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STUDY OF *PARAVATADI AGAD* WITH SPECIAL REFERENCE TO ITS ANALYTICAL STUDY

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ABSTRACT

Scorpion sting is an endemic public health problem in some part of the India. *Ayurveda* has explained numerous medicinal preparations for the management of *vrishchika damsha paravatadi agad* is one of them. *Paravatadi agad* comprised of *parawat shakrut, Tagar, Haritaki, Shunthi, Bijpure swaras*. Keeping above facts in mind it is aimed to standardize *Paravatadi agada* employing standard testing protocol for Ayush drugs physio - chemical studies like loss on drying at 105c, Total ash, Acid insoluble ash, water soluble ash, alcohol soluble extractive, water soluble extractive, HPTLC, were carried out as per WHO guidelines, Ayurvedic pharmacopoeia and Indian pharmacopoeia. Quality indicating physical and chemical test was done and standard values for *paravatadi agad* were recorded. Standardization test done on *dushivishari agada* helped in authenticating the polyherbal preparation also in ensuring quality of the same.

Keywords: Scorpion Sting, *Vrishchika damsha, Paravatadi Agad,*

INTRODUCTION

In Maharashtra, particularly in Vidarbha region, scorpion sting is a major health hazard especially, during paddy harvesting season immediately after rainy season leading to high mortality and morbidity rates.[1]There are around 1400 species of scorpions but only 46-50 of these are potentially lethal to humans. [2] Indian red Scorpion (Mesobuthus tamulus) belonging to Buthidae family is the most lethal amongst all the poisonous species of scorpions in India. Red scorpion venom causes massive release of neurotransmitters, which result in various clinical features symptoms such as profuse sweating, vomiting, increased salivation, bradycardia, cold extremities or transient hypertension followed by development of severe cardiovascular manifestations.[3] Morbidity and mortality are result due to acute refractory pulmonary edema, cardiogenic shock and multi-organ failure. In Scorpion bite cases, it is necessary to give immediate treatment but the patient cannot receive treatment immediately due to lack of transport facility and the Scorpion Antivenom used for Scorpion bite is not available easily in village. So the period between scorpion bite and actual

treatment increases. This may be one of the causes for poor prognosis in such circumstances. In *Ayurved* many *Agadas* are indicated for scorpion bite, but experimentally they are not proved. So now it is need of our science to introduce our medicines all over world with the help of research and experimental study. *Paravatadi Agada* is one of the drug remedy mentioned by *Vaghbhatachrya* in *Astang Hridayam*, [4]for the treatment of scorpion bite poisoning, but its efficacy has not been proved with the help of modern parameter. Present research is conducted to evaluate Physico-chemical and Phyto-chemical analysis *Paravatadi Agada*.

Aim: Study of the *Paravatadi Agad* on the basis of Physico-chemical and Phyto-chemical analysis

Objectives:

1. To assess the physico-chemical observations in *Paravatadi Agada*.
2. To assess the phyto-chemical observations in *Paravatadi Agada*.

Material and Methods

Study Design- It is an Analytical study.

Material

Collection of the drugs

Ingredients of Paravtdi Agada were collected from different regions. The samples were collected from Nagpur, Wardha, Kolhapur.

Identification and authentication of study material:-

The raw drugs were identified and authenticated from Dravyaguna department, Mahatma Gandhi Ayurveda College Hospital and Research Centre, Salod (H), Wardha.

Method

Place of Drug preparation:- Drug (*Paravatadi Agada*) is prepared in Dattatraya Rasshala MGACH & RC Salod (H), wardha.

Table 1: Ingredients of *Paravatadi Agada*:-

Sr. no.	Sample	Useful part	Quantity
1.	*Parawat shakrut* (fecal matter of pegion)	fecal matter of pegion	150gm
2.	*Tagar* (*veleriana wallichi*)	Root	150gm
3.	*Haritaki* (*terminallia Chebula*)	Fruit	150gm
4.	*Vishwabhaishajyam* (*zingiber officinale*)	Root (rhizome)	150gm
5.	*Bijpure Swaras* (Juice of Citrus Medica)	Fruit	1litre

Procedure:

Preparation of drug:-

- Ingredients number 1 to 4 were med into fine powder and sieved through 80 number mesh.
- *Bijapurak swarasa* was extracted from *Bijapurak* fruits.
- Powdered ingredients were taken in iron *Khalva Yanta* and *Bhavana* of *Bijapuraka swarasa* was given till the obtaining uniform homogenous mixture.

