



Research Article

J. Ayu. Herb. Med.
2016; 2(4): 104-111
July- August
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Quality Control Parameters of *Arogyavardhini Rasa* prepared by classical method

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ABSTRACT

Background: *Arogyavardhini Rasa* (AVR) is a well-known An Ayurvedic herbo-mineral formulation, indicated for treatment of broad spectrum chronic diseased conditions commonly used by physicians whose details of pharmaceutical processing are yet to document. Current research is first effort to document quality control parameters of this important formulation. **Aim:** To study the Pharmaceutical and physicochemical Quality Control Parameters profiles of *Arogyavardhini Rasa*. **Materials & Method:** AVR was prepared in one pilot and 3 main batches as per classical reference of *Rasaratnasamucchaya*. Its Physico-chemical parameters, qualitative tests for functional groups, Chromatography and quantitative elemental estimation were investigated. **Results & Discussion:** An average of 2500ml Swarasa was required for optimum *Mardana* for preparation of AVR from Avg. 506 gms of powdered Raw drugs, leading to an average yield, % yield (as that of powdered drugs) % wt gain of 605gm, 119.56%, 99 gm and 19.56% respectively. Functional groups Cardiac Glycosides, Alkaloids, Tannins and Phenols, Proteins, carbohydrates, steroids, Flavanoids, Saponins, Amino acids, Starch and Sugar were present. HPTLC study revealed a total of 11 and 8 bands at 254 nm and 366 nm in AVR. **Conclusion:** There is uniformity among results of observed and test parameters, among 3 batches. Pharmaceutical process, Results of pharmaceutical study, Physico-chemical tests, Presence of functional groups and HPTLC profile in present study may be considered as Standard manufacturing process of *Arogyavardhini Rasa*.

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

INTRODUCTION

Arogyavardhini Rasa is widely practiced *Kharaliya Rasaushadhi* used for the treatment of different types of *Jvara* (fever), *Kushtha* (skin disorders), *Medoroga* (obesity), *Kamala* (jaundice) and other *Yakrit vikara* (liver disorders). It has been described in text by *Rasa Vagbhatta* in 13th century^[1] and many other classical texts including Ayurvedic Formulary of India^[2]. *Rasaushadhi* (formulations containing metallominerals and mercurials) are important formulation in Ayurvedic therapeutics due to lesser therapeutic doses, enhancement of action of other ingredients of formulation, quicker action and palatability^[3] and more stability/ shelf life^[4] as compared to formulations prepared from drugs of plant or animal origin. Toxicity studies and in vitro and in vivo efficacy studies of *Arogyavardhini Rasa* has been carried out. It has been proven safe on liver, kidney, and brain through earlier studies^[5, 6]. Another study conducted on albino rats for acute, sub-acute and chronic toxicity and Hepatoprotective effect revealed non toxic effect on vital organs in therapeutic dose, even in four times higher doses and with good hepatoprotective activity against CCl₄ induced liver injury^[7]. But scientifically studied published data on Pharmaceutico analytical profile is lacking. Hence present research work was carried out to establish Pharmaceutical and analytical profiles of classical *Arogyavardhani Rasa* (AVR) .

MATERIALS AND METHODS

Collection and authentication of raw materials

Fresh roots of *Chitraka* and leaves of *Nimba* (as per requirement) were collected from botanical garden of University and other raw materials were procured from Pharmacy (Table 1). All herbal raw drugs were authenticated by Pharmacognosy laboratory. Analysis was carried out by employing different physicochemical parameters for raw material as well as finished products.

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Processing of raw materials

Roots of *chitraka* were dried and powder was prepared by pounding in iron pounding apparatus and grounded by household mixer grinder and sieved before packing. *Shodhana* of *Guggulu* (fig 1a) was carried out by *Swedana* method using *Triphala Kwatha*^[8]. Herbal ingredients were powdered by Grinding and sieving (fig 1b) and passed through sieve no # 80^[9]. Fresh *NimbaPatra Swarasa* was prepared by grinding and squeezing^[10]. *Dhatukajjali* (*Kajjali*, *LohaBhasma*, *AbhrakaBhasma* and *TamraBhasma*) was prepared by sequential *Mardana* (trituration)^[11].

Pharmaceutical Manufacturing Process of AVR

All the ingredients were accurately weighted. *Dhatukajjali* was taken in polythene bag with addition of powdered drugs and mixed thoroughly by shaking. Then mixture was added little by little in a butterfly wet-grinder initially containing mixture of *Guggulu*, *Shilajatu* and *Swarasa*. Required quantity of *Nimba patra Swarasa* for optimum levigation was added till the mixture gets immersed completely (*Samyagpluta*). 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 below 70°C and was Powdered, weighed, packaged, labeled and stored in glass container. Total three batches of AVR were prepared (Table 2) (fig 1c - 1j).

