Analytical study of Ajmodadi Churna With Different Permutations

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Abstract: For churna (powder) formulations used in Ayurveda, mixing pattern in market practices is not after looking to shloka or clues mentioned with texts, but it is at once using blender. But shloka might be leaving clues for ordered mixing, which is well attended by modern pharmaceutical science with different nature of compounds. Ingredients of Ajmodadi churna are having dominating compounds with different nature. The change in pattern of mixing brings changes in flow properties of powder, physicochemical tests and quantitative tests, however preliminary phytochemical tests show similar result in all permutations.

Keywords: Churna, powder, Ajmodadi churna, mixing pattern, shloka.....

Churna is one of formulation mentioned under panchakashaya kalpana in Charak Samhita[1] and shadvidhakashaya kalpana in Sushruta Samhita[2]. Sharanghadhara Samhita defines churna as, 'dried drugs which are powdered separately and filtered through clean cloth to get the homogenous mixture'[3]. This is also called as raja or kshodha[3]. Churna is nothing but a pharmaceutical powder and a pharmaceutical powder is defined as dry, solid substance, consisting of a large number of finely divided particles (varying 10nm-1000µm), and typically obtained by crushing, grinding or by comminuting[4]. Nature and properties of compounds inherited by herbal churna (powder) as single formulation give remedial actions. In case of formulation or mixture of more than one herbal churna, various factors including adhesion, reactions etc. work together to bring the final effect.

In Ayurveda, many of compound herbal churna are used. Ingredient drugs, method of preparation etc. are given in shloka form. For selected formulations, sequence names of herbs are not changing in any book while for some formulations sequences are observed altered as viz., Shaddharana yoga, Samangadhi churna, Shangadhi churna, Katphaladi churna, Kanaadyam churna, Krishnadhi churna, Katphaladi churna, Karanjadi churna, Karkadi beejaadi churna, Ajamodadi churna, Chitrakadi churna, Candana churna, Sitopaladi churna etc. are formulations without changes whereas Krisnadi churna, Balachaturbadra churna, Naracha churna, Talisadi churna, Hingvastaka churna etc. are formulations with changes. Ingredients pay important role in changing of pattern means according to placement of ingredients in shloka, specific clues are assumed as ordered mixing.

The ordered mixing is described in steps including breaking of agglomerated particles of one material during mixing process followed by carrier particles[5]. In herbal combinations, major ingredients may play the same role of enhancing or masking the characteristics of some compounds as adsorption of microionized sodium bicarbonate particles on to larger sucrose crystals are proved facts[6]. Mixing is dynamic equilibrium process between one adhesive and non-adhesive mixtures[7] which indicates the changing characteristics of ingredients in formulations. It means a formulation with varying dominant constituents of diversified nature should be attended for ordered mixing. Ajmodadi churna is one formulation with changing ingredients viz. Ajmoda (volatile oil and protein), Mochrasa (Gum), Shunthi (Starch and Volatile oil) and Dhataki (more sugar). Whether the quality of formulation changes by altering the sequencing depending upon the nature of constituents of drugs, their surface, cohesion, adhesion, shear forces betw-

een particles etc. The idea behind selection of this work is to prove this fact.

Materials and Methods: Mocharasa was procured from SP Kajrekar pharmacy, Hindwadi, Belgaum while Ajmoda, Dhataki and Shunthi were obtained from PKM pharmacy, Kunnur, Kerala. After confirming the genuine status based on pharmacopeial standards from Quality Control Laboratories, ALNRMAMC, Koppa, they were taken for study. Drugs were powdered by using *Preethi Platinum Mixer Grinder*. Thereafter, they were sieved through sieve number 125 to find fine powder[8]. Blending pattern was calculated alphabetically.

Permutations: 40 gm of individual drug was used in every permutation. Blending was done with mortar and pestle. The permutations were noted as AMSD, AMDS, ASDM, ASMD, ADMS, ADSM, MSDA, MSAD, MDSA, MDAS, MASD, MADS, SDAM, SDMA, SAMD, SADM, SMDA, SMAD, DAMS, DASM, DMSA, DMAS, DSAM, DSMA and all at once. These permutations were analyzed for flow properties, physicochemical parameters, preliminary phytochemical qualitative tests and quantitative tests. Among permutations, A means Ajmoda, M stands for Mocharasa, S is for Shunthi and D belongs to Dhataki.

