



PHYSICO CHEMICAL ANALYSIS OF PANCHAVAKTRA RAS: A HERBO-MINERAL FORMULATION

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ABSTRACT

Panchavaktra Ras is a rational combination of Rasadravyas and Kashaushadhis prescribed in the management of Amavata. Panchavaktra rasa has been taken into consideration for its Pharmaceutical standardization through Standard Operative procedures. In pharmaceutical study, the drug has been prepared in 3 batches adopting Khalviya Rasayana method and physico chemical analysis was carried out on these batches. This formulation was analysed by using Inductively Coupled Plasma with Optical Emission Spectroscopy (ICPOES) and found the major elements Hg, S, B, Fe, Cr, Cu, Ag, C, Pb, As from medicine. The heavy metals like As, Pb and Cd were almost within permissible limits. Organoleptic features of drug are found dark grey astringent taste and pungent odour which is made of the crude drugs within the standard range. The pH of compound was 8.93-8.99 and alkaline in nature. The phytochemical screening showed the presence of Alkaloids, Saponins, Flavanoids, Tanins and Triterpenoids in this drug. The Panchavaktra Ras was subjected to estimation of Microbial contamination which was within normal limits and it does not contain harmful microbes like Enterobacteriaceae, E.coli, Salmonella species. Efforts have been made to fix the analytical standards of Panchavaktra ras, which were not found, reported till date.

Keywords: Panchavaktra Ras, Herbo-mineral Formulation, Physico-chemical Analysis, ICPOES Analysis, EDAX Analysis

INTRODUCTION

Khalviya rasayana (mortar pestle apparatus used medicinal preparations) is the one among different preparations, Khalviya method is a basic procedure applicable to all Rasaushadhis (Mercurial medicines) before they are subjected to any specific procedure. It is a simple process to convert crude drugs i.e. macro to micro level and also gives specific Samskara (stages of processing). Purification of raw material has to be done first with specified and authentic method then after the drugs are mixed in khalva yantra and Mardhan (grinding) was done by adding liquid materials like herbal juices, certain period of well grinded material made into pills, these pills was used for the treatment purpose. Before the clinical study the final drug has to undergo some analytical procedure. Panchavaktra ras is a herbomineral compound prepared with authentic procedures was also analysed.

Physico-chemical analysis of Panchavaktra Ras was carried out in the SIRIS Impex, LAILA Impex and IMIS Pharmaceuticals, Vijayawada. Analytical parameters for finished drug were Organoleptic properties, Diameter, Hardness, Weight variation and Disintegration time and Physicochemical parameters including Loss on drying ash value, Acid insoluble ash, Water soluble extractive, Alcohol soluble extractive, pH value and Estimation of microbial contamination. ICPOES, EDAX analysis was carried out in the Defense Metallurgical Research Laboratory (DMRL), Kanchanbagh, Hyderabad, AP., India

In the name of Panchavaktra ras, various formulations are available in ayurvedic texts. This formula is taken from well known Andhra Telugu book, Basavarajeeyam in the treatment of Amavata (Rheumatoid Arthritis) disease¹.

MATERIAL AND METHODS

Panchavaktra ras consists of equal parts of purified Parada² (Mercury-Hg), Gandhaka³ (Sulphur-S), Tankana⁴ (Borax-Na₂B₄O₇·10H₂O) and Pippali (*Piper longum* Linn.), Marica

(*Piper nigrum* Linn.), the juice of Krishna dhatura leaves (*Datura metel* Linn.) is used for Bhavana (maceration). (Figures. 4 to 10)

All these ingredients were procured from the local market of Vijayawada, Andhra Pradesh, India and all the herbal and mineral materials were checked & confirmed by experts of Rasashastra, Dr. N.R.S. Govt. Ayurvedic College, Vijayawada, AP.

Kajjali (Figure. 6) is prepared initially with one part of Parada and Gandhaka then added to one part of each purified Tankana, Pippali and Maricha, Bhavana (Impregnation or Maceration) is given with Krishna Dhatura patra swarasa (Juice of Black Datura metel leaves). (Figure. 11-14) Standardization of the pharmaceutical preparation of Panchavaktra Ras by preparing in three seasons and analytical study of three samples were carried out.

