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An impact of Mardana- A comperative study on *Arogyavardhini Rasa*

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ABSTRACT

Background: *Arogyavardhini Rasa* (AVR); a Kharaliya Rasakalpa containing rasavarga dravya (metallomineral ingredients) is widely used Ayurvedic herbo-mineral formulation having broad spectrum therapeutic indications. Current research is first effort to scientifically document quality control parameters of this important formulation. **Aim:** To establish a comparative pharmaceutico - analytical profile of AVR prepared with and without its metallomineral ingredients. **Materials & Methods:** *Arogyavardhini Rasa* (AVR) and modified *Arogyavardhini Rasa* (MAVR) without addition of its metallomineral ingredients were prepared and investigated for Physico-chemical parameters, qualitative tests for functional groups, Chromatography, quantitative elemental analysis by ICP-AES. **Results & Discussion:** An average of 2500ml *Swarasa* was required for optimum *Mardana* in both formulations. HPTLC study revealed a total of 11 and 8 bands at 254nm and 366nm in each AVR and MAVR with only two similar Rf values. Samples AVR, MAVR and MAVR with addition of Rasa Dhatukajjali showed 1.5226, 0.0117 and 1.703 % of mercury respectively. **Conclusion:** Mardana play significant role in development of Kharaliya Rasakalpa Both the formulations showed different through physicochemical profiles and ICP-AES analysis. Differences in chromatographic fingerprinting of AVR and MAVR are suggestive of changes in chemical constituents of the formulation due to addition of metallo minerals.

Keywords: *Arogyavardhini Rasa*, *Bhasma*, HPTLC, Heavy metal, Organometallic, *Rasaushadhi*, Mercurials.

INTRODUCTION

Rasaushadhi (formulations containing metallo minerals) are important formulations in Ayurvedic therapeutics due to lesser therapeutic doses, enhancement of action of other ingredients of formulation, quicker action and palatability[1] and more shelf life[2] as compared to formulations prepared from drugs of plant or animal origin. *Kharaliya kalpa* are formulations prepared by the process of *Mardana* (Triturition) or *Bhavana* (levigation) in mortar and pestle. Even traces of these *Rasavarga dravya* when processed with drugs of organic origin especially by means of *Bhavana* in *Kharaliya Rasakalpa* leads to enhancement of stability of the formulation as an effect of *Samskara*; classical pharmaceutical processing leading to transformation of the inherent attributes of a substance, which leads to addition of new properties or qualitative improvement[3]. *Rasavarga Dravya* undergoes repeated *Samskara* (*Shodhana, Marana, Bhavana, Mardana* etc), which bring about changes in their characteristics and enable them safe and therapeutically effective. Although *Rasaushadhi* are used in different disease conditions since centuries without developing any noticeable side effects despite of this, concerns are being expressed on safety of traditional preparations containing *Rasavarga dravya*.

Arogyavardhini Rasa is widely practiced *Kharaliya Rasaushadhi* used in the management of *Jvara* (fever), *Kushtha* (all types of skin disorders), *Medoroga* (obesity) and other *Yakrit vikara* (liver disorders). It has been described in 13th century[4] and included in Ayurvedic Formulary of India[5]. Although Acute, subacute and Chronic toxicity studies[6,7] have been conducted, and Hepatoprotective activity has been proven[8]. In view of global acceptance, it is advisable to study the formulation without addition of metallo mineral ingredients. Although method of estimation of tannin, kutkoside and steroid in *Arogyavardhini vati* has been developed[9] still it is needed to develop its analytical profile.

The process of *bhavana* / *mardana* of *rasaushadhi* with ingredients of plant or animal origin facilitates conversion of *Nirendriya dravya* (inorganic material) to *Sendriya dravya* (Organo-metallic/Organo-mineral compound) [10] i.e. changing its Bio assimilation and thus forms basis of newer target organ drug delivery system. Hence, *Rasavarga Dravya* has significant role in *Rasaushadhi* formulation but simultaneously hamper their global acceptance as well. Hence, in present research work, pharmaceutical and analytical profiles of *Arogyavardhani Rasa* (AVR) and *Arogyavardhani Rasa* without *Rasavarga dravya* were developed to evaluate role of ingredients of metallo mineral origin.

MATERIAL AND METHODS

Collection and authentication of raw materials

Fresh Roots of *Chitraka* and leaves of *Nimba* were collected from botanical garden of University and other raw materials were procured from Pharmacy (Table 1). All herbal raw drugs were authenticated at Pharmacognosy laboratory.