1. Flow properties of permutations[9]: Bulk density, tapped density, compressibility index, hausner ratio and angle of repose were measured for all permutations by the methods mentioned in *United State Pharmacopeia*.

2. Tests based on physicochemical parameters[9,10,11]: Total ash, acid insoluble ash, water soluble extractives, and alcohol soluble extractives were given as per methods given in *Ayurvedic Pharmacopoeia of India*.

3. Preliminary Phytochemical Tests[9,10,11,12,13,14,15,16]: Fehling's test for carbohydrate, biuret test for protein, borntrager's test for glycoside, salkowoski test for triterpenoids, ferric chloride test for phenolic compounds and dragendorff's test for alkaloid were done.

4. Quantitative estimation: For all spectrophotometric estimation UV-Visible spectrophometer of *Systronic 106* was used and chemicals used were from Merck (India).

a. Protein Estimation[17]: 10 gm of each permutation taken with distilled water. It was centrifuged at 2000 rpm for 15 minutes, and then supernatant was collected. It was diluted to 1 liter. All supernatants were mixed. For standard, 1 gm of protein powder of Leochem was mixed with 1 liter of distilled water. From this mg/10 mL solution of 1, 2, 3, 4, 5, and 6 were prepared. They were added with 2.5 mL of biuret reagent. In another test-tube, 0.1 mL of drug sample was mixed with 2mg/ 10 mL of standard and then it was added with 2.5 mL of reagent. Now absorbance was read on 550 nm. From calibration curve of standard, concentration of sample was measured.

b. Total Sugar[18]: 10 gm of each permutation was dissolved with 80% ethanol for 24 hrs as cold extraction. Next day, it was centrifuged at 2000 rpm for 15 minutes and supernatants were collected. All supernatants were mixed and evaporated. Now it was dissolved in 5ml of water and was brought to 100 ml with 2.5N HCl. It was allowed to hydrolyze on water-bath for 3 hours. After hydrolysis, it was dissolved to make 1 Liter solution. 10 ml of this was taken and dissolved to make 100 ml with distilled water.

For standard solution 100 mg of glucose (Merck) was dissolved in 1 Liter of distilled water. 10 mL of this was taken to make 100 mL with distilled water. From this standard containing 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 mg/mL were prepared. 1 ml of standard (with distilled water for specified concentration) and 1 mL of material was taken in separate test-tubes. They were added with 1 mL of 5% phenol solution followed by 5 mL of concentrated sulphuric acid. They were mixed well and absorbance was noted at 490 nm.

c. Reducing Sugar[19]: 10 gm of each permutation was taken in 80% ethanol for cold extraction and after that extract was obtained. Next day, it was centrifuged at 2000 rpm for 15 minutes and supernatants were collected. All supernatants were mixed and evaporated. Now it was dissolved in 5ml of water and was brought to 1 liter. 1 ml of material was taken and it was made to 10 ml with distilled water to find the concentration.

250 mg of glucose solution was taken and volume was made to 100 ml with distilled water. Now volume was taken in different test tube make the volume 0.2-2 mg/ml. 1 ml of DNS reagent was added to Glucose sample in a lightly capped test tube (to avoid loss of liquid due to evaporation, the test tube was covered with a piece of . . . . . ^{o}C for 5-15 minutes to develop the red brown colour. Now 1 ml of a 40% potassium sodium tartrate (Rochelle salt) was added to solution to stabilize the colour. After cooling to the room temperature in a cold water bath, record the absorbance with a spectrophotometer at 550 nm.

