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Analysis of evaluation parameters of the in-house and marketed samples of Rasayana Churna

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The traditional systems of medicine are being subjected to rigours of standardization to compete with national and international markets to establish their credibility. The present paper involves an Ayurvedic formulation Rasayana churna. It comprises of the dried powders of three rejuvenating drugs *viz.,* dried stem of Guduchi (*Tinospora cordifolia* Miers.), dried fruit of Gokshur (*Tribulus terrestris* Linn.) and dried pericarp of Amalaki (*Emblica officinalis* Gaertn.). All these plants are used in rasayana therapy. Rasayana Churna plays a very important role as an immunomodulator, rejuvenator, adaptogen, antioxidant and diuretic. Other important roles of rasayana churna involve elimination of toxins through urinary system and lowering the risk in kidney and cardiac disorders. The present study aims at analysis of the evaluation parameters of the in-house and two marketed samples of rasayana churna (coded as IHRC, MRC and ZRC), for its morphological, microscopical, phytochemical, physicochemical, spectroscopical and biological evaluation aspects. The need of standardisation is justified in the range of variations obtained in the results of morphology and physicochemical parameters. The highest berberine content was found in MRC (1.19 µg/mL). The highest phenolic content was found in MRC (64.29±2.91 mg GAE/g) and highest flavonoid content was found in MRC (18.99±0.40 mg QUE/g). Samples also showed antioxidant activity in correlation with the amount of phenolic and flavonoid content present in them (MRC had the maximum activity with IC_{50} value of 204.09±14.64 µg/mL and 8.18±0.65 µg/mL for H_2O_2 and NO free radicals). The antiurolithiatic activity was studied and was found to be maximum for the aqueous extract of ZRC with 47.15% inhibition at 100 µg/mL within the first 2 min.

Keywords: Analysis, Ayurveda, Churna, Evaluation, Herbal, Rasayana

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In the recent years due to the holistic approach in treatment, safety and effectiveness, Ayurveda has been accepted in the entire world. Ayurvedic formulations are more effective, less harmful and comparatively cost effective than the modern medicine. However, the chances of adulteration and substitution give rise to the haze of doubt and speculation about these formulations. Moreover the inherit possibility of variation in the species of plants can also lead to range of values in terms of several parameters that would directly or indirectly affect the patient compliance as well as the efficacy of the treatment. The same is discounted in the purview of modern medicine as they are subjected to strict standardization protocols and the ease of having a comparative data for analysis. This philosophy is now being strongly advocated for the herbal formulations as well to increase their easy acceptability and gaining confidence in the public domain[1]. With this objective being decided the present study aimed at a significant formulation in Ayurveda, known as Rasayana Churna. Different standardisation parameters include morphological, microscopical, phytochemical, physicochemical, spectroscopical and biological evaluation parameters. Morphological data involves appearance, colour, odour, taste and texture. In herbal drug standardisation, microscopy is an important tool. Phytochemical screening, quantitative determination of chemical constituents done by chromatographic techniques and spectroscopical data also plays an important role.

Churna is a fine powder of the drug or drugs in Ayurveda system of medicine. Churna is also called as Ayurvedic spice powder mainly used to correct any imbalance in the body. It is a mixture of different herbs with various medicinal uses[2]. Rasayana churna is an

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Ayurvedic formulation, comprising of dried powders of three rejuvenating drugs *viz.,* dried stem of Guduchi (*Tinospora cordifolia* Miers.), dried fruit of Gokshur (*Tribulus terrestris* Linn.) and dried pericarp of Amalaki (*Emblica officinalis* Gaertn.)[3]. These three have significant contribution as Rasayana agents, which in general, mean rejuvenation. The activities attributed to these include as immunomodulator, rejuvenator, adaptogen, antioxidant and diuretic. Rasayana churna has a significant role in elimination of toxins through urinary system and lowering the risk in kidney and cardiac disorders. Amalaki is a rich source of vitamin C, tannins, phenols, gallic acid and ascorbic acid thus contributes in antioxidant activity[4]. Guduchi also contributes in antioxidant activity along with immunomodulatory activity as it contains alkaloids, glycosides, steroids, and sesquiterpenoids as bioactive compounds[5]. Antiurolithiatic activity has been reported in Gokshur[6].

Considering the activities shown by individual drugs; phenolic, flavonoidal, antioxidant and antiurolithiatic studies have been performed on rasayana churna in the present work along with the morphological, microscopical, physicochemical, phytochemical and microbial studies of the in-house and marketed samples of rasayana churna in order to develop a standard protocol and make a comparison between in-house and marketed samples of rasayana churna.

Methodology

Collection of samples

Dried stem of Guduchi (*Tinospora cordifolia* Miers.), dried fruit of Gokshur (*Tribulus terrestris* Linn.) and dried pericarp of Amalaki (*Emblica officinalis* Gaertn.) were collected from Gomantak Ayurveda Medical and Research Centre, Shiroda and in-house formulation (IHRC) was prepared. Two marketed formulations were bought and labelled as MRC and ZRC.

Churna preparation

Equal proportion, 100 g each of dried stem of Guduchi (*Tinospora cordifolia* Miers.), dried fruit of Gokshur (*Tribulus terrestris* Linn.) and dried pericarp of Amalaki *(Emblica officinalis* Gaertn.) were individually dried in shade, then powdered and passed through 80# sieve. All the powders were mixed together. Using a grinder, coarse powder was made[3,7].