Standardization of drug:-

1. Total Ash

Sample was taken in a weighted dish and was strongly heated in a muffle furnace at 550°C to 575°C for 3 hours. Continued heating was done until a constant weight was obtained.[5]

2. Acid insoluble Ash

Ash was taken with 25 ml dilute hydrochloric acid in a beaker of 100 ml capacity and boiled for few minutes and cooled. Then it was filtered through 41 number Whattman filter paper and washed with distilled water repeatedly till it becomes chloride free. Then the filter paper along with residue in a glass funnel was kept for drying in the oven.

Later that dried paper along with the residue was shifted to pre-weighed crucible and kept in muffle furnace and heated upto 600°C. After cooling it was weighed and from the weight of residue obtained, acid insoluble ash was calculated.[6]

3. Water soluble Ash

Boil the total ash for 5 minutes with 25 ml of water; collect the insoluble matter in a Gooch crucible or on

an ash less filter paper, wash with hot water, and ignite to constant weight at a low temperature. Subtract the weight of insoluble matter from the weight of the ash; the difference in weight represents the water- soluble ash. Calculate the percentage of water- soluble ash with reference to the air dried drug.[7]

4. PH value

The acidic or basic property of substances is measured in terms of pH. It is a measurement of the hydrogen ion concentration. pH is defined as the negative logarithm (base 10) of hydrogen ion concentration.

5. Water soluble extractive

About 5 g of accurately weighed coarsely powdered, air dried sample was transferred into a glass-stoppered, 250 mL reflux conical flask, followed by the addition of 50 mL of boiled water. The flask was well shaken, and allowed to stand for 10 minutes. It was cooled and filtered. Filtrate was transferred to an evaporating dish, which was 7.5 cm in diameter; the solvent was evaporated on water bath, allowed to dry for 30 minutes, finally dried in an oven and residue was weighed. Percentage of water-soluble extractives was calculated with reference to the air-dried drug.[8]

6. Alcohol soluble extractive

5 g of dried samples was macerated with 100 mL of alcohol in a closed flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution to minimize the loss of methanol. Evaporated 25 mL of filtrate to dryness in a tared flat bottom shallow dish dried at 105ºC and weighed. Percentage of Alcohol soluble extractive was calculated with reference to the air dried samples.[9]

7. Menthol soluble extractive

5 g of dried samples was macerated with 100 mL of menthol (40-60oC) in a conical flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution against loss of Menthol. Evaporated 25 mL of filtrate to dryness in a tared flat bottom shallow dish dried at 105ºC and weighed. Percentage of pet ether soluble extractive was calculated with reference to the air-dried samples.

8. HPTLC

Advanced and sophisticated, optimized, instrument based and more or less automated technique in comparison to simple chromatography that gives more reproducible and high resolution separations.

Principle of HPTLC

Principle remains the same as of TLC i.e. adsorption. One or more compounds are spotted in a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action (against gravitational force).

The components with more affinity towards stationary phase travels slower while those with lower affinity, moves faster. Thus the components are separated on a thin layer chromatographic plate based on the relative affinity of the components towards the stationary phase and mobile phase.

9. **HPLC analysis Experimental**

20 µl of freshly prepared stock solution of paravatadi-agada and paravat-shakrut were injected separately to the C18 column and eluted at the flow rate of 1.5 $ml.min^{-1}$. Binary composition of methanol and water including additives such as trifluoroacetic acid and ammonium formate were employed throughout analysis. 254 nm UV wavelength and ambient temperature was considered for demonstrating better peak shape and peak area.