d. Total Flavonoids[17]: 50 gm material was taken in methanol-water mixture (7:3). They were extracted by soxhlet extraction method. The extract was used for determination. The aluminum chloride method was used for the determination of the total flavonoid content of the sample extracts. Aliquots of extract solutions were taken and made up the volume 3ml with methanol. Then 0.1ml AlCl3 (10%), 0.1ml potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

e. Estimation of the Concentration of elements (Calcium, Sodium, Potassium) by Flame photometer at different stages[20]: Flame Photometer Systronic 128 and FPM Compressor 126 were used. 1000 ppm of standard solutions was prepared by sodium chloride, potassium hydroxide and calcium chloride. Sodium and Potassium were prepared for calibration at concentration of 80 ppm, 60ppm, 40ppm and 20ppm while calcium was prepared with concentration 80ppm, 60 ppm and 40 ppm. After calibration, the solution containing different concentrations of sodium chloride, potassium chloride and calcium were introduced to find the intensity of the emitted light of each solution. A calibration graph between concentration and intensity of the solutions were drawn for each case. Finally the different permutations were brought to find out the intensity of emitted radiation. From the intensity displayed with screen, the concentration of calcium, sodium and potassium were determined. For preparation of material, 10 . . . . . $_3$ was added and was refluxed for 15 minutes. Now it was dissolved to 50 ml. By this solution estimation was done. The calculation has been done by using Microsoft excel making calibration curve.

Result: 1. Flow properties of different permutations

Table Number: 1

Showing Flow Properties of Different Permutations

Permutations	Bulk density	Tapped density	Compressibility index	Hausner ratio	Angle of repose
AMSD	0.414	0.556	25.29	1.37	44.67
AMDS	0.419	0.600	29.86	1.42	49.67

Permutations	Bulk density	Tapped density	Compressibility index	Hausner ratio	Angle of repose
ASDM	0.443	0.616	28.10	1.39	51.33
ASMD	0.460	0.620	25.93	1.35	51.33
ADMS	0.463	0.662	29.81	1.43	58.33
ADSM	0.463	0.645	28.20	1.39	56.00
MSDA	0.480	0.650	26.07	1.35	55.00
MSAD	0.430	0.672	36.15	1.56	56.67
MDSA	0.460	0.655	30.06	1.43	52.50
MDAS	0.456	0.666	31.52	1.46	53.00
MASD	0.442	0.650	31.98	1.47	53.33
MADS	0.442	0.651	32.10	1.47	55.33
SDAM	0.432	0.622	30.24	1.44	54.00
SDMA	0.426	0.565	28.02	1.39	71.33
SAMD	0.413	0.626	33.75	1.51	55.00
SADM	0.418	0.615	32.08	1.47	53.00
SMDA	0.400	0.624	36.08	1.56	53.67
SMAD	0.421	0.637	33.92	1.51	69.00
DAMS	0.414	0.648	36.09	1.56	54.67
DASM	0.413	0.624	33.76	1.51	54.67
DMSA	0.400	0.624	36.00	1.56	53.33
DMAS	0.410	0.640	36.09	1.56	49.00
DSAM	0.410	0.638	35.89	1.56	52.67
DSMA	0.381	0.635	39.96	1.66	52.00
All at once	0.386	0.644	40.00	1.67	50.67

2. Tests based on physicochemical parameters

Table Number: 2

Showing Observation of Phjysicochemical Parameters

Permutations	Total ash	Acid insoluble ash	Water soluble extractives	Alcohol soluble extractives
AMSD	10.75	2.00	23.17	6.75
AMDS	10.91	2.10	21.92	5.96
ASDM	8.83	2.31	22.72	5.53
ASMD	10.25	2.10	22.49	5.57
ADMS	10.50	2.10	21.29	6.35
ADSM	10.50	2.35	21.83	6.50
MSDA	9.25	1.76	22.10	5.78
MSAD	8.75	1.95	23.08	6.45
MDSA	8.58	2.26	22.03	6.62
MDAS	10.00	2.50	21.93	5.66
MASD	10.08	2.36	22.60	5.60
MADS	11.75	2.20	22.37	6.35