Organoleptic evaluation

Appearance, colour, odour, taste and texture examination was done on the samples[3].

Microscopical evaluation

Samples were cleared by boiling with chloralhydrate and later on placed in phloroglucinol and Conc. HCL for staining. It was mounted on a slide with a drop of glycerine and observed under 40x magnification using inverted microscope attached with camera[8].

Phytochemical evaluation

Samples were subjected for various phytochemical tests using qualitative reagents as per the standard procedures[8].

Physicochemical evaluation

Micromeritic properties like bulk density, tapped density, angle of repose, hausner's ratio and carr's index were evaluated[8]. The ash (total ash, acid insoluble ash, and water soluble ash), extractive values (water soluble, alcohol soluble, and ether soluble) and moisture content (loss on drying) were evaluated as per the standard procedures[9].

Preparation of alcoholic extract for TLC, HPTLC, total phenolic content, total flavonoid content, antioxidant and antiurolithiatic studies

Modified protocols were used for the analysis of TLC and HPTLC techniques[3]. 1 g of each sample was extracted with 10 mL of methanol for 24 h by the maceration process. Afterwards centrifugation was done and the supernatant liquid was taken and used further to make dilutions as required.

Thin layer chromatography

Using n-propanol: formic acid: water (90: 1: 9 v/v) as a solvent system, R_f value of standard berberine and other samples was determined[10].

High performance thin layer chromatography

Quantitative determination of berberine was done by HPTLC. 100 µg/mL of standard solution was prepared. 2, 4, 6 and 20 µL of standard and sample solution were applied using micro syringe respectively on TLC silica gel 60 F_{254} plate (6 cm x 9 cm; 0.2 mm thickness) with a band width of 7 mm and 6 mm distance between the two bands. Plate was developed in a solvent system of n-propanol: formic acid: water (90: 1: 9 v/v) in a twin trough chamber (100 mm x 100 mm) and observed under UV at 254 and 366 nm[10].

Total phenolic content

It was determined by modified folin-ciocalteau method. In this 1 mL of each concentration of the sample (20, 40, 60, 80, 100 µg/mL) was taken and to it 2.5 mL of 10% folin-ciocalteau and 2.5 mL of 7.5% sodium bicarbonate reagents were added. This mixture was incubated for 45 min and then absorbance was taken at 760 nm using UV-visible spectrophotometer. A control tube containing 1 mL methanol, 2.5 mL of 10% folin-ciocalteau reagent and 2.5 mL of 7.5% sodium bicarbonate reagent was used as a blank. Using gallic acid as a standard, a standard curve was plotted with 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/mL as different concentrations of gallic acid. Triplicate readings were taken and expressed as mg GAE/g[11].

Total flavonoid content

It was determined by modified aluminium chloride colorimetry method. In this 2 mL of each concentration of the sample (200, 400, 600, 800, 1000) was taken and to it 2 mL of 2% aluminium chloride reagent was added. This mixture was incubated for 45 min and then absorbance was taken at 415 nm using UV-visible spectrophotometer. A control tube containing 2 mL methanol, 2 mL of 2% aluminium chloride reagent was used as a blank. Using quercetin as a standard, a standard curve was plotted with 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 µg/mL as different concentrations of quercetin. Triplicate readings were taken and expressed as mg QUE/g[11].

Antioxidant assays

DPPH radical scavenging assay

In this method, DPPH reagent is employed. A standard curve of ascorbic acid was plotted using 2-20 µg/mL concentrations. 1 mL of the sample concentration, 1 mL methanol and 1 mL of DPPH solution were mixed and kept in dark for 30 min. Absorbance was measured at 517 nm using UV-visible spectrophotometer. A control tube contained 2 mL methanol and 1 mL DPPH solution. Later on percentage inhibition and IC_{50} values were calculated[12].

% DPPH radical scavenging activity
$= [(A_{control} - A_{test})/ A_{control}] \times 100$

Hydrogen peroxide radical scavenging assay

In this method, 1.5 mL of each sample concentration, 0.3 mL of 5 mM ferrous ammonium sulphate and 0.1 mL of 1 mM hydrogen peroxide were mixed and kept in dark for 5 min. Afterwards 1.5 mL of 1 mM 1,10-phenanthroline solution was added. This mixture was incubated in dark for 15 min. A control tube contained all reagents and methanol instead of the sample concentration. Absorbance was measured at 510 nm. A standard curve of gallic acid was plotted with 2-20 µg/mL concentrations. Later on percentage inhibition and IC_{50} values were calculated[13].

% Hydrogen peroxide radical scavenging activity
$= [A_{test} / A_{control}] \times 100$

Nitric oxide radical scavenging assay

This method was determined using griess reagent. A standard gallic acid curve was plotted using 2-20 µg/mL concentrations. 1 mL of sodium nitroprusside was taken and to it 1 mL of sample concentration was added. This was kept in dark for 150 min at RT and then 2 mL of griess reagent was added. It was again incubated for 20 min in dark and then absorbance was measured at 540 nm. A control tube contained all the reagents except sample concentration. Percentage inhibition and IC_{50} values were calculated[14].