Physico Chemical Standardization

Organoleptic Characters

The organoleptic characters of Ayurvedic drugs are evaluating the qualities of preparation by color, touch, fineness, taste, odor, etc. were noted through Jyanendriya and it is providing the idea about the quality of different formulations without using chemical tests. The colour taste and odour was depending on the ingredients and bhavana drugs which are added in the formulations.

The present drug is having the dark grey colour, astringent taste and pungent odour because of the Pippali, Marica and juice of Black Datura metel leaves which is added. The final product was made tablet form, which was biconvex round uncoated, plain on both sides. (Figure. 15)

Physico Chemical Analysis⁵⁻¹²

Physico-chemical parameters such as Total- ash, Acid-insoluble ash, Alcohol- soluble extractives, Water-soluble extractive, Volatile matter, Moisture content, Ph value & Loss on drying methods were followed according to the

Ayurvedic pharmacopeia and Indian Pharmacopoeia. (Table 1)

Table 1: Physico-chemical parameters

Parameter	Sample-1	Sample-2	Sample-3
Total- ash	24.01% w/w	22.21% w/w	24.09 w/w
Acid- insoluble ash	1.20% w/w	2.15% w/w	1.82% w/w
Alcohol- soluble extractive	6.40% w/w	7.23% w/w	6.88% w/w
Water-soluble extractive	15.05% w/w	16.48% w/w	17.24% w/w
Volatile matter	10.11% w/w	8.96% w/w	7.33% w/w
Moisture content	9.84% w/w	8.31% w/w	7.05% w/w
Ph value (1% w/v)	8.96	8.99	8.93
Loss on drying at 105°C	10.02% w/w	9.01% w/w	7.92% w/w

Pharmaceutical analysis¹³⁻¹⁷

Table 2: Pharmaceutical analysis

Test	Sample-1	Sample-2	Sample-3
Average weight test (Wt. in mg.)	149.10	149.10	145
Hardness (kg/ cm ²)	2.5 -3.0	1.0 -1.5	1.0 -1.5
Friability (% friability)	0.37	0.34	0.44
Diameter (in mm)	6.08	6.24 - 6.30	6.19 - 6.29
Disintegration time (DT in min.)	35 - 41	6 -12	15 -19

Phytochemical screening

Weigh accurately about 5 gm of sample into round bottom flask reduced pressure and partitioned by sequential extractions with various solvents and quantitative analysis was performed Alkaloids, Saponins, Flavanoids, Tanins, Triterpenoids¹⁸⁻²².

Table 3: Phytochemical screening

Components	Sample-1	Sample-2	Sample-3
Alkaloids	0.23% w/w	0.31% w/w	0.25% w/w
Saponins	17.98% w/w	22.50% w/w	20.95% w/w
Flavanoids	3.05% w/w	3.0% w/w	3.25% w/w
Tanins	0.79% w/w	0.50% w/w	0.63% w/w
Triterpenoids	9.19% w/w	11.50% w/w	10.71% w/w

Estimation of Microbial Contamination²³

Using petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the preparation and about 15 ml of plate count agar M 091 (HIMEDIA) medium at not more than 45°C. Alternatively spread the preparation on the surface of the solidified medium in a petri dish of the same diameter. If necessary, dilute the preparation so that a colony count of not more than 300 may be expected. Prepare at least two such petri dishes using the same dilution and incubate at 35 ± 2°C for 72 hours. Count the numbers of colonies that are formed. Calculate the results using the plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation. (Figure. 16)

The results shows in which the pathogens are within the limits as per mentioned in Ayurvedic Pharmacopeia.

