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Research Article

Standardization of Rasayana churna – A classical ayurvedic formulation

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ABSTRACT

Standardization is necessary to ensure quality and purity of the herbal drugs. It is equally important to establish system of standardization for every plant used in the herbal formulation to develop safe and quality based herbal formulation. Present study is initiated with to establish a standard methods and quality parameters for Rasayana Churna. It includes Authentication, Organoleptic properties, physic-chemical parameters, assay of active, heavy metal analysis, microbial load and qualitative HPTLC analysis. All results were compared with Ayurvedic Pharmacopoeial standard. The obtained values should be helpful to develop pharmacopoeial standards of Rasayana Churna. Simultaneous comparative data of Rasayana Churna showed presence of each and every ingredient in formulation with their unique R_f value. This data will be helpful to overcome batch to batch variations in traditional preparation of Rasayana Churna.

Keywords: Rasayana Churna; Ayurvedic Pharmacopoeial Standard; Standardization

INTRODUCTION

Standardization is the process of evaluating the quality and purity by means of various parameters like microscopical, physical, chemical and biological (Agrawal S *et al.*,2007). Evaluation is necessary to ensure quality and purity of the herbal drugs. It is equally important to establish system of standardization for every plant used in the herbal formulation to develop safe and quality based herbal formulation. Ayurvedic / Herbal medicines are widely used and accepted by about 60 per cent of the world's population for primary health care (Seth S *et al.*, 2004). For standardization of Ayurvedic drugs it is equally important to understand Ayurvedic concept of the treatment.

Ayurveda is concerned with healthy living along with curative measures that synchronize an individual physically, mentally and spiritually. In this modern era, it is getting accepted as a self-care system for individual well being. Focused primarily towards correcting imbalances before they develop into diseases, it is a solution, for all those who acknowledge responsibility for their own health and want a healthy and long life. One of the important concepts in Ayurveda is categorised under the heading of Rasayana or Rasayana therapy (P.V. Sharma, 1985).

Churna is a fine powder of a drug or drugs which is prepared by mixing clean, finely powdered, and sieved drugs (Ajay K *et al.*, 2010). Rasayana Churna is an Ayurvedic classical formulation mainly used for Urinary tract problems, stress related condition and immunosuppressive problems and it contains ingredients mentioned in Table 1. (H. Sharma, 1997).

MATERIALS & METHODS

Preparation of Rasayana Churna

The Rasayana churna was prepared as per the general method describe in Ayurvedic Formulary of India. All the ingredients were shade dried and powdered separately, passed through 80 # sieve, and then mixed together in equal proportions to get uniformly blended churna. and made into coarse powder with the help of a grinder. They were finally powdered and sieved (80#) separately. (Anonymous, 2003).

Collection & Authentification of Raw materials

Raw materials were collected from Atarsumba forest department, Vireshwar, Sabarkantha, Gujarat during the winter season. Authentication of raw materials was carried out at Department of Botany, The H.N.S.B. Ltd. Science College, Himmatnagar, Gujarat.

Determination of Foreign Matter

Weigh 100 g of the drug sample to be examined and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (6x). Separate and weigh it and calcu-

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Table 1: Composition of Rasayana Churna

Sanskrit Name	Scientific Name	Part used	Quantity
Guduchi	*Tinospora cordifolia* (Willd.) Miers.	Dried stem	1 Part
Gokshur	*Tribulus terrestris* Linn.	Dried fruit	1 Part
Amalaki	*Emblica officinalis* Gaertn.	Pericarp of dried fruit	1 Part

Table 2: Voucher specimen number of raw materials

Sr. No.	Name of the Raw material	Voucher Specimen Number
1	Guduchi (*Tinospora cordifolia* (Willd.) Miers)	HKS/02/05012010
2	Gokshur (*Tribulus terrestris* Linn.)	HKS/03/05012010
3	Amalaki (*Emblica officinalis* Gaertn.)	HKS/01/05012010

Table 3: Presence of Foreign matter in Raw materials

Sr. No.	Name of the raw material	Foreign Matter (% w/w)	
		Limit*	Result
1	Guduchi	NMT 2	1.2
2	Gokshur	NMT 2	1.5
3	Amalaki	NMT 3	1.78

*Limits are mentioned as per Ayurvedic Pharmacopoeia of India (API); NMT – Not More Than;

Table 4: Organoleptic properties of Raw materials & Rasayana Churna

Sr. No.	Name of the Sample	Organoleptic properties				
		Appearance	Texture	Colour	Odour	Taste
1	Guduchi	Powder	Fine	Brown	Characteristic	Bitter
2	Gokshur	Powder	Fine	Light Green	Characteristic	Characteristic
3	Amalaki	Powder	Fine	Brown	Characteristic	Astringent
4	Rasayana Churna	Powder	Fine	Greenish brown	Characteristic	Bitter & Astringent

late the percentage. (Ayurvedic Pharmacopeia of India (API), 2001)

Organoleptic Parameters

Organoleptic parameters like colour, odour and taste were carried out for all raw materials. These parameters helped in visual identification of raw materials.