Processing of raw materials

Shodhana of *Guggulu* was carried out by *Swedana* method using *Triphala Kwatha*[11]. Herbal ingredients were powdered by Grinding and sieving and passed through sieve no # 80[12]. Fresh *Swarasa* of *Nimba Patra* was prepared *by* grinding and squeezing[13]. *Rasa Dhatukajjali* (*Kajjali*, *Loha Bhasma*, *Abhraka Bhasma* and *Tamra Bhasma*) was prepared by sequential *Mardana* (trituration) [14].

Preparation of *Arogyavardhini Rasa* (AVR)

All the ingredients (Table-1) were accurately weighed. Rasa *Dhataukajjali* was Mixed thoroughly along with other powdered ingredients in polythene bag till homogeneous mixing. It was then added little by little to a butterfly wet - grinder containing mixture of *Guggulu*, *Shilajatu* and *Swarasa.* Required quantity of *Nimba patra Swarasa* for optimum levigation was added till the mixture gets immersed completely. Approximately 200 ml *Swarasa* was added 4 hrly. Grinding and soaking was carried out for 12hr */d for 2 days. Final material was subjected for complete drying in an oven bellow 70^0C and was powdered, weighed, packaged, labeled and stored in a glass container. Three batches of AVR were prepared (Fig.1).

Figure 1: (a) Powder preparation in multimill (b) Raw materials (c) Butterfly wet grinder (d) *Nimba patra swarasa* in wet grinder (e) Wet grinding process (f) Spread blend for drying (g) Dried *Arogyavardhini* (h) finished product powder of AVR and MAVR in glass bottle.

Table 1: Ingredients used for the Preparation Formulation

S. No.	Name of the Ingredients	Latin/ English Name (AVR)	MAVR	Part / Type use	Prportion
1.	*Shuddha Parada*	Processed Mercury	-	-	1Part
2.	*Shuddha. Gandhaka*	Processed Sulphur	-	-	1Part
3.	*Lauha Bhasma*	Calcinated Iron	-	-	1Part
4.	*Abharaka Bhasma*	Calcinated Mica	-	-	1Part
5.	*Tamra Bhasma*	Calcinated Copper	-	-	1Part
6.	*Triphala Churna* *Haritaki* *Bibhitaka* *Amalaki*	 *Terminalia chebula Retz* *Terminalia bellirica (Gaertn.) Roxb* *Phyllanthus emblica L*	+	 Dried Fruit Rind Dried Fruit Rind Dried Fruit Rind	 2*3 part
7	*Shuddha.Shilajatu*	Processed Black Bitumen	+	Processed product	3part
8	*Shuddha. Guggulu*	Processed *Commiphora wightii* (Arn.) Bhandari	+	Processed *Niryasa* (Resinous gum)	4part
9	*Chitraka Moola*	*Plumbago zeylanica linn*	+	Dried Root	4part
10	*Katuki Moola*	*Picrorhiza kurroa* Royle ex Benth	+	Dried Rhizome	22part
11	*NimbaPatra Swarasa*	*Azadirachta indica A. Juss*	+	Leaf Juice (for 2 days *Mardana* (wet Lavigation))	Q. S

(-) Not present (+) Present

Preparation of Modified *Arogyavardhini Rasa* (MAVR)

Arogyavardhini rasa without addition of *Rasa Dhatukajjali* (RD) was prepared with similar process as above. Three batches of Modified AVR were prepared. (Table 2)

Table 2: Showing Results of AVR & MAVR preparation

Parameter	MAVR I	MAVRII	MAVRIII	AVR I	AVR II	AVR III
Swarasa utilized (ml)	2500	2500	2500	2500	2500	2500
Duration of levigation for each Batch	12hr * 2 day	12hr * 2 day	12hr * 2 day	12hr * 2 day	12hr * 2 day	12hr * 2 day
Duration of Soaking for each Batch	12hr * 2 nights	12hr * 2 nights	12hr * 2 nights	12hr * 2 nights	12hr * 2 nights	12hr * 2 nights
Initial Weight of product	501	501	501	506	506	506
Final weight of product (gm)	567	564	580	605	608	602
Weight gain(gm)	66	63	79	99	102	96
% of Weight gain	13.173	12.574	15.768	19.565	20.158	18.972
Colour of final product	Dark Brown	Dark Brown	Dark Brown	Browish black	Browish black	Browish black

Preparation of MAVR with addition of *Dhatukajjali*

RD mixed with MAVR (same proportion of RD and mixture of rest of the ingredients as in AVR). In this sample same percentage of RD was mixed with MAVR as that RD present in each 10 Gm of *Arogyavardhini rasa.* This mixture was only prepared to identify effect of *Mardana/Bhavana* on leaching behavior of predominant composition of inorganic elements like Hg, Fe, Cu, Si in the formulation.