% Nitric oxide radical scavenging activity
$= [(A_{control} - A_{test})/ A_{control}] \times 100$

Antiurolithiatic activity

According to Burns and Finlayson method, the artificial urine was prepared as follows: 105.5 mM of sodium chloride, 32.3 mM of sodium phosphate, 3.21 mM of sodium citrate, 3.85 mM of magnesium sulphate, 16.95 mM of sodium sulphate, 63.7 mM of potassium chloride, 4.5 mM of calcium chloride, 0.32 mM of sodium oxalate, 17.9 mM of ammonium hydroxide, 0.0028 mM of ammonium chloride were mixed together and the volume was made upto 1000 mL with distilled water. pH was adjusted to 6.0. For the sample reading, 1 mL of artificial urine and 0.5 mL of sample concentration were taken in the cuvette. Then 0.5 mL of 0.01 M sodium oxalate was added to the above solution and absorbance was measured immediately after every 2 min for 10 min period at 620 nm. For blank reading sample concentration was replaced with distilled water[15,16].

% inhibition $= [(A_{control} - A_{test})/ A_{control}] \times 100$

Biological activity

A suspension of the sample was made using buffered sodium chloride-peptone solution and then cyclomixed. For the viable count of bacteria; 1 mL of suspension, 20 mL of liquefied casein-soyabean digest agar were cyclomixed together and then poured

in the petriplate. The plates were incubated at 30-35°C for about 2-3 days and the number of colonies were counted. For the viable count of fungi; 1 mL of suspension, and 20 mL of liquefied saboraud dextrose agar (with streptomycin) were cyclomixed together and then poured in the petriplate. The plates were incubated at 20-25°C for about 5 days and the number of colonies were counted[9].

Results and Discussion

Standardisation is essential to maintain the quality of the products and this can be done by using analytical techniques that are validated. In the present study rasayana churna containing three majour ingredients, which contains three ingredients, *viz.,* dried stem of Guduchi (*Tinospora cordifolia* Miers.), dried fruit of Gokshur (*Tribulus terrestris* Linn.) and dried pericarp of Amalaki *(Emblica officinalis* Gaertn.) was prepared in-house using standard protocol. A comparison was done between in-house and marketed samples of rasayana churna in the ambit of several aspects of evaluation like morphology, microscopy, phytochemical, physicochemical and activity studies.

For the morphological assessment appearance, colour, odour, taste and texture of the samples were determined using the standard protocols[3]. IHRC is brown in colour, MRC is buff in colour and ZRC is buff-brown in colour. All the powders have a characteristic odour and bitter-sour taste. IHRC is coarse in appearance whereas MRC and ZRC are fine in appearance. Texture of IHRC is grainy whereas MRC and ZRC are smooth in texture. The results of IHRC are more in collaboration with the literature published so far on Rasayana churna, matching to the description of greenish-brown colour, characteristic odour, bitter-astringent taste, fine appearance and powdery texture[3]. Microscopical evaluation revealed different characteristics of IHRC, MRC & ZRC which are given in Fig. 1 (a-j), Fig. 2 (a-h) & Fig. 3 (a-i) respectively. IHRC showed presence of cork cells,

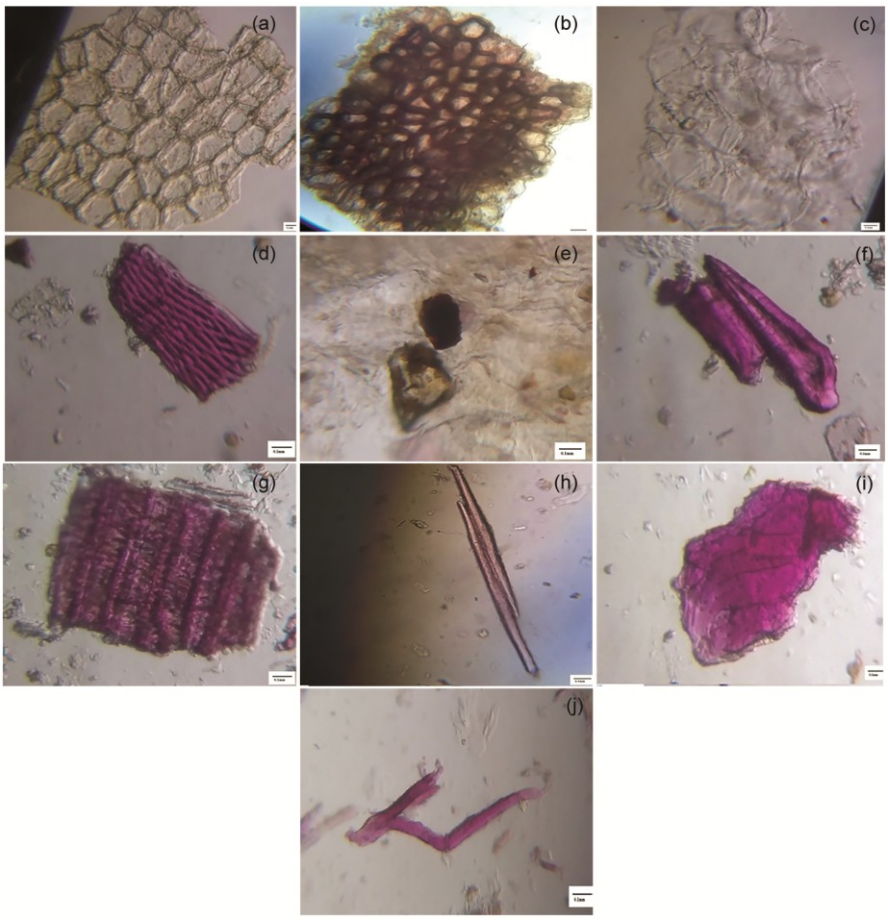

Fig. 1 — Powder microscopy of IHRC – (a) Cork cells of Guduchi (b) Collenchyma cells of Guduchi (c) Parenchyma cells of Guduchi (d) Scalariform xylem vessel of Guduchi (e) Prismatic calcium oxalate crystal of Amalaki (f) Stone cells of Amalaki (g) Spiral xylem vessels of Amalaki (h) Lignified fibres of Amalaki (i) Brachy sclereid of Gokshur (j) Lignified trichomes of Gokshur