Determination of Physicochemical parameters

It includes determination of parameters like Moisture content (Loss on Drying), Total ash, Acid insoluble ash, Water soluble ash, Alcohol soluble extractive, Water soluble extractive. (API, 2001)

Determination of pH

1% solution of samples were prepared in distilled water and pH was determined using pH meter SYSTRONICS DIGITAL pH METER, MK VI.

Estimation of active compounds

Estimation of Total Saponin (V. Rajpal, 2002a), Total Tannin (V. Rajpal, 2002b), Bitter Residue (V. Rajpal, 2002c) was carried out in raw materials and Rasayana Churna.

Heavy Metal Analysis

Heavy metal analysis was performed using Shimadzu AA-6300. Sample digestion was carried out by CEM MARS Express microwave digestive system. Weigh 0.5g of sample and add 8ml of 69% Nitric acid in the Teflon PFA 75ml vessels. Parameters used for the digestion were Power 400 W with 100%, Ramp time was 20 minutes to attempt temperature 150^{O}C and hold for 10 minutes. After digestion process completed the sample was diluted up to 50ml by distilled water and filter through whatman filter paper No. 1. The standards of Lead (Pb), Cadmium (Cd), Arsenic (As) and Mercury (Hg) were prepared and the calibration curve was developed for each of them. Samples were analyzed by using these standard curves. The permissible limit for Heavy Metal content was mentioned in Ayurvedic Pharmacopoeia of India.

Microbial Limit Test

Microbial analysis was carried out as per standard procedure **(Indian Pharmacopoeia, 2010)**. It included Total bacterial count, Total Fungal Count, Presence of *Escherichia coli, Salmonella ebony, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* Pure culture of *Escherichia coli* (NCIM: 2065; ATCC: 8739), *Salmonella ebony* (NCIM: 2257 NCTC: 6017), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6358) were obtained from NCIM Pune. The media used for the microbial limit test were of HiMedia Pvt. Ltd.

Table 5: Moisture content and pH value of Raw materials & Rasayana Churna

Sr. No.	Name of the Sample	Tests			
		Moisture Content (% w/w)		pH Value (1%w/v Soln.)	
		Limit*	Result#	Limit*	Result#
1	Guduchi	NMT 8	4.44 ± 0.10	NA	5.93 ± 0.03
2	Gokshur	NMT 8	3.36 ± 0.05	NA	6.43 ± 0.06
3	Amalaki	NMT 8	4.58 ± 0.04	NA	4.00 ± 0.05
4	Rasayana Churna	NA	3.53 ± 0.04	NA	5.07 ± 0.08

*Limits are mentioned as per Ayurvedic Pharmacopoeia of India (API); NA -Not Available; NMT – Not More Than; # Data represent in Mean ± SEM where n=3.

Table 6: Results of Ash in Raw materials & Rasayana Churna

Sr. No.	Name of the Sample	Tests					
		Total Ash (% w/w)		Acid Insoluble Ash (% w/w)		Water Soluble Ash (% w/w)	
		Limit*	Result#	Limit*	Result#	Limit*	Result#
1	Guduchi	NMT 16	8.41 ± 0.11	NMT 3	1.23 ± 0.05	NA	6.90 ± 0.14
2	Gokshur	NMT 15	9.72 ± 0.24	NMT 2	1.05 ± 0.03	NA	7.45 ± 0.11
3	Amalaki	NMT 7	4.39 ± 0.12	NMT 2	0.81 ± 0.02	NA	3.01 ± 0.08
4	Rasayana Churna	NA	6.56 ± 0.07	NA	1.10 ± 0.03	NA	5.18 ± 0.13

*Limits are mentioned as per Ayurvedic Pharmacopoeia of India (API); NA -Not Available; NMT – Not More Than; # Data represent in Mean ± SEM where n=3.