Table 4: Microbiological Analysis

Pathogens	Sample-1	Sample-2	Sample-3
Total microbial plate count (TPC)	2200 CFU/gm	3600 CFU/gm	3800 CFU/gm
Total Moulds & Yeast	10 CFU/gm	22 CFU/gm	30 CFU/gm
Total Enterobacteriaceae	Absent	Absent	Absent
Escherichia coli	Absent	Absent	Absent
Salmonella spp.	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent	Absent
Staphylococcus aureus	Absent	Absent	Absent

Elemental analysis

Inductively Coupled Plasma with Optical Emission Spectroscopy (ICPOES)

It is a powerful tool for the determination of trace metals in a various sample. In this technique, liquid samples are injected into a radiofrequency (RF)-induced argon plasma using one of a variety of nebulizers or sample introduction techniques. The sample mist reaching the plasma is quickly dried, vaporized, and energized through collisional excitation at high temperature. The atomic emission emanating from the plasma is viewed in either a radial or axial configuration, collected with a lens or mirror, and imaged onto the entrance slit of a wavelength selection device. Single element measurements can be performed cost effectively with a simple monochromator/photomultiplier tube (PMT) combination, and simultaneous multi element determinations are performed for up to 70 elements with the combination of a polychromator and an array detector. The analytical performance of such systems is competitive with most other inorganic analysis techniques, especially with regards to sample throughput and sensitivity.²⁴

In the present three samples Hg, S, B, Pb, C, Fe, Cr, Cu, Ag, As elements are present in various ratio. (Table 5)

Table 5: Elemental analysis

Elements	Sample-1	Sample-2	Sample-3
As	0.25 ppm	0.223 ppm	0.62 ppm
Hg	15.54 %	14.34 %	16.56 %
Se	ND	ND	ND
Zn	ND	ND	ND
Pb	0.098 %	0.024 %	0.107 %
Co	ND	ND	ND
Cd	ND	0.0006 %	0.0011 %
Sn	ND	ND	ND
B	20.02 %	22.98 %	24.29 %
Fe	0.36 %	0.55 %	1.64 %
Cr	0.034 %	0.023 %	0.03 %
Cu	0.003 %	0.0003 %	0.0026 %
Ag	0.29 %	0.55 %	0.63 %
C	17.4 %	17.6 %	17.5 %
S	15.57 %	15.3 %	15.2 %

Energy Dispersive X-Ray Analysis

A SEM may be equipped with an EDX analysis system to enable it to perform compositional analysis on specimens. EDX analysis is useful in identifying materials and contaminants, as well as estimating their relative concentrations on the surface of the specimen.

EDAX has been used to detect the elements present a considerable trace amount. Table 6-8 shows chemical compositions of Panchavaktra ras using EDAX.

EDAX Analysis of 3 Samples of Panchavakra ras

EDAX Analysis of Panchavakra Ras sample-1

SEM Quant results. Listed at 11:58:56 AM On 4/9/2008.

Operator: D.M.R.L. Hyderabad.

Client: All ISIS USERS.

Job: Demonstration data SiLi detector.

Spectrum lable:

System resolution = 123 eV.

Quantitative method: ZAF (3 iterations).

Analysed all elements and normalized results.

2 peaks possibly omitted: 0.00, 8.02 keV.

Panchavakra Ras Sample -1

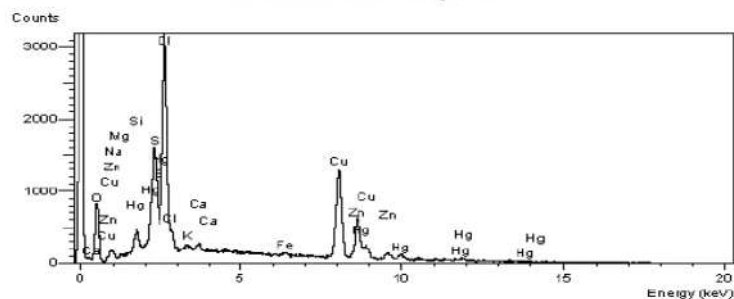


Figure 1: EDAX graph of Sample 1

Table 6: Elemental Composition of Sample 1

Standards:

O	K	Quartz 01 / 12 / 93
Na	K	Albite 02 / 12 / 93
Mg	K	Mgo 01 / 12 / 93
Si	K	Quartz 01 / 12 / 93
S	K	FeS2 01 / 12 / 93
Cl	K	KCl 15 / 02 / 94
K	K	MAD 01 / 12 / 93
Ca	K	Wollas 23 / 11 / 93
Fe	K	Fe 01 / 12 / 93
Hg	M	HgTe 01 / 12 / 93

Results:

Elements	Spect. Type	Element %	Atomic %
O K	ED	46.68	69.31
Na K	ED	0.76	0.78
Mg K	ED	0.56	0.55
Si K	ED	1.81	1.53
S K	ED	7.56	5.60
Cl K	ED	28.90	19.37
K K	ED	0.88	0.54
Ca K	ED	1.13	0.67
Fe K	ED	0.87	0.37
Hg M	ED	10.86	1.29
TOTAL		100.00	100.00

*=<2 Sigma

EDAX Analysis of Panchavakra Ras sample-2

SEM Quant results. Listed at 12:26:23 PM On 4 / 9 / 08

Operator: D.M.R.L. Hyderabad.

Client: All ISIS users

Job: Demonstration data SiLi detector.

Spectrum lable:

System resolution = 123 eV.

Quantitative method: ZAF (3 iterations).

Analysed all elements and normalized results.

2 peaks possibly omitted: 0.00, 8.02 keV.

Panchavakra Ras Sample - 2

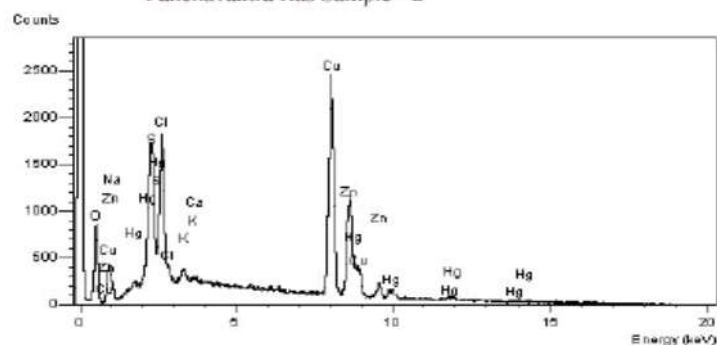


Figure 2: EDAX graph of Sample 2

Table 7: Elemental Composition of sample 2

Standards:

O K	Quartz 01 / 12 / 93
Na K	Albite 02 / 12 / 93
S K	FeS2 01 / 12 / 93
Cl K	KCl 15 / 02 / 94
K K	MAD 01 / 12 / 93
Ca K	Wollas 23 / 11 / 93
Hg M	HgTe 01/12/93

Result:

Elements	Spect. Type	Element %	Atomic %
O K	ED	51.32	76.24
Na K	ED	1.36	1.41
S K	ED	8.86	6.57
Cl K	ED	18.39	12.33
K K	ED	1.54	0.94
Ca K	ED	0.67	0.39
Hg M	ED	17.86	2.12
TOTAL		100.00	100.00

*=<2 Sigma

EDAX Analysis of Panchavaktra Ras sample-3

SEM Quant results. Listed at 1: 00: 12 PM On 4 / 9 / 08

Operator: D.M.R.L. Hyderabad.

Client: All ISIS users

Job: Demonstration data SiLi detector.

Spectrum table :

System resolution = 124 eV.

Quantitative method: ZAF (3 iterations).

Analysed all elements and normalized results.

2 peaks possibly omitted: 0.00, 8.02 keV.

Panchavaktra Ras Sample - 3

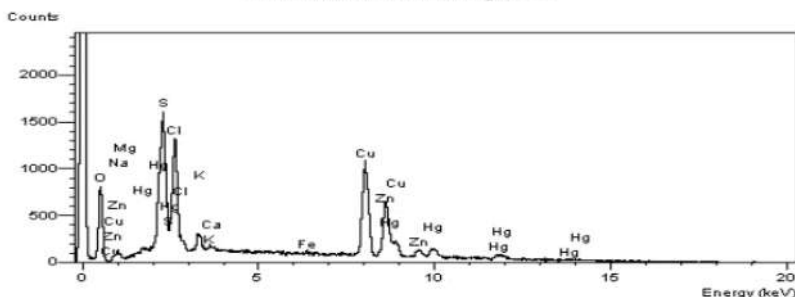


Figure 3: EDAX graph of sample 3

Table 8: Elemental Composition of sample 3

Standards:

O K	Quartz 01 / 12 / 93
Na K	Albite 02 / 12 / 93
Mg K	Mgo 01/12/93
S K	FeS2 01 / 12 / 93
K K	MAD 10 02 / 12 / 93
Ca K	Wollas 23 / 11 / 93
Fe K	Fe 01 / 12 / 93
Hg M	HgTe 01/12/93

Results:

Elements	Spect. Type	Element %	Atomic %
O K	ED	54.46	83.30
Na K	ED	1.24	1.32
Mg K	ED	0.07*	0.07*
S K	ED	12.24	9.34
K K	ED	3.30	2.07
Ca K	ED	0.63	0.39
Fe K	ED	0.28*	0.12*
Hg M	ED	27.77	3.39
TOTAL		100.00	100.00

* = <2 Sigma

RESULTS AND DISCUSSION

The mass of Panchavaktra Ras has been sent for making tablets in IMIS Pharmaceuticals each tablet contained 150 mg. medicine which includes excipient (starch, binding material, etc.). In the 1st sample values of Disintegration time and Hardness, sample values are slightly high, so in the later 2 samples the above values comes in normal range because the quantity of binding agent was reduced.

In the Panchavaktra Ras tablets average weight were found in (mean $\pm$ SD) 149.4 mg. 99% tablets were within acceptable range of weight. Panchavaktra Ras was found to have 1.0-1.5 kg/cm² hardness and 6-19 min. disintegration time which was noticed within accepted limits. Both hardness and disintegration time interfere with the bioavailability of drug. Panchavaktra Ras contains moisture content 7.05-9.84 % w/w. If it is not protected in humid atmosphere, the water activity increases and it will cause degradation of the tablet.

Preparation of Panchavaktra ras



Figure 4: Purified Parada



Figure 5: Purified Gandhaka



Figure 6: Samaguna Kajjali



Figure 7: Purified Tankana



Figure 8: Marica



Figure 9: Pippali



Figure 10: Dattura leaf Juice



Figure 11: Ingredients with Dattura Juice



Figure 12: Mardhana in Khalva yantra



Figure 13: Final Sample before dry



Figure 14: Final Sample after dry



Figure 15: Panchavaktra ras in Tablet form



Figure 16: Microbiological Analysis Using petri dishes

Excess of water in drug encourage microbial growth, presence of fungi or insects and deterioration following hydrolysis.

Ash values are the criteria to judge the identity and purity of crude drug, where total water soluble and acid insoluble ashes are considered. Panchavaktra Ras contained 22.21-24.01%w/w of total ash and 1.20-2.15%w/w acid insoluble ash. The 15.05-17.24%w/w of water soluble and 6.40-7.23% w/w of methanol soluble extractive were present in Panchavaktra Ras indicating that the drug is having good solubility in water.

Panchavaktra Ras after preparation of mass has been subjected to chemical analysis in the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) in which about 13 elements (Table 6) have been tried for apart from Mercury and Sulphur. Less than 0.1% of chromium (Cr), 0.001% of Cadmium (Cd), 0.09% of Lead (Pb), 0.02% of Arsenic (As), 0.003% of Copper (Cu), apart from 15% each Mercury, Sulphur, 20- 24% of Boron are detected in the mass. Elements which are presents Panchavaktra ras also detected by using the EDAX Analysis, in this elements O, Ca, Cl, Fe, Hg, K, Mg, Na, S, Si are present.

CONCLUSION

As far as Panchavaktra Ras is concerned, this showed almost same values for all 3 batches of the medicine indicating for the fixation of standards for this particular compound. Since the instruments used in ICPOES, EDAX etc. the values of this analysis can be taken as standard for further practicals in this regard. The estimation of physico chemical values and Alkaloids, Saponins, Flavanoids, Tannins, Triterpenoids gives a clear picture about characterization of Panchavaktra Ras indicating the catholic adherence to the textual instruction in all three batches confirms the standardization of this formula. The Microbial contamination moulds and yeasts are within acceptable normal limits while the absence of Enterobacteriaceae, *E.coli*, Salmonella species, *Pseudomonas aeruginosa*, and *S. aureus* indicate the perfect preparation of this medicine even accepted by the international standards. Efforts have been made to lay down analytical standards of Panchavaktra ras, which were not found, reported till date. All the parameters are within acceptable range.

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