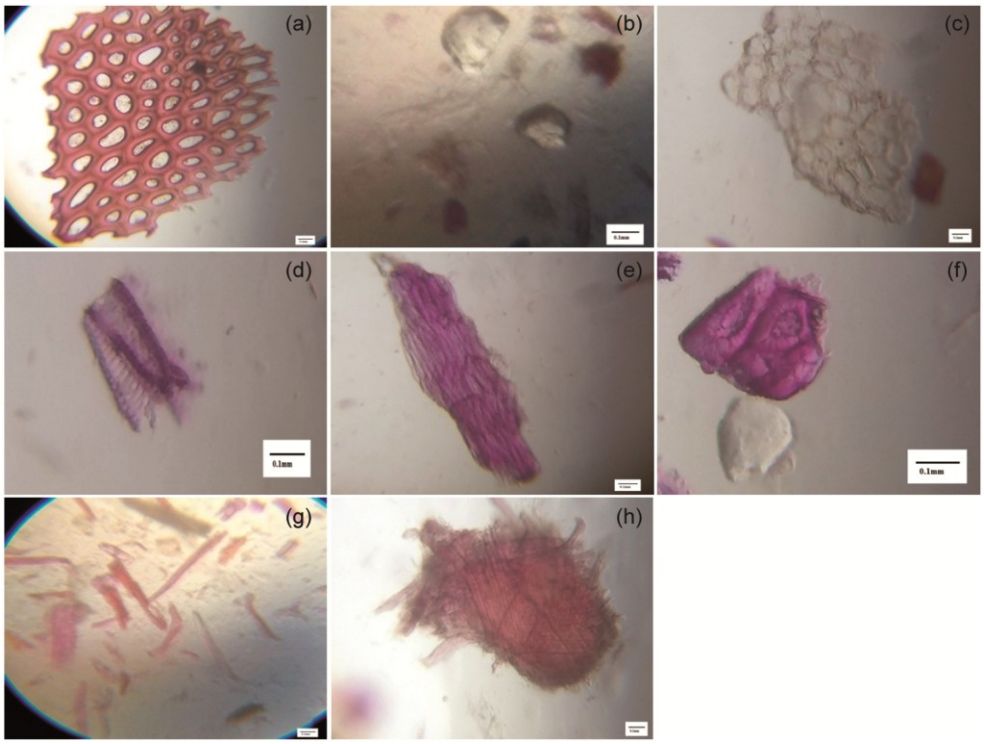

Fig. 2 — Powder microscopy of MRC – (a) Collenchyma cells of Guduchi (b) Calcium oxalate crystals of Amalaki (c) Epidermal cells of Amalaki (d) Spiral xylem vessels of Amalaki (e) Vascular bundle of Amalaki (f) Stone cells of Gokshur (g) Lignified trichomes of Gokshur (h) Brachy sclereid of Gokshur

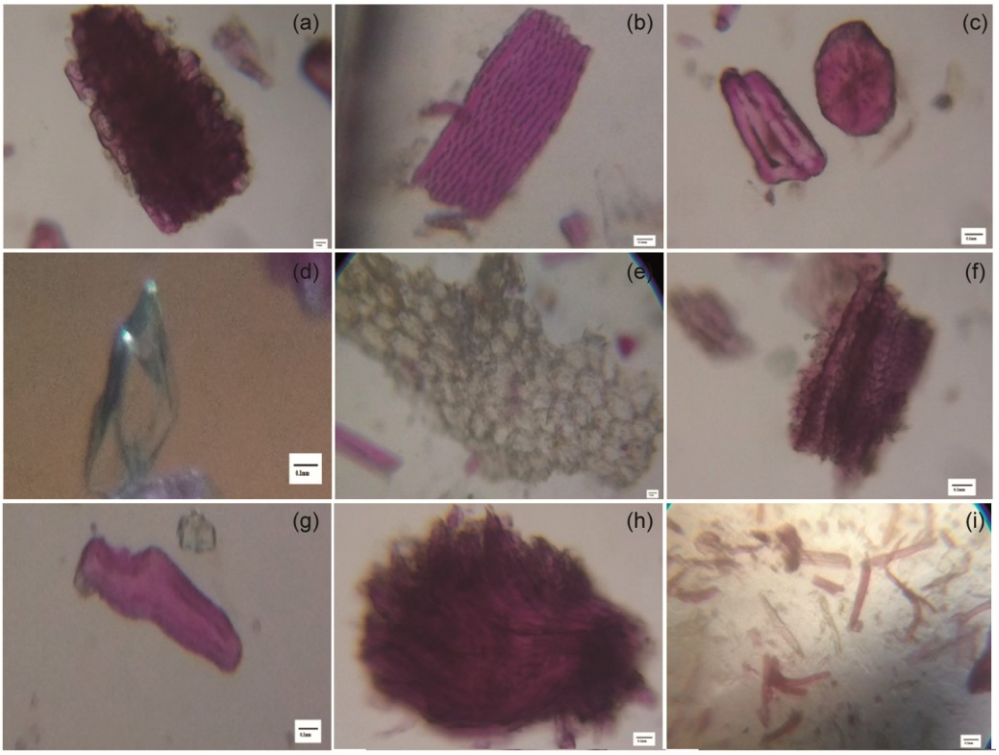

Fig. 3 — Powder microscopy of ZRC- (a) Collenchyma cells of Guduchi (b) Scalariform xylem vessel of Guduchi (c) Stone cells of Guduchi (d) Prismatic calcium oxalate crystal of Amalaki (e) Epidermal cells of Amalaki (f) Spiral xylem vessels of Amalaki (g) Sclereid of Amalaki (h) Brachy sclereid of Gokshur (i) Lignified trichomes of Gokshur