Table 7: Results of Alcohol Soluble and Water Soluble Extractive Value

Sr. No.	Name of the Sample	Tests			
		Alcohol Soluble Extractive (% w/w)		Water Soluble Extractive (% w/w)	
		Limit*	Result#	Limit*	Result#
1	Guduchi	NLT 3	18.52 ± 0.20	NLT 11	22.63 ± 0.18
2	Gokshur	NLT 6	28.80 ± 0.09	NLT 10	25.63 ± 0.08
3	Amalaki	NLT 40	54.87 ± 0.11	NLT 50	68.88 ± 0.09
4	Rasayana Churna	NA	40.38 ± 0.09	NA	58.79 ± 0.09

* Limits are mentioned as per Ayurvedic Pharmacopoeia of India (API); NA -Not Available; NLT – Not Less Than

Data represent in Mean ± SEM where n=3.

Table 8: Percentage of Major constitutes in Raw materials & Rasayana Churna

Sr. No.	Name of the Sample	Bitter Residue # (% w/w)	Total Saponin # (% w/w)	Total Tannin # (% w/w)
1	Guduchi	**4.93 ± 0.17**	2.04 ± 0.05	3.19 ± 0.03
2	Gokshur	0.93 ± 0.05	**21.57 ± 0.22**	5.87 ± 0.21
3	Amalaki	0.26 ± 0.03	7.33 ± 0.11	**36.40 ± 0.16**
4	Rasayana Churna	3.86 ± 0.16	16.27 ± 0.15	30.12 ± 0.06

Data represent in Mean ± SEM where n=3.

HPTLC Analysis of Raw materials vs. Rasayana Churna

***Selection of plate and adsorbent*:** Pre-coated aluminium plates with Silica Gel $60F_{254}$ (E. Merck, India) of 6 x 10 cm and 0.2 mm thickness, were used for detection. The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography.

Sample Preparation: For raw materials - Accurately weighed 1g of sample, dissolved in 20ml methanol and & reflux it on water bath at 90-100^{0}C for 15 min. Filter and evaporate up to 5ml in porcelain dish. Take the solution for TLC/HPTLC Profiling.

Formulation - Accurately weighed 3g of Rasayana churna, dissolved in 20ml methanol and & reflux it on water bath at 90-100°C for 15 min. Filter and evaporate up to 5ml in porcelain dish. Take the solution for TLC/HPTLC Profiling.

Application of sample: Sample application was carried out by automatic device "CAMAG LINOMAT V". Each band was of 8 mm width and 10µl sample was applied.

Development: The plate was developed in CAMAG glass twin-through chamber (10x10 cm) previously saturated with the solvent for 60 min (temperature 25

Table 9: Results of Heavy Metal Analysis

Sr. No.	Name of the Sample	Heavy Metal			
		Lead (10 ppm) *	Cadmium (0.3 ppm) *	Arsenic (3 ppm)*	Mercury (1 ppm) *
1	Guduchi	1.22	0.125	1.89	Absent
2	Gokshur	2.26	0.165	2.89	Absent
3	Amalaki	1.59	0.235	1.56	Absent
4	Rasayana Churna	2.05	0.198	1.96	Absent

* Limits are mentioned as per Ayurvedic Pharmacopoeia of India (API)

Table 10: Results of Microbial Load

Sr. No.	Microbial Analysis	Limit*	Guduchi	Gokshur	Amalaki	Rasayana Churna
1	Total bacterial count	NMT 10^5 CFU/mL	$22x10^3$	$89x10^3$	$65x10^3$	$32x10^3$
2	Total yeast and mould	NMT 10^3 CFU/mL	$14x10^1$	$5x10^2$	$22x10^1$	$18x10^1$
3	*E. coli*	Absent	Absent	Absent	Absent	Absent
4	*S. spp.*	Absent	Absent	Absent	Absent	Absent
5	*S.aureus*	Absent	Absent	Absent	Absent	Absent
6	*P.areuginosa*	Absent	Absent	Absent	Absent	Absent

*Limits are mentioned as per Ayurvedic Pharmacopoeia of India (API); NMT – Not More Than; CFU/mL – Colony forming unit / millilitre