SDAM	9.50	2.35	22.60	5.93
SDMA	9.25	2.13	22.11	5.73
SAMD	8.75	2.43	22.78	6.84
SADM	11.83	2.35	22.52	5.50
SMDA	8.75	2.23	23.96	5.92
SMAD	8.58	2.11	22.40	5.68
DAMS	9.08	2.08	22.05	6.40
DASM	9.70	2.42	22.04	5.85
DMSA	9.25	2.26	22.10	6.00
DMAS	9.83	2.00	21.67	5.90
DSAM	10.75	2.10	21.88	5.20
DSMA				

3. Preliminary Phytochemical Tests:

Permutations	Carbohydrate	Protein	Glycosides	Triterpenoids	Phenols	Alkaloids
AMSD	+	+	+	+	+	+
AMDS	+	+	+	+	+	+
ASDM	+	+	+	+	+	+
ASMD	+	+	+	+	+	+
ADMS	+	+	+	+	+	+
ADSM	+	+	+	+	+	+
MSDA	+	+	+	+	+	+
MSAD	+	+	+	+	+	+
MDSA	+	+	+	+	+	+
MDAS	+	+	+	+	+	+
MASD	+	+	+	+	+	+
MADS	+	+	+	+	+	+
SDAM	+	+	+	+	+	+
SDMA	+	+	+	+	+	+
SAMD	+	+	+	+	+	+
SADM	+	+	+	+	+	+
SMDA	+	+	+	+	+	+
SMAD	+	+	+	+	+	+
DAMS	+	+	+	+	+	+
DASM	+	+	+	+	+	+
DMSA	+	+	+	+	+	+
DMAS	+	+	+	+	+	+
DSAM	+	+	+	+	+	+
DSMA	+	+	+	+	+	+
All at once	+	+	+	+	+	+

(Continued to next edition.....)

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4. Quantitative estimation:

Table Number: 4

Showing Quantity of Protein, Total Sugar, Reducing Sugar and Flavonoids in Different Permutations

Permutations	Protein (gm/100gm)	Total Sugar (gm/100gm)	Reducing Sugar (gm/100gm)	Flavonoids (mg Quantity Equivalent/gm)
AMSD	1.25	12.80	8.25	191.26
AMDS	1.20	13.75	8.37	173.25
ASDM	0.71	13.90	8.40	161.25
ASMD	1.10	12.77	8.30	186.45
ADMS	1.15	13.10	8.30	175.80
ADSM	1.14	13.05	8.35	163.55
MSDA	1.01	12.10	8.10	180.10
MSAD	0.95	12.25	8.15	183.20
MDSA	0.85	12.50	8.14	179.56
MDAS	1.06	12.55	8.20	163.65
MASD	1.10	11.85	8.05	180.17
MADS	1.35	12.20	8.10	183.59
SDAM	0.99	13.75	8.45	162.34
SDMA	0.80	13.35	8.35	178.90
SAMD	0.72	13.26	8.20	184.56
SADM	1.65	13.65	8.40	178.76
SMDA	5.01	11.28	7.95	185.50
SMAD	0.74	11.21	8.05	191.53
DAMS	1.69	12.89	8.25	159.20
DASM	1.09	14.10	8.76	162.35
DMSA	0.92	13.13	8.55	169.95
DMAS	1.02	13.35	8.43	167.85
DSAM	1.42	13.79	8.40	160.45
DSMA	0.63	13.65	8.35	174.50
All at once	1.21	13.10	8.28	177.54

Table Number: 5

Showing Quantity of Sodium, Potassium and Calcium in Different Permutations

Permutations	Sodium (mg/500mg)	Potassium (mg/500mg)	Calcium (mg/500mg)
AMSD	2.54	1.71	0.40
AMDS	1.05	1.00	0.30
ASDM	0.64	2.52	0.21