collenchyma cells, parenchyma cells, scalariform xylem vessel of Guduchi; prismatic calcium oxalate crystal, stone cells, spiral xylem vessels, lignified fibres of Amalaki; brachy sclereid, lignified trichomes of Gokshur. Thus it can be concluded that IHRC has all the ingredients present. MRC showed presence of collenchyma cells of Guduchi; calcium oxalate crystals, epidermal cells, spiral xylem vessels, vascular bundle of Amalaki; stone cells, lignified trichomes, brachy sclereid of Gokshur. This shows the presence of all ingredients in MRC. ZRC showed presence of collenchyma cells, scalariform xylem vessel, stone cells of Guduchi; prismatic calcium oxalate crystal, epidermal cells, spiral xylem vessels, sclereid of Amalaki; brachy sclereid, lignified trichomes of Gokshur. This indicated presence of all ingredients in ZRC. The three samples have been identified microscopically due to the presence of all the reported features like the prisms of calcium oxalate, sclerides, and stone cells of the stems of Guduchi, lignified trichomes significant of Gokshur and spiral xylem vessels and lignified fibres of Amalaki[3].

Phytochemical screening indicated the presence of a range of phytoconstituents in the Rasayana churna. As can be corroborated from the literature Amla is a rich source of tannins and glycosides along with alkaloids and carbohydrates[17]; Guduchi is a rich source of alkaloids and steroids along with the presence of glycosides[18]; whereas Gokhru is herb with copious amounts of saponins, flavonoids, glycosides along with alkaloids and amino acids[19]. All the three samples showed presence of all these phytochemicals.

Physicochemical tests showed some variation in the results of the three samples (Table 1). With the advancement of the Ayurvedic and herbal industry and the related market, there is more of large scale manufacture that is prevalent presently. This in turn leads to handling of large quantities of raw materials passing through different automated equipment containing size range of pipes, orifices and dies, typically like those of the modern medicine. Though this particular aspect was not much studied thus far in the case of herbal raw materials (powders), an array of physicochemical tests were performed in the present study to assess the range of powder characters of the churna samples. Micrometric analysis was performed to understand several powder characters for the samples and based on the observations the flow properties and porosity could be assessed[20]. Micromeritic studies like bulk density, tapped density, Carr's index, Hausner's ratio and Angle of repose were performed. The bulk density and tapped density were determined to calculate the Carr's index and Hausner's ratio which are influenced by size and shape, surface area, moisture content, and cohesiveness of materials. These features affect the flowability of the churnas. The range stretches from <10% to >38% for excellent and very poor flow properties with reference to the Carr's index and <1.00 to >1.60 for Hausner's ratio. From the results, Carr's index: IHRC (29.20±0.00), MRC (35.60±0.00), ZRC (39.15±0.00), Hausner's ratio: IHRC (1.41±0.00), MRC (1.55±0.00), ZRC (1.64±0.00), it was observed that all the three churnas were having poor flow ability. This can be attributed to coarseness of the powders due to different tissues present in the churna observed in the microscopy. Angle of repose is a characteristic related to interparticulate friction or resistance to movement between particles. The range varies from <30º to >65º for excellent and very poor flow properties. Angle of repose values: IHRC

Table 1 — Physicochemical characteristics of IHRC, MRC & ZRC

	Parameters	IHRC	MRC	ZRC
Micromeritic characteristics	Bulk density	0.43±0.00	0.35±0.00	0.36±0.00
	Tapped density	0.60±0.00	0.55±0.00	0.59±0.00
	Carr's index	29.20±0.00	35.60±0.00	39.15±0.00
	Hausner's ratio	1.41±0.00	1.55±0.00	1.64±0.00
	Angle of repose	38.86±0.00	48.64±0.00	47.98±0.00
Ash values (% w/w)	Total ash	22.59±1.58	14.87±1.13	33.87±1.14
	Water soluble ash	3.64±0.06	3.17±0.04	3.14±0.02
	Acid insoluble ash	1.48±0.02	1.22±0.01	1.15±0.02
Extractive values (% w/v)	Water soluble extractive	10.75±0.31	11.12±0.41	9.45±0.91
	Alcohol soluble extractive	9.70±0.10	10.48±0.16	9.32±0.23
	Ether soluble extractive	4.17±0.03	3.87±0.03	2.97±0.02
Moisture content (% w/w)		8.84±0.02	4.01±0.04	4.62±0.03

All values are expressed as mean ± S.E.M., n=3.

(38.86º), MRC (48.64º) and ZRC (47.98º) were in the range of passable to poor flow powder characteristics[18]. The inorganic content and the soluble organic content of the churnas was determined using ash values and extractive values respectively. Standard protocols were followed for the tests[7]. However, there is as such no standardized reference for these values available to indicate their limits. From the results obtained it was observed that among the three samples of the chruna, MRC possessed the minimum inorganic content as reflected by its lower ash values and higher soluble organic content in the respective solvents of water, alcohol and ether as can be deduced from the extractive values.

