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INTERNATIONAL AYURVEDA PUBLICATIONS

RESEARCH ARTICLE *ISSN 2456-0170*

PHYTOCHEMICAL EVALUATION OF *MAHA AGADA*

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ABSTRACT

Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles, physical and chemical standards. Standardization of the poly herbal formulation is possible by following modern scientific quality control procedure both for raw material and the finished product. The phytochemical constituents found to be present in the raw material used for the preparation of "Maha Agada" possibly facilitate the desirable therapeutic efficacy of standardized medicinal formulation as a whole, and also could help in knowing the underlying mechanisms of the pharmacological action. The article reports on standardization of polyherbal formulation used to the snake bite, scorpion bite, spider, rodent and insect bite etc. They were also examined for evaluation of phytochemical parameters and their quality control standards.

KEYWORDS: Phytochemical, Standardization, Herbal, *MahaAgada.*

INTRODUCTION

Acharay Charaka mentioned *Agada* in twenty four Upakram. *Agada*s are the anti-poisonous remedies which are used in various types of poisoning indicated in the snake bite, scorpion bite, spider, rodent and insect bite etc. According to them *Agadas* have broad spectrum action and nullify the effect of various type of poison. Various *Agada* are mentioned in classic for different poisoning cases along with it can be prescribed in the condition other than poisonous incidence.

Maha Agada is one of type of *Agada*. *Maha Agada* is mentioned in *Sushurta Samhita, kalpsthan*[1] and *Astang Sangraha, uttartantra*[2]. It is a poly herbal combination indicated in treatment of snake, scorpion, rats and spider bite. So this poly herbal formulation can be very useful in poisoning cases. But, the most important challenges faced by these formulations arise because of their lack of complete evaluation. So evaluation is necessary to ensure quality and purity of the herbal product. The process of evaluating the quality and purity of crude drugs by means of various parameters is called standardization.[3] Standardization is an essential factor for polyherbal formulation in order to assess the quality of the drugs based on the concentration of their active principle. It is very important to establish a system of standardization for every plant medicine in the market.

AIM & OBJECTIVES

Aim: Phytochemical analysis of *Maha agad*

Objectives: To detect presence of alkaloids, flavonoids and steroids (phytosterols) as constituents in *Maha Agad.*

MATERIAL AND METHODS

To obtain good quality of final finished product, it is essential to develop reliable specific and sensitive quality control methods using a combination of classical and modern instrumental method of analysis. The "evaluation" of the drug needs to be validated by its identification and determination to ascertain its quality and accuracy and its pattern of adulteration.

1. COLLECTION OF RAW DRUG:

Ingredients of *Maha Agad* were collected from Dattatraya Rasashala Wardha and local Market Nagpur.

Table No. 1: Ingredients of *Maha Agad*[4]

Sr. No.	Ingredients	Useful parts	Quantity
1	*Trivrt*	Root	50g
2	*Langali*	Root	50g
3	*Yastimadhu*	Stem	50g
4	*Daruharidra*	Root, Stem	50g
5	*Haridra*	Rhizome	50g
6	*Manjistha*	Root	50g
7	*Shunthi*	Rhizome	50g
8	*Marich*	Fruit	50g
9	*Pippali*	Fruit	50g
10	*Saindhav lavana*	---	50g
11	*Samudra lavana*	---	50g
12	*Sauvarchal lavana*	---	50g
13	*Audbhid Lavana*	---	50g
14	*Vid Lavana*	---	50g

2. IDENTIFICATION AND AUTHENTICATION OF STUDY MATERIAL

The herbal drugs were identified and authenticated from Dravyaguna department and panchalavana was identified and authenticated from Rasashastra department, Mahatma Gandhi Ayurveda College Hospital and Research Center Salod (H), Wardha.

3. PREPARATION OF *MAHA AGAD*[5]

- Individual ingredients were made into fine powder in khalvayantra.
- The fine powder was mixed together in equal quantity.
- This mixture was then triturated with 100ml of Ajamutra.
- This mixture was then allowed to dry.
- After drying it was kept for analytical test.