OBSERVATION AND RESULTS:

Analysis of *Paravatadi Agada*

Quantity of sample taken –100gms

Table 2: Analytical observations of *Paravatadi Agada*

Parameters	Observations
Macroscopic / Organoleptic	
Color	Brown
Odor	Irritant odor
Physiochemical	
Loss on drying at 105^0 C	3.9%
Total ash	12%
Water soluble ash	4.5%
Acid Insoluble ash	4%
Ethanol-soluble extractive	5%
Methanol-soluble extractive	12.5
Water –Soluble extractive	26%
pH	5.44

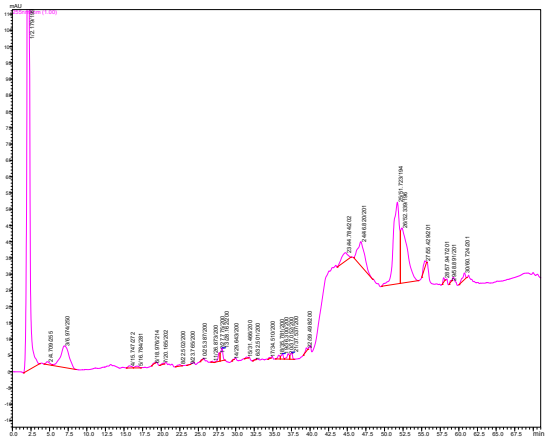

Fig. 1: HPLC of *Paravatadi Agada*

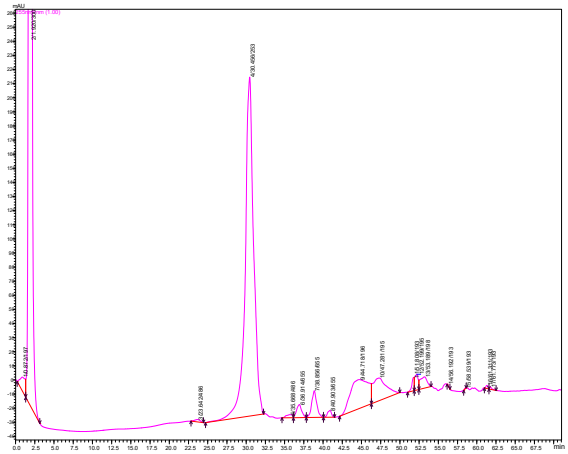

Fig. 2: HPLC of *Paravatadi Shakruta*

DISCUSSION

Paravatadi Agad is one among such formulations mentioned in *Ashtanga Sangraha*. Therefore to standardize this formulation present study has been conducted which includes physico-chemical and phytochemical analysis of *Paravatadi Agad*.

All ingredients of *Paravatadi Agada* were authenticated after procurement and then utilized for *Paravatadi Agada* preparation. As the quantity of ingredients is not mentioned in reference, all ingredients were taken in equal quantity according to principle of *Anukta mana grahan*. *Bhavana* of *Bijapuraka nimbu* was given by using edge runner as it facilitate for uniform grinding with uniform force and pressure. Both force and pressure are important for friction and homogenous mixing of constitutes of ingredients. Moreover using modern instrument for *Bhavana* facilitates large scale production in future use. Obtained quantity of *Paravatadi Agada* is slightly higher than total weight of ingredients due to addition of few constituted from *Bijapuraka Swarasa Bhavana*.

The residue remaining left after incineration of the crude drug is designated as ash. The residue obtained usually represents the inorganic salts naturally occurring in the drug and adhering to it. It varies with in definite limits according to the soils. It may also include inorganic matter deliberately added for the purpose of adulteration. Hence, an ash value determination furnishes the basis for judging the identity and cleanliness of any drug and gives information relative to its adulteration/contamination with inorganic matter, thus ash values are helpful in determining the quality and purity of drug. The total ash of a crude drug also reflects the care taken in its preparation. The acid insoluble ash is a part of the total ash that is insoluble in dilute hydrochloric acid. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is higher. Total ash values of *Shunthi*, *Tagar* and *Haritaki* are observed within limit prescribed in API. However such limit is not mentioned for *Paravatadi Shakrut* and the observed total ash value of this sample is considered as standard. *Paravatadi Shakrut* is an organic material which contain few minerals hence its total ash is higher than herbal drugs. The same reason is applicable to total ash value of *Paravatadi agad* as it contain *Paravatadi Shakrut* as a main ingredient.