All the Batches of AVR and MAVR and were analyzed by employing bellow cited analytical parameters.

Organoleptic Evaluation

Evaluation of texture, color, odor, taste etc of both formulations was carried out[15,16].

Physico-chemical evaluation:

The routine physico-chemical parameters mentioned in API were carried out for the evaluation of drug.

Preliminary Qualitative tests for functional groups

Qualitative tests for the presence of functional groups, which plays very important role in the expression of biological activity of both the formulations, were done [18,19].

ICP-AES Analysis[20]

Detection of element in sample of AVR, MAVR and MAVR mixed with RD using ICP-AES (Inductive Coupled Plasma-Atomic Emission Spectrometer) of Model: Optima 3300 RL at SAIF, IIT, Powai, Mumbai.

High performance Thin layer chromatography (HPTLC) [21]

HPTLC fingerprint for Methanol extract of samples of AVR and MAVR was performed by using CAMAG Linomat V applicator and CAMAG TLC SCANNER‐III in reflectance absorbance mode at 254 nm and 366 nm equipped with Win‐CAT software.

RESULTS

The study inferred that initially 1500ml *Swarasa* was required to levigate 500 gm of raw material and 5 times of volume of *Swarasa* was required for optimum levigation for two days (Table 2). During levigation colour of *Swarasa* started changing after addition of mixture of raw material. Characteristics of optimum *Mardana (Subhavita Lakshana)* were observed on third day. After complete trituration formulations exhibited semisolid non sticky mass, characteristic smell, bitter taste and light brown colour which turned to blackish brown during drying. No foul smell was noted during wet grinding or drying. While drying, part of formulations exposed to air in wet state turned comparatively more blackish. Bitter test was felt in mouth and throat while powdering and sieving finished product.

Organoleptic parameters:

Observed organoleptic characters of raw material used for the preparation of AVR and MAVR and final product of prepared test drug sample are presented in Table 3.

Physicochemical parameters:

The results of Physiochemical analysis of media i.e *Nimba Patra Swarasa* shows in table 4 and final product of AVR shows average pH 3.5, LOD 4.85, ash value 15.34%, water insoluble ash 8.89% where MAVR revealed- pH 3.56, LOD 3.14%, ash value 6.15%, water insoluble ash 2.37%, acid insoluble ash 0.75%, as shown in Table 5. The average percentage of carbon disulphide soluble extractive of AVR was found to be 1.118 %w/w.

Preliminary Qualitative Tests

Qualitative Tests of both the formulations revealed presence of Cardiac glycosides, Alkaloids, Tannins, Steroids, Flavanoids, Carbohydrates, Starch and sugar (Table 6)

ICP-AES Analysis

Elemental analysis (ICP-AES) in sample of AVR, MAVR and MAVR mixed with Rasa Dhatukajjali result shows Percentage of mercury as 1.5226, 0.0117 and 1.703 respectively and other values are shown in Table 7.

HPTLC

In HPTLC of finished products, 11 bands were seen at 254 nm, while 8 bands were seen at 366 nm.(fig 2a, 2b). Out of total 11 Rf values observed at 254 nm, in each of the finished products i.e. AVR & MAVR, only two Rf values (**0.40** and **0.45)** were found to be similar. All 8 Rf values seen at 366nm in either formulations were dissimilar (Table 8).

Table 3: Oragnoleptic characters of finished product

Parameter	Sparsha (touch)	Rupa (colour)	Rasa (taste)	Gandha (odour)
AVR	Fine	Browish black	Bitter	Characteristic
MAVR	Fine	Dark Brown	Bitter	Characteristic