4. ANALYTICAL STUDY

Analysis of *Maha Agada* was done by using following parameters.

4.1 PHYSICOCHEMICAL ANALYSIS

A. Total Ash[6]

Sample was taken in a weighted dish and was strongly heated in a muffle furnace at 550ºC to 575ºC for 3 hours. Continued heating was done until a constant weight was obtained. The dish was cooled in desiccators and weighted. Percent of total ash with reference to air dried sample was calculated as-

Total Ash = [100 × (weight of ash)] / weight of sample taken for test

B. Acid Insoluble Ash[7]

The acid insoluble ash content test was conducted to access the percentage of inorganic content of the sample which is insoluble in dilute acid.

Procedure:

Ash was taken with 25ml dilute hydrochloric acid in a beaker of 100ml capacity and boiled for few minutes and cooled. Then it was filtered through 41 numbers 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- weighted crucible and kept in muffle furnace and heated upto 600ºC. After cooling, it was weight and from the weight of residue obtained, acid insoluble ash calculated.

A. Water soluble extractive[8]

About 5gm of accurately weighted coarsely powdered, air dried sample was transferred into a glass-stoppered, 250ml reflux conical flask, followed by the addition of 50ml 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 7.5cm in diameter, the solvent was evaporated on water bath, allowed to dry for 30 minutes, finally dried in an oven and residue was weighted. Percentage of water-soluble extractives was calculated with reference to the air-dried drug.

B. Alcohol soluble extractive

5gm of dried sample was macerated with 100ml 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 25ml of filtrate to dryness in a tared flat bottom shallow dish dried at 105°C and weighted. Percentage of alcohol soluble extractive was calculated with reference to the air dried samples.

4.2 PHYTO CHEMICAL ANALYSIS

HPLC Analysis Procedure

20 µl freshly prepared stock solution of *Maha Agad* was injected directly to the C18 column (250 x 4.6 mm id) and eluted at the flow rate of 1.5 ml. min^{-1}. Binary composition of methanol and water including ammonium acetate as additives was used throughout HPLC analysis. The separation was carried out for 125 minute run time to elute all components from *Maha-Agada*. For this HPLC separation, 254nm UV wavelength and 25°C temperature were considered for getting good peak shape and peak area. Furthermore, the same HPLC separation technique was utilised for class separation studies. In this class separation, only acid-base strength components were isolated from whole mixture of *Maha-Agada*. Similarly, the phenol class of compounds like polyphenols, flavonoids or antioxidants were separated from a whole mixture of *Maha-Agada*. Likewise, only phytosterols and tocopherols were isolated from a whole mixture of *Maha-Agada*. In class separation, isocratic elution mode at 245 nm wavelength, at 24ºC was considered.

RESULTS AND OBSERVATION

Table No. 2: Analytical observation of *Maha Agada*

Parameters	Observation
Organoleptic	
Colour	Pale Brown
Odour	Characterstic
Taste	Tikta-Katu
Physicochemical	
Loss on drying at 105°C	10.0%
Total Ash value	18.0%
Acid insoluble ash	11.0%
Water soluble extractive	13%
Alcohol soluble extractive	4.0%
pH	7.6%
Microbiological specifications	
Total viable count	Absent
Enterobacteriaceae	Absent
Total fungus count	Absent
E-coli	Absent
Salmonella	Absent
Staphylococcus aureus	Absent
Pseudomonas aueruginosa	Absent