All the Batches of AVR were analyzed by employing various analytical parameters.

Organoleptic evaluation

Observed organoleptic characters (texture, color, odor, taste etc.^[12, 13]) of raw materials and final product of AVR are presented in Table.

Physico-chemical evaluation

Physico-chemical parameters for the evaluation of drug mentioned in Ayurvedic pharmacopeia of India were carried out^[14]. The results of Physicochemical analysis of procured raw material, media and final product AVR are mentioned in the Table below.

Preliminary Qualitative tests for functional groups^[15, 16]

Common functional groups in ingredients of formulation were studied.

Elemental analysis^[17]

Percentage of major inorganic elements present in ingredients of *Arogyavardhini Rasa* were analyzed by Inductively Coupled Plasma method at Sophisticated Analytical Instrumental facility, IIT Powai, Mumbai.

High Performance Thin Layer Chromatography (HPTLC)

Methanolic extract of sample of AVR was spotted on Precoated Silica Gel GF254 aluminium plate (20 cm × 10 cm with 250 μm thickness) using Camag Linomate V sample applicator fitted with a 100μ L Hamilton syringe. The plate was developed using optimized mobile phase using solvent system Toluene :Ethyl acetate: Formic acid(7: 2: 0.5 v/v) in twin trough development chamber. After development, Densitometric scanning was performed with a Camag TLC scanner III in reflectance absorbance mode at 254 nm and 366 nm equipped with Win-CATsoftware^[18].

OBSERVATION AND RESULTS

The pilot study inferred that Initially 1500 ml *Swarasa* was required to levigate 500 gm of raw material and approximately 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. *Subhavita Lakshana* were observed on third day after complete trituration with Characteristic smell, taste and colour i.e light brown ultimately turned to blackish brown with semisolid consistency and finally mass became non sticky. During wet grinding process no foul smell was noted. While drying, part in contact to air was turned darker in colour than that of one which was in contact with tray. Total time taken in drying process depends on season, in summer it dries fast in comparison to winters and rainy season. Bitter test was felt in mouth and throat while powdering and sieving of finished product. Percent yield of AVR was found to be increased than that of initial weight of material (Table 2). An average of 2500 ml *Swarasa* was required for optimum *Mardana* for preparation of AVR from Avg. 506 gms of powdered Raw drugs, leading to an average yield, % yield (as that of powdered drugs), wt gain and % wt gain of 605gm, is 119.56%, 99 gm and 19.56% respectively.

Organoleptic Evaluation

Organoleptic characters of raw material used for the preparation of AVR and final product of test drug sample are presented in (Table 3).

Physicochemical parameters:

The results of Physicochemical analysis of media and Procured bhasmas are mentioned in the Table 4 and Table 5 respectively where as Final product of AVR mentioned in Table 6.

Preliminary Qualitative Tests

Evaluation of preliminary Qualitative tests for functional groups in AVR revealed the presence of Cardiac glycosides, Alkaloids, Tannins, Steroids, Flavonoids, Carbohydrates, Starch and sugar etc in the sample of AVR (Table 7).

Elemental analysis

Elemental analysis of major inorganic elements present in ingredients of *Arogyavardhini Rasa* revealed 1.5226%, 0.59%, 1.086% and 1.656% of Hg, Si, Cu and Fe respectively (Table 8).

HPTLC

In HPTLC of finished products of AVR where 11 Rf bands were seen at 254 nm, while 8 Rf bands were seen at 366 nm (fig 2a, 2b) (Table 9).

DISCUSSION

In the present study ingredients of *Arogyavardhini Rasa* are same in all available references except *chitrakamula* (*Plumbago zeylanica* linn) whose interpretation differs as per AFI^[19] apart from other commentators and proportion of ingredients varies among classical texts and commentaries. Method of *Mardana* in view of persistency of trituration (2 days) is not clearly mentioned in textual reference^[20]. 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. Percent yield of AVR found to be increased by 19 % (as that of raw material) which may be due to lavigation with *Nimba patra swarasa* whose total solid content (Table 5) help to increase the weight of final product.