Thin layer chromatography was performed using n-propanol: formic acid: water (90: 1: 9 v/v) as a solvent system. Berberine is one of the prime alkaloid present in the three samples, contributed by the portion of Guduchi[10]. This is taken up as the standard as its a coloured alkaloid which can be easily detected in both the visible and UV range after resolution of the TLC plant. Since its a coloured alkaloid it does not require any further derivatisation or spraying agent to be observed. The R_f value of standard berberine and other samples is given in Table 2 and Fig. 4 (a), (b). Tracks 1, 2, 3, 4 stand for standard berberine, IHRC, MRC & ZRC respectively. High performance thin layer chromatography was carried out for quantitative determination of berberine in the samples. Mobile phase of n-propanol: formic acid: water (90: 1: 9 v/v) resulted in a good resolution of berberine with a R_f value of 0.56 and is reported in Table 2 & Fig. 4 (c), (d). Tracks 1, 2, 3, 4 stand for standard berberine, IHRC, MRC & ZRC, respectively. The overlay of the HPTLC densitogram indicated that MRC has the highest content of berberine (Table 3 & Fig. 4(e)).

The efficiency of a drug as a curative agent at large is attributed to two major pools of phytoconstituents: phenolics and flavonoids. Hence it became imperitive in the standardization protocols to incorporate the estimation of total phenolic (TPC) and total flavonoid content (TFC) of a drug. Most referred *in vitro* protocols for these estimations include modified versions using Folin Ciocalteau reagent and Aluminium trichloride methods[12]. Total phenolic content was found to be higher incase of MRC (64.29±2.91 mg GAE/g) than ZRC (40.64±2.14 mg

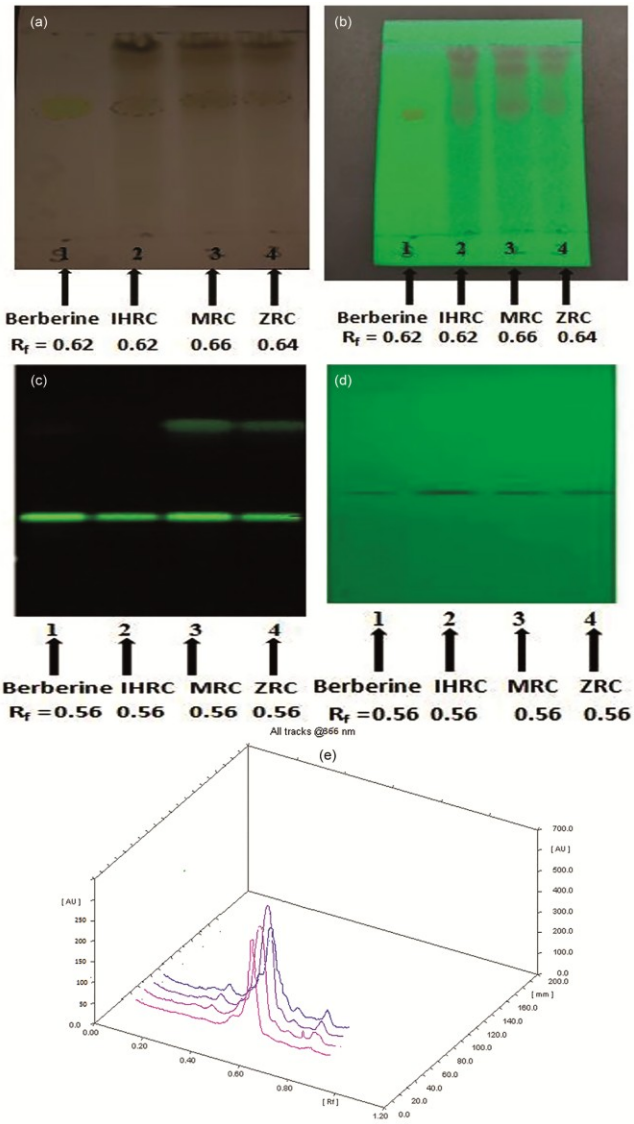

Fig. 4 — (a) TLC under white light (b) TLC under UV light (c) HPTLC under UV 366 nm (d) HPTLC under UV 254 nm (e) Overlay of HPTLC densitogram at 366 nm

Table 2 — TLC and HPTLC profile of methanolic extract of IHRC, MRC & ZRC

Sr. No.	Sample name	R_f by TLC	R_f by HPTLC	Observed berberine in solution in µg/mL
1.	Berberine	0.62	0.56	0.20
2.	IHRC	0.62	0.56	0.91
3.	MRC	0.66	0.56	1.19
4.	ZRC	0.64	0.56	0.98

Table 3 — Overlay of HPTLC densitogram at 366 nm

Sr. No.	Sample Name	Amount applied (µL)	Area	Observed Berberine in solution
1	Standard	2	585.13	0.2 µg
2	IHRC	20	530.02	0.91 µg/mL
3	MRC	20	702.08	1.199 µg/mL
4	ZRC	20	572.19	0.98 µg/mL

GAE/g) and IHRC (32.21±2.45 mg GAE/g). Total flavonoid content was found to be higher in case of MRC (18.99±0.40 mg QUE /g) than ZRC (13.39±5.07 mg QUE /g) and IHRC (8.93±0.87mg QUE /g) (Table 4).

