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COMPARATIVE PHYSICO CHEMICAL ANALYSIS OF VYOSHADIVATI– AN AYURVEDIC POLYHERBAL FORMULATION W.S.R TO MARKET SAMPLES

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ABSTRACT: The quality control assessment of herbal formulations is of great significance in order to justify their acceptability in modern system of medicine though the drug may be therapeutically potent. Ayurvedic formulations prepared by several manufacturers are guaranteed to carry out the quality control test as per the standards mentioned in the Ayurvedic Formulary of India. Though the standards have been followed, still the variability in their results has been observed when compared between same formulations. *Vyoshadivati* is one such polyherbal formulation consists of 13 drugs and treated for ailments viz. *Pinasa* (coryza), *Pratishaya* (Rhinitis), *Swarabheda* (Hoarseness of voice) etc. An attempt is made here to compare Vyoshadivati prepared by GMP certified pharmacies with In house preparation. Results revealed that all the samples differ in their organoleptics, pH, and physicochemical properties. Thin layer chromatographic study showed sample B, D and E have almost similar number of bands at the wavelength of 255nm and 365nm. Major difference was seen in disintegration time and hardness of sample A i.e. hardness is 7.78 but disintegrates in 22min whereas sample D hardness 2.63 but disintegrates in 78min.The physicochemical data of this comparative study assists in maintaining the standard limits of *Vyoshadivati*.

INTRODUCTION: Ayurveda the oldest and alternative system of medicine in the present scenario has gained its popularity and demand globally due its effective and efficacious results witnessed in various diseases and syndromes in the recent era. Herbal drugs are the core of this system of medicine and these drugs possess all the quality required to prevent and cure the disease which is the prime motto of Ayurveda.

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The principles to standardize the drugs that were developed in ancient period were subjective and are based on the scientific background prevailing in those days.

Now they are to be viewed and answered looking towards the advancement of science and technology in present scenario. Hence there is prime need to validate Ayurvedic formulations with the aid of modern sophisticated instrumental and analytical techniques explained in the context of herbal medicine to justify the quality of the products. In order to meet the needs of population enormous numbers of manufacturing companies have come into existence. These manufacturers though prepare the similar formulation fails to meet the standard quality control parameters when compared.

Vyoshadivati [1] is one such polyherbal formulation explained in the Ayurvedic classics which is a combination of 13 drugs and has its efficacy over *Pinasa* (coryza), *Pratishyaya* (Rhinitis), *Swarabheda* (Hoarness of voice), *Kasa* (cough), *Shwasa* (asthma) [1, 2] etc. most of which simulate to symptoms of upper respiratory tract infection. Based on the above rationale the present study is designed to compare *Vyoshadivati* prepared in house with different market samples based onprimary quality control parameters.

MATERIALS AND METHODS:

Pharmaceutical Part

Raw Material Procurement

Market samples

Four samples of *Vyoshadivati* manufactured by GMP certified pharmacy were collected from the Belgaum market and given the code as Vv-A, Vv-B, Vv-C, and Vv-D.

IN HOUSE SAMPLE PREPARATION

Plant Material

Vyoshadivati consists of 13 herbal ingredients. All the drugs of *Vyoshadivati* were procured from GMP certified KLE Ayurveda Pharmacy Khasbag Belgaum, Karnataka and were authenticated at AYUSH approved Central Research Laboratory of KLE University's Shri B.M. Kankanwadi Ayurved Mahavidhyalaya Belgaum, Karnataka, India.