Arogyavardhini rasa whose data on Pharmaceutico analytical standardization is lacking. Although study by Padhar *et al* evaluated Pharmacognostic and phytochemical parameters of "Arogyavardhini compound" but this is not classical formulation and the parameters of standardization would be significantly different from classical formulation as it levigated with equal amount of Garlic^[21]. Physico-chemical analysis data of AVR indicates an average LOD in sample was 4.85% w/w, which shows that the value of moisture content is within the limit. Excess of water in drug encourage microbial growth, presence of fungi or insects and deterioration following hydrolysis. pH value is one of the main factor which may influence the quality of drug. pH of 5% aqueous solution shows 3.5 which indicates acidic nature of AVR while of media used *Nimba patra Swarasa* and *Triphala Kwatha* were found to be basic and acidic in nature with total solid content of 3.95% and 16.98% respectively. Ash values are the criteria to judge the identity and purity of crude drug, where total, water soluble and acid insoluble ashes are considered as parameter to be carried out. Analysis shows AVR contained average of 15.34% w/w of total ash, 2.59 % w/w acid insoluble ash and 8.89% w/w water soluble ash. The result revealed that AVR is free from unwanted organic and inorganic compounds and sample free from dust and other soil matters etc. Water-soluble extractive and alcohol-soluble extractive were found to be 31.40 % w/w, 23.82% w/w respectively which reveals that the drug have fairly good solubility in water and the average percentage of carbon disulphide soluble extractive was found to be 1.118 %w/w. which found to be decrease as that of initial qty. used in formulation i.e. 2.2727%. Thus this % decrease of sulpher may be formation of new chemical moieties due to *Mardana* of drug ingredients with liquid

media. Qualitative tests are used to detect the presence of functional groups, which plays very important role in the expression of biological activity thus tests for functional groups in AVR revealed the presence of Cardiac glycosides, Alkaloids, Tannins, Steroids, Flavonoids, Carbohydrates, Starch and sugar etc in the sample.

Elemental analysis (ICP-AES) of major inorganic elements in sample of AVR, shows presence of mercur, silicon, copper and iron which is obvious and percentage of inorganic elements; Hg, Si, Fe and Cu is helpful for differentiation from other Ayurvedic formulations with ingredients of only Plant or mineral origin. Mamata N.G carried out a study in *Arogyavardhini Vati* by Chromatographic, Spectroscopic techniques (AAS) and X-ray diffraction to find *paradedosha* (impurities) remain in *Rasaushadhi*^[22]. In a one analytical study a rapid, simple, accurate and specific HPTLC method for estimation of tannin, kutkoside and steroid present in the Rhizome of *Picrorrhiza kurroa* and *Arogyavardhinivati* has been developed^[23]. In HPTLC profile of AVR shows 11 Rf bands at 254 nm and 8 Rf bands at 366 nm. These possible compounds of the matrix which may possess its therapeutic effect. These findings may help to generate qualitative and quantitative standards to determine the quality and purity of the drug formulation.

CONCLUSION

There is uniformity among results of observed test parameters, among 3 batches. An average of 2500 ml *Swarasa* was required for optimum *Mardana* for preparation of AVR from an Avg. of 506 gms of powdered Raw drugs, leading to an average yield, % yield (as that of powdered drugs), wt gain and % wt gain as- 605gm, 119.56%, 99 gm and 19.56% respectively.

Pharmaceutical process, Results of pharmaceutical study, Physico-chemical tests, Presence of functional groups and HPTLC profile in present study may be considered as quality parameter in further study and also in manufacturing process of *Arogyavardhini Rasa*.

Table 1: Ingredients used in the Preparation of *Arogyavardhini Rasa*

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

Table 2: Results of AVR preparation

Parameter	Pilot Batch	AVR Batch I	AVR Batch II	AVR Batch III
Swarasa utilized (ml)	2400	2500	2500	2500
Duration of levigation for each Batch	12hr * 2 day	12hr * 2day	12hr * 2day	12hr * 2 day
Duration of Soaking for each Batch	12hr * 2 nights	12hr * 2 nights	12hr * 2 nights	12hr * 2 nights
Initial Weight of product	506	506	506	506
Final weight of product (g)	600	605	608	602
weight gain(g)	94	99	102	96
% of Weight gain	18.577	19.565	20.158	18.972
Colour of final product	Browish black	Browish black	Browish black	Browish black