Free radicals are produced deep down in the cellular biochemical processes, during various pathological conditions and these initiate chain reactions leading to cascade of events that manifest themselves ultimately as visible signs and symptoms. The effectiveness of the medicine in treating many of the ailments is directly attributed for its ability to counter free radicals. Hence determination of the antioxidant potential of a drug substance has become a part of the protocol for standardization[12-14]. Antioxidant activity was determined using three different methods *viz.,* DPPH radical scavenging activity, Hydrogen peroxide radical scavenging activity and Nitric oxide radical scavenging activity. The IC_{50} values are reported in Table 5. In DPPH radical scavenging activity, when DPPH (radical), violet in colour, reacts with a hydrogen donor of the antioxidant, it results in the formation of the reduced (molecular) form *viz.,* DPPH with the disappearance of the violet colour. Thus there is a decrease in absorbance linearly as there is an increase in the concentration of antioxidant[12]. In this antioxidant activity of samples is compared with standard ascorbic acid. Lesser the IC_{50} value, greater is the antioxidant activity. As per the results, IHRC (104.69±0.61 µg/mL) has a higher antioxidant activity as compared to MRC (115.72±1.35 µg/mL) & ZRC (107.07±2.63 µg/mL).

Hydrogen peroxide radical scavenging activity is a very important assay used in determination of antioxidant activity. Hydrogen peroxide (H_2O_2) is a weak oxidizing agent. It can cross cell membranes rapidly and react with Fe^{2+} and Cu^{2+} ions to form hydroxyl radical. Thus it results in toxic effects. If scavenger has a good scavenging activity and scavenges H_2O_2 then it won't allow the conversion of ferrous to ferric ion forming the reddish-orange complex. But if the scavenger has poor scavenging activity then H_2O_2 will bring about oxidation of ferrous to ferric ion resulting in the loss of reddish-orange colour. Thus the intensity of the colour produced is directly proportional to the concentration of the scavenger and the efficient scavenging activity[13]. In this antioxidant activity of samples is compared with standard gallic acid. As per the results, MRC (204.09±14.64 µg/mL) has a higher antioxidant activity as compared to IHRC (232.35±18.46 µg/mL) & ZRC (248.17±19.00 µg/mL). This means that the constituents present in sample possessing good scavenging activity, scavenge H_2O_2 thus do not allow conversion of ferrous to ferric ion forming the reddish-orange complex.

In nitric oxide radical scavenging assay, Griess reagent is used. Sodium nitroprusside at physiological pH (7.2) in the aqueous media spontaneously decomposes to produce nitric oxide. This nitric oxide reacts with oxygen and produces nitrate and nitrite ions (stable). The presence of these nitrite ions is determined by Griess reagent[14]. In this antioxidant activity of samples is compared with standard gallic acid. As per the results, MRC (8.18±0.65 µg/mL) has a higher antioxidant activity as compared to IHRC (24.66±1.00 µg/mL) & ZRC (48.29±1.39 µg/mL). This means that the constituents present in sample possessing antioxidant activity do not allow formation of nitrite by competing with oxygen. In the later two methods IC_{50} of MRC was found to be less than IHRC and ZRC. This indicates that MRC has a higher antioxidant activity as compared to IHRC and ZRC. This result corroborated with the results obtained for TPC and TFC.

Amalaki is rich in hydrolysable tannins like Emblicanin A and B, chebulinic acid, chebulagic acid, corilagin, phenolic compounds like gallic acid, ellagic acid, amino acids like aspartic acid, vitamins like ascorbic acid, flavonoids like quercetin, kaempferol[17]; Guduchi contains alkaloids like tinosporine, palmatine, berberine, isocolumbin, terpenoids like tinosporide, furanolactone diterpene, flavonoids like (-)epicatechin[18] and Gokshur contains flavonoids like kaempferol, rutin, isorhamnetin, quercetin, alkaloids

Table 4 — Total phenolic and total flavonoid content of IHRC, MRC & ZRC

Sr no	Sample name	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QUE/g)
1	IHRC	32.21±2.45	8.93±0.87
2	MRC	64.29±2.91	18.99±0.40
3	ZRC	40.64±2.14	13.39±5.07

All values are expressed as mean± S.E.M., n=3.

Table 5 — IC_{50} values of IHRC, MRC & ZRC (µg/mL)

Sr no	Sample name	DPPH assay	H_2O_2 assay	Nitric oxide assay
1	Standard	10.97±0.52	28.72±3.66	4.69±0.99
2	IHRC	104.69±0.61	232.35±18.46	24.66±1.00
3	MRC	115.72±1.35	204.09±14.64	8.18±0.65
4	ZRC	107.07 ±2.63	248.17±19.00	48.29±1.39

All values are expressed as mean± S.E.M., n=3.

like β- carboline alkaloid *viz.,* tribulusterine, steroidal saponins like spirostanol and furostanol[19]. These phytoconstituents contribute in antioxidant activity. Phenolic and flavonoid content directly contributes to the antioxidant activity and thus a positive correlation can be reported between phenolic, flavonoid and antioxidant activity. MRC showed higher amount of phenolic and flavonoid content thus possessing higher antioxidant activity than IHRC & ZRC.