METHOD OF PREPARATION –

TABLE 1: INGREDIENTS OF *VYOSHADIVATI*

Sl.no.	Ingredients	Latin Name [3]	Part used	Quantity
1.	Pippali	*Piper longum*Linn	Fruit	1part
2.	Marich	*Piper nigrum* Linn	Fruit	1part
3.	Shunthi	*Zingiberofficinale*Roxb	Rhizome	1part
4.	Chavya	*Piper cheba*Hunter	Stem	1part
5.	Chitrak	*Plumbagozeylanica* Linn	Root	1part
6.	Jeerak	*Cuminumcyminum*Linn	Fruit	1part
7.	Talisapatra	*Abbes webbiana*Lindl	Leaves	1part
8.	Amlavetasa	*Rhumeemodi*Wall. ex Meissn	Stem	1part
9.	Tintidika	*Tamarindusindica*Linn.	Fruit	1part
10.	Twak	*Cinnamomumzeylanica*Blume	Stembark	1/4th part
11.	Ela	*Elettariacardmomum*Maton	Seed	1/4th part
12.	Tamalapatra	*Cinnamomumtamala*Ness	Leaves	1/4th part
13.	Guda(Jaggery)	-	-	20 parts

Instruments and Equipment's

Weighing machine, Analytical balance, Pulveriser, Clean cotton cloth, Steel vessel, Mask, Cap, Apron, Sieve no 85 and 120, Gas and stove.

Preparation of *Churna*

The *Churna* (powder) was prepared as per the procedure explained in Ayurvedic Formulary of India. All drugs (except *Tamarindus indica* Linn) were made into fine powder in a pulveriser. These *churna* are passed first through 85# mesh followed by 120 # sieve individually and then all are mixed together in specified proportions to get uniformly blended homogenous mixture.

Preparation of *Vyoshadi Vati* [4]

Step 1 – Weigh the jaggery in specified quantity and pound it.

Step 2 – 1part of Tamarind soaked in 6parts of water for an hour later macerated and filtered through muslin cloth.

Step 3 –Jaggery syrup was prepared by heating the jaggery and q.s (10ml) water along with tamarind juice till it gets 2 thread consistency.

Step 4 – Stop heating and add the homogenous mixture of *churna* to Jaggery syrup with continuous stirring.

Step 5 –Round pillswere prepared and dried in shade.

Step 6 –Vati's are then stored in air tight container. In house sample has given the code Vv-E.

ANALYTICAL PART:

To carryout Physico-chemical analysis, standard parameter has been applied as per Standards of Ayurvedic Pharmacopoeia of India. Analytical

study was carried out in AYUSH approved Central Research Laboratory of Shri B.M.K. Ayurveda Mahavidyalaya Belgaum. Microbial Limit Test was carried out in Microbiology Laboratory of KLE University's Shri B.M. Kankanwadi Ayurveda Mahavidhyalaya Belgaum, Karnataka, India.

The samples had been analyzed for Organoleptic characters, Moisture content, Extractive values, Ash values,[5, 6] Qualitative Estimation through TLC [5], Physical test for Tablet i.e. Hardness, Disintegration and Uniformity of weight[7], Phytochemical analysis and Microbial Limit Test.[8]

RESULTS AND DISCUSSION:

Physicochemical analysis of *Vyoshadivati* market samples and in house preparation has been carried out and the results are shown in tables. Ayurvedic formulations claimed to be made according to CCRAS guidelines are effective but it is very difficult to maintain uniformity in formulations which is may be due to natural heterogeneity, the quality of herbal starting material obtained from wild collection shows more and more fluctuations which can be depicted from our experimental data[9].

ORGANOLEPTIC STUDY Organoleptic of samples reveals adequate differences observed in the presentation of the formulation and their taste i.e. sample A and B are punched tablet indicates about addition of some binders and are light brown in colour whereas sample D and E are handmade round pills and are dark brown and brown in colour. Drawback of Sample C is its irregular shape for which the analysis of physical test for tablet doesn't apply. This might be because as some references in classic mention the dosage form as *Vataka* which is synonym for both *Vatikalpana* and *Avalehakalpana*.

MOISTURE CONTENT Moisture content in a drug is an important tool for a stability of any formulation. If moisture is high, it provides healthy environment for microbial growth. Sample C and E have least and sample B has high moisture content i.e. 12%.

ASH VALUE- Ash value represents amount of non-physiological components present in the drug [10]. Lesser the amount ash, less the impurities. Sample E and sample C has lesser ash value when compared to other samples i.e. 4.597%w/w and 3.53% w/w.