Table 3: Organoleptic characters of raw materials and finished product

Parameter	Sparsha (touch)	Rupa (colour)	Rasa (taste)	Gandha(odour)
Katuki	Smooth	Dustygrayish	Bitter (++)	Characteristic
Chitrak	Smooth	creamy	Katu (pungent)	Characteristic
Triphala	Smooth	greenish brown	Kashaya Amla	Characteristic
Guggulu	Hard Lumps	Black	Tikta,Katu	Aeromatic
Neem swarasa	Slippery	Green	Bitter	Characteristic
Shilajatu	Hard	Black	Tikta	Characteristic
Tamra	Fine	Dark blue/Black	Tasteless	Not specific
Abhrakh	Fine	Brick red	Tasteless	Odourless
Loha	Fine	Blood red	Tasteless	Odourless
Kajjali	Fine	Black	Tasteless	Odourless
AVR	Fine	Brownish black	Bitter	Characteristic

Table 4: Physicochemical parameters of Bhasma (Arogyavardhini Raw material)

	Test parameter	Tamra Bhasma	Abhraka Bhasma	Loha Bhasma	Kajjali
Physico-chemical parameters	LOD at 105 ⁰ C (%)	0.97	0.66	00.31	0.39
	Total Ash (%)	97.42	99.18	99.63	0.56
	Acid insoluble ash (%)	2.29	33.74	27.80	96.18
Ayurvedic Parameter	Lusterless (Nischandrika)	+ve	+ve	+ve	+ve
	Fineness (fine enough to enter in lines of finger – Rekha purratva)	+ve	+ve	+ve	+ve
	Floats on water (Varitara)	+ve	+ve (partially)	+ve	+ve
	Tasteless (Niswadu)	-ve, (metallic taste +)	+ve	+ve	-
	Special test Dadhi amla Pariksha	colour change +	NA	NA	NA

(+ve) – Positive, (-ve) – Negative, (-) – Not done, and (NA)- Not applicable

Table 5: Physicochemical parameters of media

Parameters	Nimba patra Swarasa	Triphala Kwatha
Total solid content (%)	3.95	16.98
pH	6.23	3.7
Sp. Gravity	1.0013	1.0522
Reaction with Litmus Paper	Basic	Acidic

Table 6: Physicochemical parameters of finished product

S. No.	Parameter (%w/w)	AVR			
		Batch 1	Batch 2	Batch 3	Avg
1	pH (5% aqueous sol)	3.4	3.6	3.5	3.5
2	LOD (at 110oC)	3.96	5.02	5.58	4.85
3	Ash value	15.94	14.90	15.17	15.34
4	Water soluble ash	8.77	9.07	8.92	8.92
5	Acid insoluble ash	1.21	3.98	2.58	2.59
7	Water soluble extractive	33.23	29.13	31.85	31.40
8	Alcohol soluble extractive	24.76	24.77	21.95	23.82
9	Percentage of Sulfur	0.78	1.88	0.695	1.118

Table 7: Results of qualitative test for various functional groups

S. No	Functional gp.	Test/ Reagent	Observation	AVR
1.	Cardiac glycosides	Legal's test	Color change	+ve
		Brontager's test	Fluorescence	+ve
2.	Alkaloids	Dragendorff's reagent	Orange Brown ppt	+ve
		Wagner's reagent	Reddish brown ppt	
		Hager's reagent	Yellow ppt	
3.	Tannins and Phenols	5% FeCl ₃ sol.	Deep blue black colour	+ve
		Lead acetate sol.	White ppt	+ve
		Acetic acid sol.	Red colour sol.	+ve
		Potassium dichromate sol.	Red ppt	+ve
		Iodine sol.	Red colour	+ve
4.	Proteins	Biuret reagent	No color change	-ve
5.	Carbohydrates	Molish's test	Violet ring is formed at the junction	+ve
6.	Steroids	Liebermann-buchard	First red, then blue and finally green color appears	-ve
		Salkowoki	Greenish yellow fluorescence	+ve
7.	Flavanoids	Shinoda test	Yellow ppt	+ve
		Vanillin HCl test	Pink colour	-ve
8.	Saponins	Shaking in test-tube	Frothing with honeycomb appearance	+ve
9.	Amino acids	Ninhydrin test	Purple or bluish colour observed	-ve
10.	Starch	Iodine test	Bluish colour appeared	+ve
11.	Sugar	Fehling test	Red ppt	+ve

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

Table 8: Heavy metal analysis (ICP - AES)

Sample/Element (%)	Mercury (Hg)	Silicon (Si)	Copper (Cu)	Iron (Fe)
AVR	1.5226	0.59	1.086	1.656

Table 9: HPTLC Finger print Profile

Toluene : Ethyl acetate: Formic acid (7: 2 : 0.5 v/v) as solvent system				
S. No.	Samples	Conditions	No. of spots	Rf Value
1	<i>Arogyavardhini rasa</i>	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
		Long UV – 366 nm	8	0.09, 0.22, 0.25, 0.32 0.40, 0.63, 0.73,0.93