Table 4: Physicochemical parameters of finished product

Parameter	AVR				MAVR			
	Batch 1	Batch 2	Batch 3	Avg	Batch 1	Batch 2	Batch 3	Avg
pH (5% aqueous sol)	3.4	3.6	3.5	3.5	3.6	3.2	3.9	3.56
LOD at 110°C(%w/w)	3.96	5.02	5.58	4.85	2.05	3.47	3.90	3.14
Ash value (%w/w)	15.94	14.90	15.17	15.34	4.21	7.03	7.21	6.15
Water insoluble ash (%w/w)	8.77	9.07	8.92	8.92	1.28	1.25	4.59	2.37
Acid insoluble ash (%w/w)	1.21	3.98	2.58	2.59	0.69	0.84	0.73	0.75
Water soluble extractive (%w/w)	33.23	29.13	31.85	31.40	28.44	33.60	35.10	32.38
Alcohol soluble extractive (%w/w)	24.76	24.77	21.95	23.82	27.16	21.95	29.94	26.35
Percentage Sulphur (%w/w)	0.78, ,	1.88	0.695	1.118	-	-	-	-

Table 5: Results of qualitative test for various functional groups

Functional group	Test/ Reagent	Observation	AVR	MAVR
Cardiac glycosides	Legal's test	Color change	+ve	+ve
	Brontager's test	Fluorescence	+ve	+ve
Alkaloids	Dragendorff's reagent	Orange Brown ppt	+ve	+ve
	Wagner's reagent	Reddish brown ppt		
	Hager's reagent	Yellow ppt		
Tannins and Phenols	5% $FeCl_3$ sol.	Deep blue black colour	+ve	+ve
	Lead acetate sol.	White ppt	+ve	+ve
	Acetic acid sol.	Red colour sol.	+ve	+ve
	Potassium dichromate sol.	Red ppt	+ve	+ve
	Iodine sol.	Red colour	+ve	+ve
Proteins	Biuret reagent	No color change	-ve	-ve
Carbohydrates	Molish's test	Violet ring is formed at the junction	+ve	+ve
Steroids	Liebermann-buchard	First red, then blue and finally green color appears	-ve	-ve
	Salkowoki	Greenish yellow fluorescence	+ve	+ve
Flavanoids	Shinoda test	Yellow ppt	+ve	+ve
	Vanillin HCl test	Pink colour	-ve	-ve
Saponins	Shaking in test-tube	Frothing with honeycomb appearance	+ve	+ve
Amino acids	Ninhydrin test	Purple or bluish colour observed	-ve	-ve
Starch	Iodine test	Bluish colour appeared	+ve	+ve
Sugar	Fehling test	Red ppt	+ve	+ve

(+Ve)- Positive, (-ve)-Negative test for Functional group (-)- not done

Table 6: Results of Heavy metal analysis (ICP - AES)

Sample/Element	AVR	MAVR	Mix MAVR + Dhatu kajjali
Mercury (%Hg)	1.5226	0.0117	1.703
Silocon (%Si)	0.59	0.289	0.358
Copper (%Cu)	1.086	ND	1.299
Iron (%Fe)	1.656	0.0813	1.654

ND means less than 0.01 ppm

Table 7: Chromatographic HPTLC of AVR & MAVR

Toluene : Ethyl acetate: Formic acid **(7: 2 : 0.5 v/v)** as solvent system					
S. No.	Samples	Conditions	No. of spots	Rf Value	Common Rf
1	***Arogyavardhini Rasa***	Short UV - 254 nm	11	0.09, 0.2, 0.32 ,0.35 **0.40**,**0.45**, 0.51, 0.68 0.73, 0.84, 0.93	0.09 0.32 0.40 0.45 0.51 0.73
		Long UV – 366 nm	8	0.09, 0.22, 0.25, 0.32 0.40, 0.63, 0.73,0.93	
2	***Modified Arogyavardhini Rasa***	Short UV - 254 nm	11	0.1, 0.22, 0.33, 0.36 **0.40**, **0.45** 0.49 0.63 0.67 0.72 0.94	
		Long UV – 366 nm	8	0.11, 0.17,0.23,0.27 0.33, 0.62, 0.72,0.92	