Antiurolithiatic activity is performed on the aqueous, methanolic extracts of the samples and standard drug allopurinol. The graphs showing their antiurolithiatic activity are shown in Fig. 5 (a), (b), (c) respectively. Aqueous extract of IHRC, MRC & ZRC have shown maximum inhibition at 200 µg/mL *i.e.,* 53.84%, 52% and 63.21% respectively within starting 2 min. Alcoholic extract of IHRC has shown maximum inhibition of 26.10% at 100 µg/mL after 10 min, alcoholic extract of MRC has shown maximum inhibition of 42.03% at 200 µg/mL after 10 min whereas alcoholic extract of ZRC has shown maximum inhibition of 47.51% at 100 µg/mL within starting

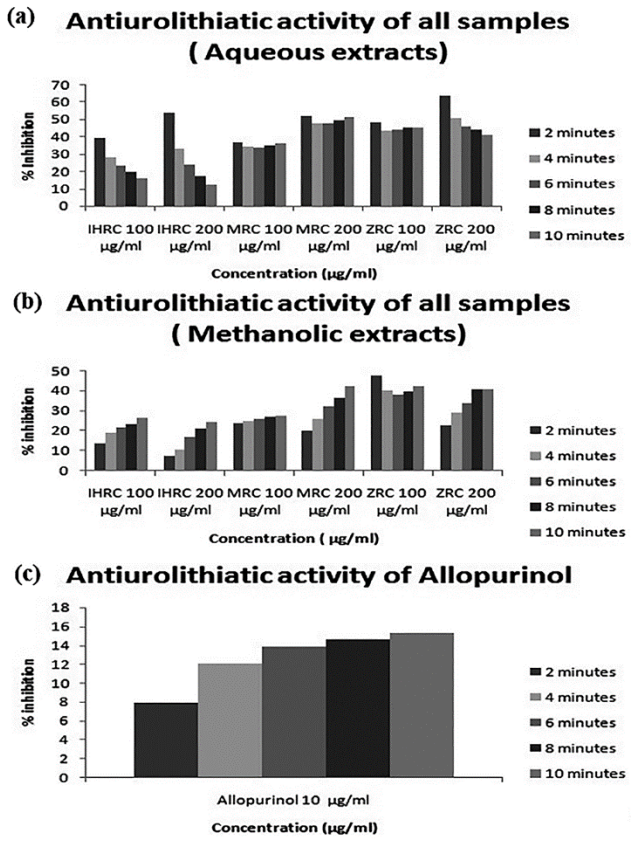

Fig. 5 — (a) Antiurolithiatic activity of all samples (aqueous extracts) (b) Antiurolithiatic activity of all samples (methanolic extracts) (c) Antiurolithiatic activity of standard drug Allopurinol

2 min. Standard allopurinol has shown maximum inhibition of 15.34% at 10 µg/mL after 10 min. This indicates that all the samples have antiurolithiatic activity as they inhibit the formation of crystals in urine thereby reducing turbidity. Flavonoids have diuretic activity and saponins have anti-crystallization activity. Thus these phytochemicals contribute in the antiurolithiatic activity.

Total microbial load is found within WHO limits for churna formulations.

Conclusion

Traditional knowledge is slowly but steadily regaining a strong foothold in the present day health care. The ability of the Ayurvedic formulations to counter several chronic ailments gave boost to the confidence of the patients. However, there is a scope given to speculate the content of the active drugs present in a formulation due to the variations observed in different sources of raw materials. This aspect can be well justified with the help of standardised protocols for various formulations[1]. A variety of research is being undertaken to encompass several fields of evaluation parameters. The present study was one such attempt compiling a range of data for the Ayurvedic formulation-Rasayana Churna. This formulation has a significance in benefitting the health with respect to vision, kidney function, hearth function and overall as a rasayana[2,3]. The present work involved three different samples of the same formulations, one in house and two marketed for comparison. The purpose of comparison was primarily to establish the variations observed in the final product and to define some limits for all the parameters. The assessment encompassed different types of evaluation *viz.,* morphological, microscopical, phytochemical, physicochemical, spectroscopical and biological investigation. Morphological, microscopical and phytochemical data helped to build a qualitative perspective of the formulation; whereas, physicochemical and spectroscopical data along with Chromatography helped in quantitative estimation of the formulation. One of the primary activities of the churna as an antiurolithic has also been estimated which can be used as reference to judge the effectiveness of the formulation.

Traditional medicine has regained its popularity by able to counter several chronic ailments. Being herbal in origin, it is tough to create a stringent set of limits for all the evaluation parameters for its formulations. However, there are guidelines devised by WHO for all

the procedures right from the stage of cultivation, collection, processing and packing of the raw materials. Implementation of these would give rise to robust circumstances for development of this domain of health care system. In the present analysis, the marketed formulation MRC was found to be significant in its activity as compared to the in-house preparation IHRC and another marketed formulation ZRC. The variations can be well attributed to the difference in the original raw material used which would vary based on several parameters *viz*., climate, soil, processing etc. Analyzing samples from different manufacturers and making a comparision would assist in designing a range of values for all parameters. Developing a measurable range is the need of the hour, with which the final products can be compared with. This signifies the role of standardisation as an important tool in the quality maintenance of the products.

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Conflict of Interest

There are no conflicts of interest.

Author Contributions

Sharavari Kamat Shankwalkar has carried out all experimental part and writing of this manuscript. Mythili Krishna Jeedigunta has supervised the entire experiment and manuscript writing. Prachi Korpardekar and Lalit Paranjape have helped in experimentation. Bishnupriya Mohanty has helped in the concept and literature of the experiment.

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