Fig:1(a) Swedana of Guggulu in Triphala kwatha



Fig :1(b) Expression of Nimbapatra swarasa



Fig:1(c) Powder of Raw materials



Fig:1(d) Rasadhathu kajjali



Fig:1(e) Mixing of Powdered ingredient



Fig:1(f) Nimba swarasa in Wet Grinder



Fig: 1(g) Wet grinding process



Fig:1(h) Drying Process



Fig: 1(i) Dry Arogyavardhini



Fig: 1(j):Powders of AVR

Figure 1: AVR preparation

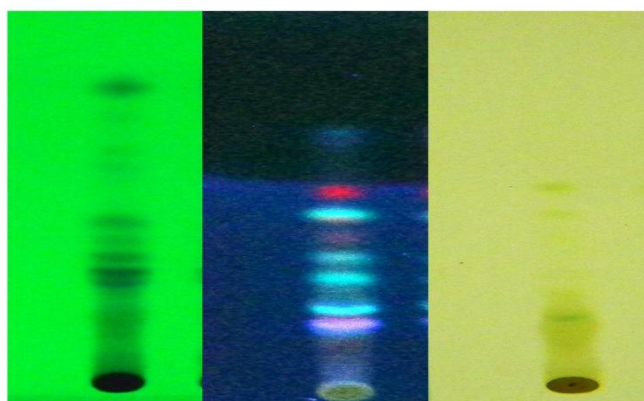
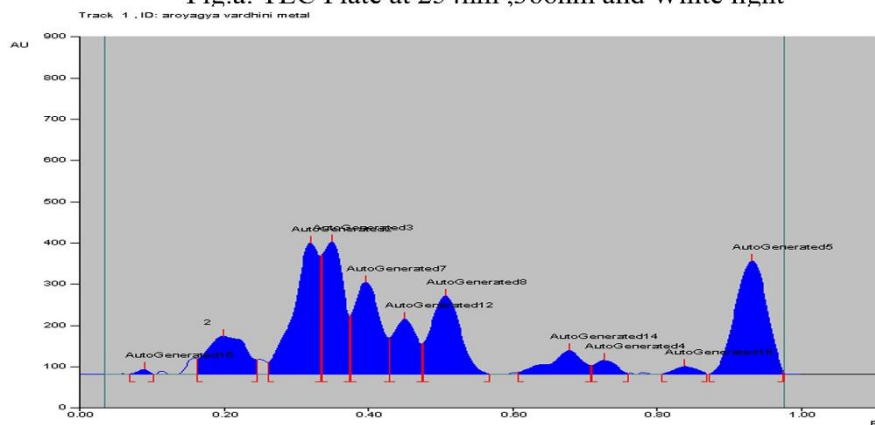


Fig.a: TLC Plate at 254nm ,366nm and White light



.Fig .b: AVR peak display at 254nm

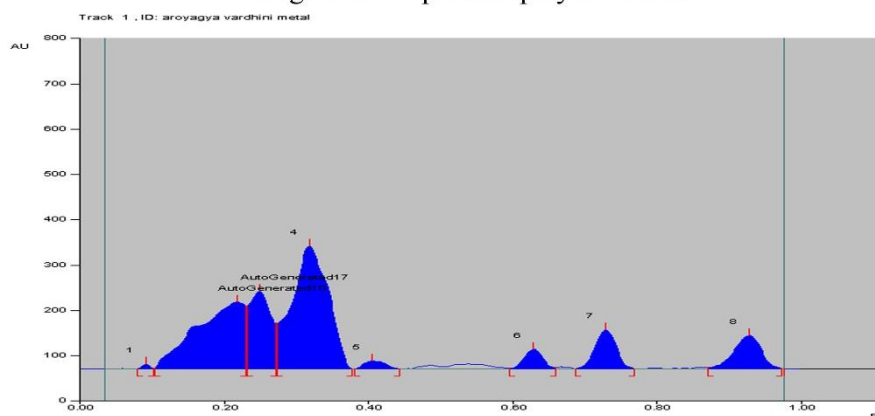


Fig. c: AVR peak display at 366nm

Figure 2: TLC profile and AVR peak at different range

Source of support

IPGTRA, Gujarat Ayurved University, Jamnagar, India.

Conflict of interest

None declared.

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HOW TO CITE THIS ARTICLE

Sapkota YR, Bedarkar P, Shukla VJ, Prajapati PK. Quality Control Parameters of *Arogyavardhini Rasa* prepared by classical method. J Ayu Herb Med 2016;2(4):104-111.