Loss on drying at 105°C indicates presence of moisture content. If moisture content is more than permissible limit then the formulation is more likely to get infected by fungal growth. Moreover unwanted changes can also occur due to presence of more moisture. In the prepared batches moisture content is much less i.e. this formulation has more stability. All samples showed loss on drying values within standard limit which indicates all samples were authentic.

Acid insoluble ash represents presence of inorganic content which is not expected in herbal formulation. The obtained value of Acid insoluble ash in all the batches is also within considerable range which con-

firms the raw materials were standard. The physical parameter such as pH was determined to determine basic nature of sample as more or less value of pH is responsible for irritation of skin. *Paravatadi shakruta* has maximum pH value i.e. 7.13 which is basic in nature. *Paravatadi Agada* is the final product and its pH value matters most. Observed pH of *Paravatadi Agada* is 5.44 which is acceptable for its therapeutic utilization.

As all the batches were prepared by taking required hygienic care and utilizing sterilized instruments, thus result of microbial content study showed absence *of Enterobacteriaceae, fungus count, E-coli, Salmonella*, *Staphyliococus aureus* and *Psudomonas aueruginosa*. The pharmaceutical standards for *Paravatadi Agada* are not available hence the analytical results of present study may prove exposure towards establishing analytical standards for *Paravatadi Agada.*

In HPTLC analysis, after comparative studies performed for *Paravatadi-agada* and *Paravat-shakrut*, different chemical composition and nature of active principles were observed. Initially, the separation was carried out for *Paravatadi-shakrut* including TFA as mobile phase additives. It was displayed that most of its constituents were eluted with the solvent front, followed by emerge of small peak. This small peak could be the acid strength or very polar fraction of *Paravat-shakrut*. Furthermore, very small fragments were observed upto retention time 40 minutes. It represents very minute quantity of basic nitrogenous compounds as well as polyphenols like antioxidants present in the sample of *Paravat-shakrut*. Between 40 to 60 minutes run time many fraction were resolved. Presumably, all these fragments might be the neutral polar steroids or alternatively hydrophobic steroids which get metabolized into polar fragments by conjugation with polar fragments. Above all, except acid and polar steroidal constituents, no other ionic and phenolic molecules were detected.

Subsequently, keeping the same separation parameters, the fresh sample of *Paravatadi-agada* was analyzed (Fig. 1). Similar to the *Paravatadi-shukrut*, first initial peak represents the un-adsorbed fragments in C18 column (Fig. 2). Surprisingly, no acid compound was detected in *Paravatadi-agada*. Importantly, compared with *Paravatadi-shakrut*, one fraction of basic ionizable constituents was detected and its concentration was maximum among all separated fractions. This basic compound was followed with many other polyphenols; however, their concentration is quite low. Even they were not resolved properly and their peak shapes were observed to be erratic and irreproducible.

In spite of these, collectively, the chemical composition of *Paravatadi-Agada* is quite different than that of *Paravatadi-Shakrut* and hence they might have different biological activities on human body.

CONCLUSION

Authentication of raw drugs and standard preparation of *Paravatadi Agada* is most important as it is indicated scorpion bite which is sever condition, however standard parameters for *Paravat Shakrut* are not found established. Hence the analytical result of *Paravatadi Shakrut* obtained in this study can be considered for future research. *Paravatadi Agada* prepared by following standard operating procedure should have Loss on drying at 105^{0} C, Total ash, Water Insoluble ash, Acid insoluble ash, Ethanol-soluble extractive, Methanol-soluble extractive, Water –Soluble extractive and pH value 3.9%, 12%, 4.5%, 4%, 5%, 12.5, 26% and 5.44 respectively. Present study has established analytical specification of *Paravatadi Agada* and filled a gap between ancient and modern knowledge of *Paravatadi Agada*. However to access the efficacy of this formulation, experimental studies on in-vivo, in-vitro and clinical grounds are necessary.

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