Result of HPLC: All components were well separated and distinguished into independent classes. There might be few other important Phytoamines/Polyphenols which were overlapped at 30-35 min interval. One important aliphatic phytoamine was characterised at 14.79 min (figure no.2). There might be the chances of presence of phytoster. Further expansion of chromatogram, resulted there are three components either overlapped or disintegrated into three multiple peaks. Including it, there are 2-3 more alkaloids were identified. Many important polyphenols like flavonoids, Steroids, Tocopherols were detected (figure no.3). This separation was carried out at 254 nm. HPLC analysis for Maha Agad sample was performed for the determination of Acid-Base compounds. Exactly 5 ionisable compounds from the whole mixture of Maha Agad sample were separated. Possibly, the fractions pk 9, 10, 11, and 12 separated at RT values 30.21, 31.59, 37.86, 39.53 min (figure no.4) respectively are the aliphatic amines. Exactly 11 polyphenols from the whole mixture of Maha Agad sample. Were detected; where maximum concentration of fraction 9; RT Value 12.199 was detected (figure no.5). Along with these components, there are more other potential neutral but moderately more lipophilic polyphenols were observed. HPLC analysis for Maha Agada sample was performed for the determination of Polyphenols like flavonoids and antioxidants.

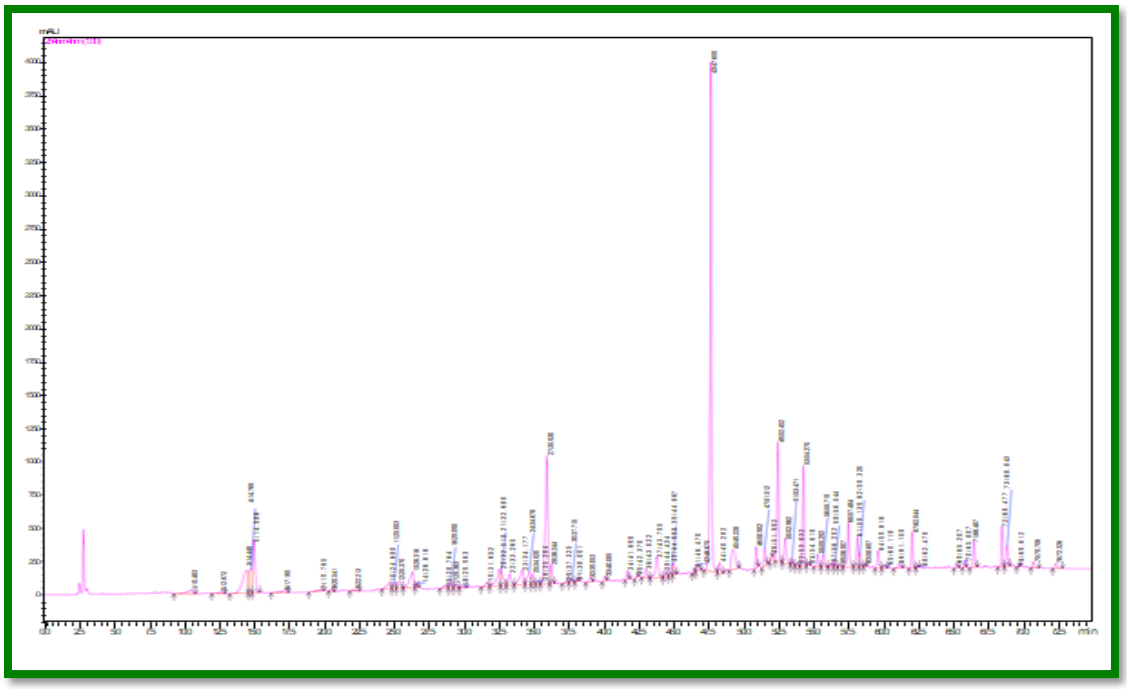
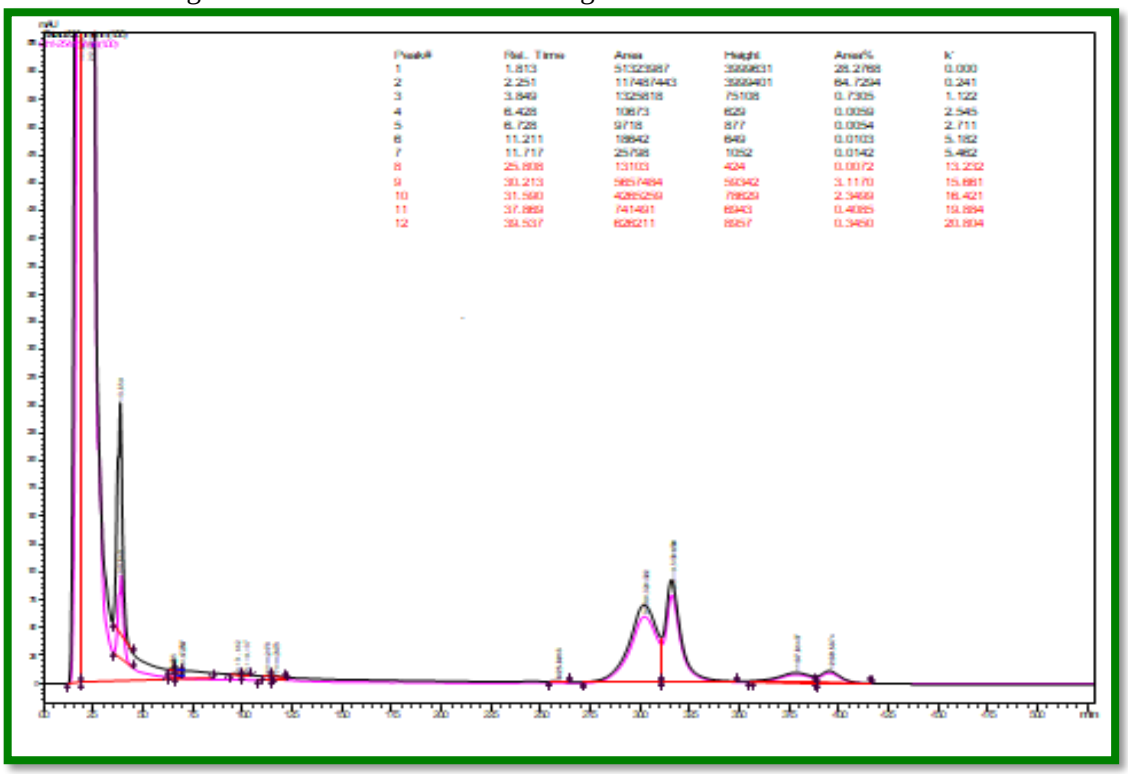