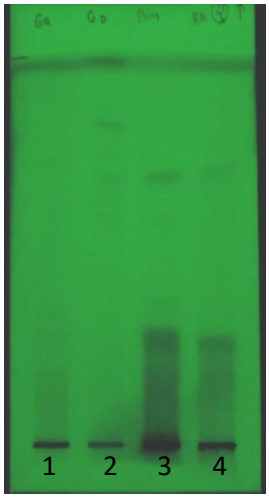

Figure 1: Image of plate @ 254nm

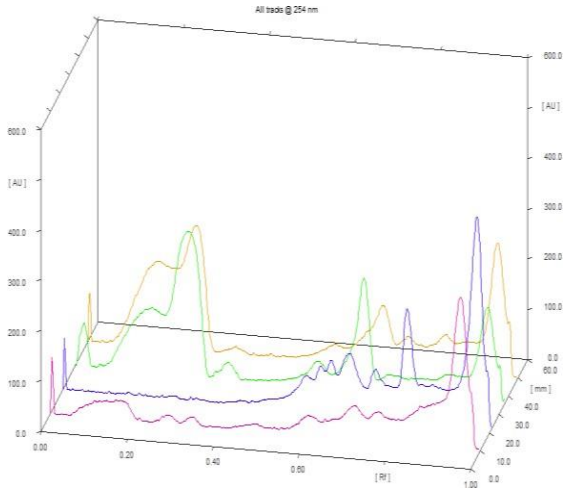

Figure 2: 3D Chromatogram @ 254 nm

Track 1: 10 µl Methanolic extract of Guduchi; Track 2: 10 µl Methanolic extract of Gokshur; Track 3: 10 µl Methanolic extract of Amalaki; Track 4: 10 µl Methanolic extract of Rasayana Churna

°C, relative humidity 40%). The development distance was 8 cm.

Mobile Phase: Toluene: Ethyl acetate: Glacial acetic acid (10:3:1 v/v)

Visualization: At 254nm, 366nm and Visible (after spray of Anisaldehyde Sulphuric acid reagent)

RESULT AND DISCUSSION

All raw materials were collected from hygiene place and authenticated at Department of Botany, The H.N.S.B. Ltd. Science College, Himmatnagar, Gujarat. The authentication certificate number / voucher specimen number of raw materials are mentioned in Table 2.

The raw materials were properly examined for adulterants and foreign matter like sand, insect, rodent contamination which was found to be in specified limit as per Ayurvedic Pharmacopoeia of India. The results are shown in Table 3.

Raw materials & Rasayana Churna was subjected to various analytical techniques. Botanical parameters revealed appearance, texture, colour, odour and taste (Table 4).

Results revealed that moisture content of all raw materials were in prescribed limit. Limits of pH value of raw materials are not available in Ayurvedic pharmacopoeia. Moisture content and pH value of Rasayana Churna was 3.53 ± 0.04 %w/w and 5.07 ± 0.08 respectively (Table 5).

Table 11: Rf values of chromatogram at 254nm

Rf Values @ 254nm			
Track 1 (Guduchi)	**Track 2 (Gokshur)**	**Track 3 (Amalaki)**	**Track 4 (Rasayana Churna)**
0.16	---	0.16	0.17
---	---	0.35	0.35
0.47	---	---	0.47
---	0.60	---	0.60
---	0.80	---	0.80

--- (No similar R_f value detected)

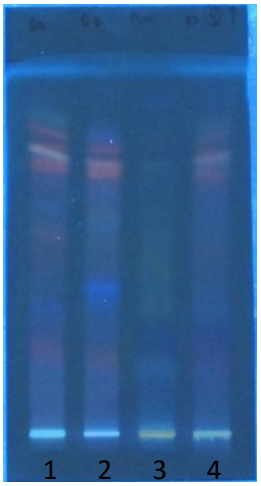

Figure 3: Image of plate @366 nm

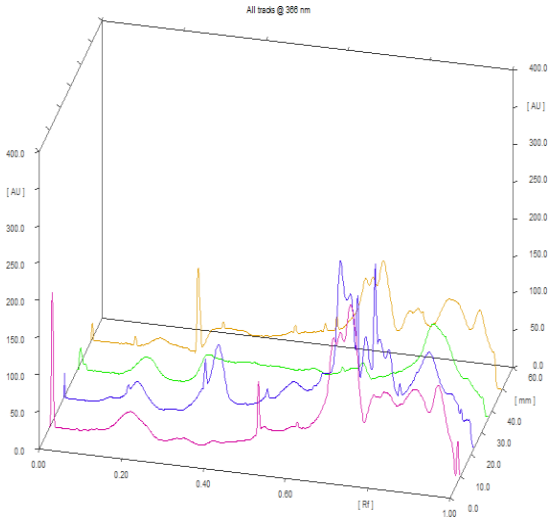

Figure 4: 3D Chromatogram @ 366 nm

Track 1 : 10 µl Methanolic extract of Guduchi; Track 2: 10 µl Methanolic extract of Gokshur; Track 3: 10 µl Methanolic extract of Amalaki; Track 4: 10 µl Methanolic extract of Rasayana Churna

Table 12: Rf values of chromatogram at 366nm

Rf Values @ 366nm			
Track 1 (Guduchi)	**Track 2 (Gokshur)**	**Track 3 (Amalaki)**	**Track 4 (Rasayana Churna)**
0.20	---	---	0.20
---	0.34	0.34	0.34
---	---	0.61	0.61
0.69	0.68	0.69	0.69

--- (No similar R_f value detected)

Total ash, Acid insoluble ash and Water soluble ash is the indicator for a presence of inorganic matter. Results showed presence of inorganic content inn all raw materials and Rasayana Churna (Table 6).