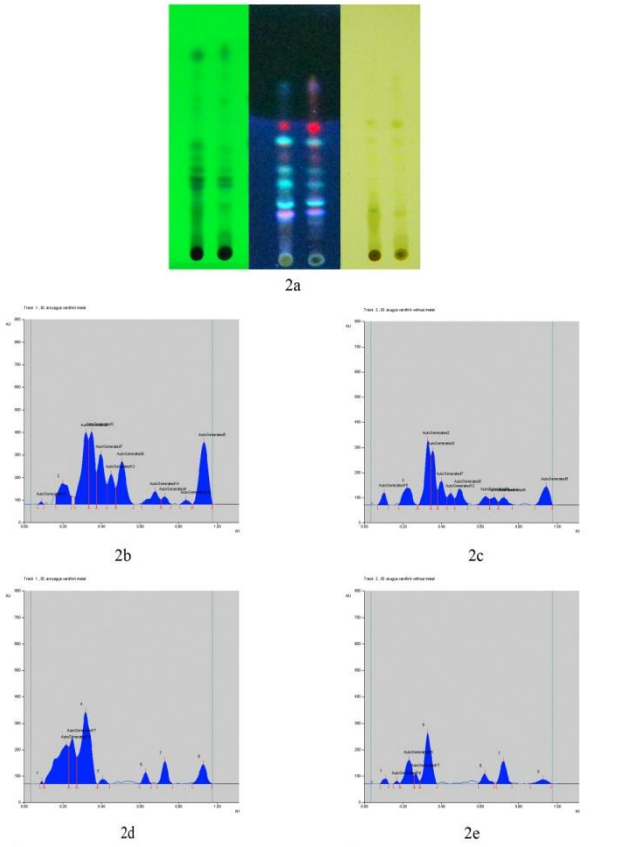

Figure 2: (a) TLC Plate at 254nm, 366nm and White light, (b) Densitogram curve of methanol extract of AVR at 254 nm (c) Densitogram curve of methanol extract of MAVR at 254nm (d) Densitogram curve of methanol extract of AVR at 366 nm (e) Densitogram curve of methanol extract of AVR at 366 nm

DISCUSSION

Arogyavardhini rasa is *Kharaliya Rasayana*. It's ingredients are same in all available references except *chitramula* whose interpretation differs as per AFI apart from other commentators and proportion of ingredients varies among classical texts and commentaries. Method of *Mardana* in view of persistency of triturition (2 days) is not clearly mentioned in textual reference. As liquid media is prescribed for *Mardana*, hence the process can be correlated with *Bhavana* (wet levigation). In this study, 12 hr is considered as a day; hence the process of *Mardana* (including duration of staged immersion) was completed in 48 hrs where total duration of *Mardana* was 24 hrs. Method of preparation of liquid for *Bhavana* is not mentioned; however maximum commentators take it as fresh leaf juice of *Azadirachta indica*. In *Bhavana*, staged levigation is mentioned ie levigation in sunlight and keeping mixture standstill at night hours.

Analytical study was carried out to identify physico-chemical changes due to *Mardana* with *Rasavarga Dravya*. Significant changes were observed between the parameters of both the formulations. Average LOD, Ash value, water soluble ash & acid insoluble ash of AVR were found to be increased in comparison to MAVR while MAVR showed increase in average value of water and methanol soluble extractive in comparison to AVR. These changes match with presence or absence of *Rasavarga Dravya* in the formulation. Carbon disulphide soluble extractive value in AVR shows presence of free sulphur. While no significant changes were observed in the qualitative test of AVR & MAVR. As there wasn't change in qualitative tests for functional groups among both formulations, it can be stated that chemical ingredients with these functional group are not completely changed after the process of *Mardana*.

Results of element Analysis (ICP-AES) in sample of AVR, MAVR and *Dhatukajjali* mixed with MAVR showed that percentage of mercury was found higher in mixture of MAVR with dhatukajjali than that of AVR. Comparatively less percentage of mercury in AVR may be due to formation of organo inorganic complexes of mercury during

the process of *Bhavana* leading to poor extraction in solvent. Copper is not within the detection limit in MAVR. In HPTLC, 11 and 8 spots were noted at 254nm and 366nm. Similarity in only 2 Rf values against dissimilarity in 9 Rf values favors generation of new chemical moieties due to probable factors like Hydrolysis, oxidation etc of chemical constituents of ingredients.

CONCLUSION

It can be concluded that Mardana/ Bhavana (triturition /levigation) has significant impact a classical approach of formulation preparation in ayurveda. Less percentage of mercury in AVR as that of mixture of its ingredients without *Mardana* signifies importance of *Mardana* process. Both the formulations classical *Arogyavardhini Rasa* and AVR by modified method without *Rasavarga dravya* shows differences through analysis of drug by physico chemical ,ICP-AES and HPTLC posses different chromatographic profile which is suggestive of generation of newer chemical ingredients by *Mardana* with metallomineral ingredients. In *Arogyavardhini Rasa*, *Mardana* (wet triturition) and metallo-mineral ingredients are responsible for exhibition of specific different analytical profile and may have important role in its pharmacological properties.

Conflict of interest

None.

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