Figure No. 1. RP-HPLC of Maha Agada in run time 72 min



Peak#	Ret. Time	Area	Height	Area%	k'
1	1.813	51322587	3999831	28.2788	0.000
2	2.251	117487443	3999401	64.7294	0.241
3	3.849	13258518	75108	0.7305	1.122
4	6.428	108673	829	0.0059	2.545
5	6.728	9718	877	0.0054	2.711
6	11.211	18842	849	0.0103	5.182
7	11.717	25758	1052	0.0142	5.462
8	25.808	131103	424	0.0072	13.232
9	30.213	5657484	56342	3.1170	15.881
10	31.560	4286256	78829	2.3469	16.421
11	37.889	741491	8943	0.4085	19.894
12	39.537	626211	8857	0.3450	20.804

Figure No. 2: class separation of alkaloids in whole time

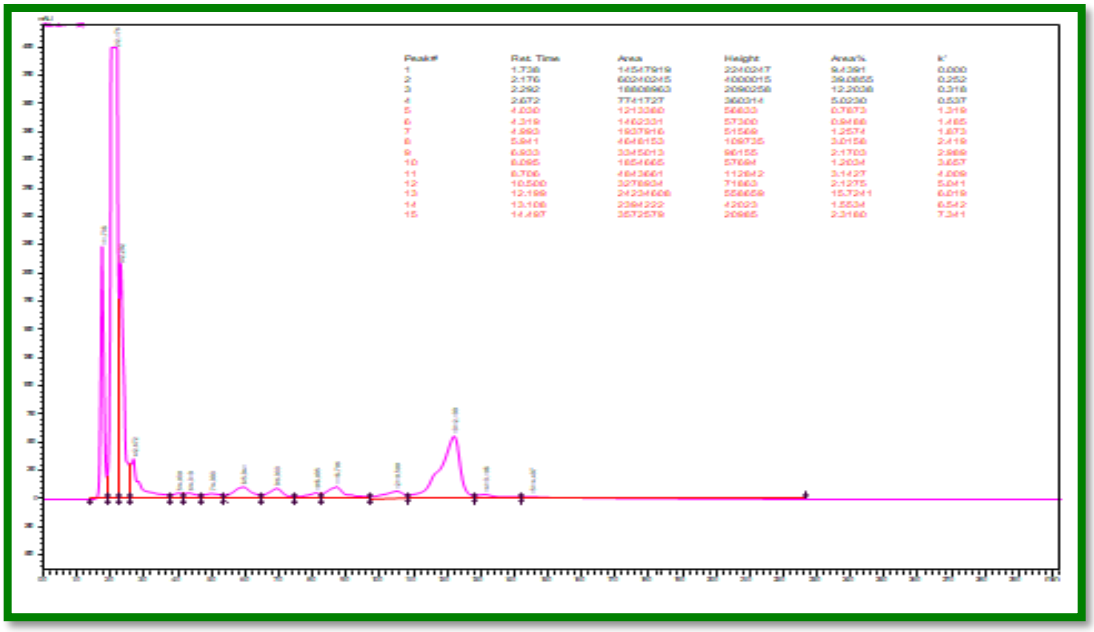


Figure 3: class separation of poly phenol

DISCUSSION

HPLC analysis of *Maha agada* indicates that it is combination of aliphatic phytoamine, few other important Phytoamines / Polyphenols, potential neutral but moderately more lipophilic polyphenols, possibility of presence of phytosterols, are 2-3 more alkaloids and presence of flavonoids, Steroids, Tocopherols. Few antioxidants are also detected in *Maga Agada.* It is reported that polyphenols, especially flavonoids and tannins of plants are the key phytochemicals effective against several snake venoms. The protective effects of polyphenols including tannic acids have been already established against the toxicity of snake. That the binding ability of polyphenols with the venom led to the precipitation of the venom protein, thus inhibiting the effects of venom on Polyphenols is a major source of antioxidants consumed by human. Polyphenols possess not only antioxidant properties but also antiviral, antibacterial, anti-inflammatory and anti-carciogenic effects. A polyphenol extract was found to have an inhibitory effect on cholera toxin induced diarrhea. So this basis Maha Agada is effective on its indicated disease. Many phyto-constitutes are observed in peaks but they were identified due to lack of information of standards. It is evident that *Maha Agada* is combination of poly-herbal and multi mineral (*Pancha Lavana*) components thus identifying all constitute is much more hence would need further investigation on independent component through require higher analytical methods**.** Therefore focus was given on establishing primary analytical parameters which are common and mostly applied for analysis of ASU drugs.

CONCLUSION

Standard parameters for Physico-chemical analysis of *Maha Agada* should have Loss on drying at 105ºC, Total Ash value, Acid insoluble ash, Water soluble extractive, Alcohol soluble extractive and pH values in

the range 10.0%, 18.0%, 11.0%, 13%, 4.0% and 7.6% respectively. *Maha Agada* is combination of phyto-constitutes such as aliphatic phytoamine, few other important Phytoamines / Polyphenols, potential neutral but moderately more lipophilic polyphenols, possibility of presence of phytosterols, are 2-3 more alkaloids and presence of flavonoids, Steroids, Tocopherols and Antioxidents.

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Source of support: Nil,
Conflict of interest: None Declared

Cite this article as
Kamlesh Soanare : Phytochemical Evaluation of Maha Agada; ayurpub; IV(4): 1279-1285