Determination of extractive value is an important parameter to understand percentage solubility of the substance in specific solvent. It is used to develop extraction procedure of herb(s). Results indicate that Alcohol and water soluble extractive of raw materials were higher than specified limit which indicates good solubility of raw materials. Rasayana churna has more than 50% water soluble extractive value (Table 7).

Active compounds / groups plays major role in the efficacy of the drug. Quantification of active compounds is necessary to standardize batch to batch variation and affected efficacy value due to same. In this study, estimation of Bitter residue, Total Tannin & Total Saponin were carried out for raw materials and Rasayana Churna. Bitter residue, Total Tannin & Total Saponin content was found more in Guduchi, Gokshur & Amalaki respectively. The percentage of active compounds in the Rasayana churna was proved presence and quality of raw materials. (Table 8)

Absence and presence of contaminants like Heavy metal and Microbial load was carried out in all raw materials & Rasayana Churna. Results of contaminants for raw materials & Rasayana Churna were in specified limit. Heavy metal and Microbial load results are tabulated in Table 9 and Table 10 respectively.

Simultaneous comparative HPTLC analysis of Rasayana Churna was carried out with respect to individual ingredients to establish the present of each ingredient in

Figure 5: Image of plate@ 540 nm

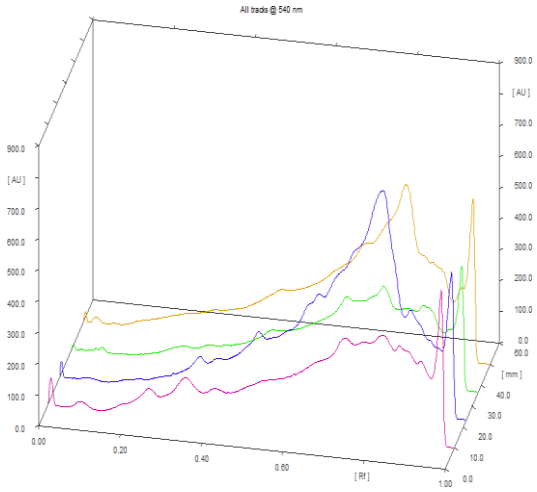

Figure 6: 3D Chromatogram @ 540 nm

Track 1: 10 µl Methanolic extract of Guduchi; Track 2: 10 µl Methanolic extract of Gokshur; Track 3: 10 µl Methanolic extract of Amalaki; Track 4: 10 µl Methanolic extract of Rasayana Churna

Table 13: Rf values of chromatogram at 540nm

Rf Values @ 540nm			
Track 1 (Guduchi)	**Track 2 (Gokshur)**	**Track 3 (Amalaki)**	**Track 4 (Rasayana Churna)**
0.25	---	---	0.25
0.34	0.34	0.34	0.34
---	0.50	0.49	0.49
---	0.70	---	0.70
0.80	---	0.80	0.80
---	---	0.86	0.86

--- (No similar R_f value detected)

Rasayana Churna. Common mobile phase was established to carry out qualitative determination of Rasayana churna. Detection was carried out at three different wavelengths i.e. @254nm, @366nm and @540nm OR visible (after spray with Anisaldehyde sulphuric acid).

HPTLC chromatogram of Rasayana Churna against individual ingredients showed presence of each and every ingredient in formulation on the basis of comparative R_f values. Plate images, 3D chromatograms and Rf value tables are showed below at different wavelengths i.e. @254nm (Fig.1 & 2, Table 11), @366nm (Fig.3 & 4, Table 12)and @ 540 (Fig.5 & 6, Table 13).

CONCLUSION

On the basis of available data we can conclude that all raw materials used in the preparation of Rasayana Churna were good quality and results were found as per pharmacopoeia limits. We were also established specification for Rasayana Churna with respect to quality based raw materials. Simultaneous comparative data of Rasayana Churna showed presence of each and every ingredient in formulation with their unique R_f value.

Hence, these parameters and the developed methods may be considered as a tool for assistance to the scientific organization and manufacturers to establish standards. This will also help to produce uniform standard products, which will retain faith in Ayurvedic Medicines.

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