ASMD	1.39	3.54	0.39
ADMS	1.45	0.92	0.47
ADSM	1.77	0.89	0.56
MSDA	1.15	1.44	0.30
MSAD	0.78	1.13	0.20
MDSA	0.91	1.05	0.20
MDAS	1.15	1.32	0.29
MASD	0.60	1.20	0.12
MADS	1.62	2.72	0.40
SDAM	0.90	1.15	0.20
SDMA	0.66	1.73	0.26
SAMD	0.42	0.58	0.64
SADM	1.84	1.27	0.15
SMDA	0.31	1.42	0.26
SMAD	0.53	1.74	0.29
DAMS	0.69	0.83	0.38
DASM	0.73	1.06	0.33
DMSA	0.62	1.01	0.52
DMAS	1.09	1.19	0.53
DSAM	0.81	1.43	0.19
DSMA	0.33	0.47	0.41
All at once	0.62	1.39	0.39

Discussion: Mixing means to make homogeneous product for similarity in appearance and quality. The process follows a "disorder to order" concept. This is termed as ordered mixing. Ordered mixtures are frequently more homogenous than random mixtures and, in certain cases, may offer a better approach to practical mixing problems[21]. This might be idea behind similar sequence of component drugs mentioned for formulation in Shloka forms depending upon formulations. Same sequence is not true for all cases, but with specific formulations with herbs having different nature of compounds viz., resin with volatile oil, gum with volatile oil etc. So for present study, problem was selected screening through nature of compounds as Ajmoda is rich in volatile oil, while mocharasa in gum. Shunthi is combination of volatile oil, starches etc. whereas Dhataki pushpa is having more of carbohydrates.

Bulk density (BD) is mass of many particlesof the material divided by the total volume they occupy. The [22]. It is freely settled volume of specific mass. The changes with bulk density was observed while changing the permutations. Maximum BD was for permutation MSDA while minimum was for DSMA. Even after the similar component drugs of same qualities the BD changes, it may be due to change in intermolecular forces after altering the sequence as the nature of compounds is different. Such changes might be due to adhesion and cohesion i.e, tendencies of dissimilar and similar particles to cling together.

The tapped density (TD) is an increased bulk density attained after mechanical tapping of container containing the powder sample[22]. It differed among the permutations. The tapped density was maximum in MSAD and minimum in AMSD. Other than cohesion and adhesion, stress and strain are basic reasons after the compaction. It might be due to elastic, plastic, or fracture properties of molecules or complexes formed in between. Elastic deformation is reversible but plastic deformation and fractures are permanent and irreversible. Typical stress-strain behavior includes initial elastic deformation, yielding, and plastic deformation, followed by hardening and eventual fracture. Brittle fra-

cture occurs when applied stress exceeds the elasticity limit[23].

Based on both bulk density and tapped density, Hausner ratio (HR) and Carr's index (CI) are measured. A Hausner ratio of 1.25 indicates poor flow ability[22] whereas a Carr index greater than 25 is considered to be an indication of poor flowability, and below 15, of good flowability[22]. The angle of repose (AoR) or the critical angle of repose of a granular materialis the steepest angle of descent or diprelative to the horizontal plane to which a material can be piled without slumping. This function is somewhat accurate for piles where individual objects in the pile are minuscule and piled in random order[23]. From flow properties point of view, AoR value of less than 30° is most desirable while greater than 55° may indicate serious flow problems[24].

Total ash, acid insoluble ash, and extractive values (both alcohol and water soluble) were not differing too much. Even qualitative tests did not show promising differences.

In present formulation, the quantity of protein was maximum in SMDA being 5.01gm/100 gm while minimum in DSMA as 0.63 gm/100gm. There was not much differentiation in total sugar and reducing for all permutation. Even flavonoid contents differed slightly among the permutations in mg quantity equivalent/gm.

Electrolytes become important as they are greater cause behind the balancing of absorption and secretion. Sodium concentration was maximum with classical reference (AMSD) being 2.55 mg/500mg while it was minimum with SMDA being 0.30 mg/500 mg. For potassium, maximum concentration was observed with ASMD and minimum was for DSMA. For calcium, not much differentiation was noted among the different permutation.

Looking to the composition of each ingredient of selected sample before and after formulation, differentiation among the values for selected parameters were noted. Classically suggested permutation leaves better clues with comparatively better performance based on chemical analysis. Pharmacological works with similar idea may give more strength to reason behind such shloka for ordered mixing.

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