytical findings of present study can be considered as reference standard for SA. It is a combination of many phyto-constitutes. But the percentage and the specific identification of these compound needs further detailed study so that it will be proved on experimental ground as an antisnake venom property.

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