EXTRACTIVE VALUES Extractive value explains the amount of constituents that are extracted from a drug in a given solution. As the *Vati*is administered along with water as a common *anupana*, maximum extraction must be observed in aqueous extract. All the samples have shown high aqueous extractive value.

PH VALUE pH determines acidity or alkalinity of a drug. The pH for sample C is 5.52 whereas sample E had 4.05 and other samples are between D and EpH. Sample E is acidic compared to other samples.

PHYSICAL CHARACTERISTICS FOR TABLETS The physical test for tablets i.e. Weight variation test where 20 tablets are randomly selected and weighed. The mean and standard deviation was calculated. Here in this study sample 1 and 2 had shown less deviation whereas sample 4 and 5 has shown significant difference in their weight. This might be due to the pills prepared were handmade. The disintegration time and hardness of tablet are important tools for physical stability and absorption rate.

Both procedures must be directly proportional to each other. But in this study the hardness of sample A is 7.78kg/cm^3 but disintegration time is22min. Whereas hardness of sample D is 2.63kg/cm^3 and disintegration time is 78min. Disintegration test of both samples were done for 18 tablets. Such change affects the bioavailability of the drug to withstand in the body and show its efficacious results. Hardness of sample E is2.1kg/cm^3 and disintegration time is 15min.

THIN LAYER CHROMATOGRAPHY

Qualitative analysis i.e. TLC study is carried out on 60F_{254}pre-coatedTLC plates under the solvent system Toulene and Ethyle acetate in the ratio 7:3 after various trial and errors. Ethanol extracts of all the samples have been taken and visualized under UV light chamber at the range of 255nm and 365nm. This parameter gives idea about qualitative estimation presence of various components of drugs. Results of TLC are shown in **Table 6**.

Sample E has shown highest number of bands i.e. at 255 nm 10 bands and at 365nm 18 bands. When compared sample B, D and E having almost similar number of bands in long wavelength.

MICROBIAL LIMIT TEST: Microbial limit test has been carried out for all the samples and study reveals all samples were within the limits as per Indian Pharmacopeia Standard.

TABLE 2: ORGANOLEPTIC CHARACTERS

Sl.no.	Sample	Colour	Odour	Taste	Form
1	Vv-A	Light Brown	Characteristic	Sweet, Pungent	Punched Tablet
2	Vv-B	Light Brown	Characteristic	Pungent, Slight Bitter	Punched Tablet
3	Vv-C	Browm	Characteristic	Sweet, Pungent	Irregular shape
4	Vv-D	Dark Brown	Characteristic	Sweet, Bitter, Pungent	Round pills
5	Vv-E	Brown	Characteristic	Sweet, Sour, Pungent	Round pills

TABLE 3: DETERMINATION OF MOISTURE CONTENT, ASH VALUES AND EXTRACTIVE VALUES

Sl.no.	Samples	Moisture content	Total Ash	Acid Insoluble Ash	Alcoholic Extract	Aqueous Extract	pH
1	Vv-A	9%w/w	10.33%w/w	7%w/w	23.2%w/w	69.6%w/w	4.50
2	Vv-B	9.5%w/w	13.67%w/w	8%w/w	36.8%w/w	49.6%w/w	4.97
3	Vv-C	3.49% w/w	4.597%w/w	1.087% w/w	17.6% w/w	76.4% w/w	5.52
4	Vv-D	12% w/w	7.65% w/w	2.522% w/w	25.9% w/w	41.4% w/w	4.45
5	Vv-E	4.09% w/w	3.53% w/w	0.846% w/w	34.16% w/w	86.9% w/w	4.05

TABLE 4: STATISTICAL ANALYSIS OF WEIGHT VARIATION TEST

Sl. No.	Samples	Number of Samples	Mean	S.D.	S.E.M
1	Vv-A	20	0.3000gms	0.007947	0.001777
2	Vv-B	20	0.2905gms	0.009987	0.002233
3	Vv-D	20	0.4265gms	0.01424	0.003185
4	Vv-E	20	0.5335gms	0.02796	0.006252

S.D – Standard deviation, S.E.M – Standard error mean

TABLE 5: DETERMINATION OF TABLET DISINTEGRATION AND HARDNESS

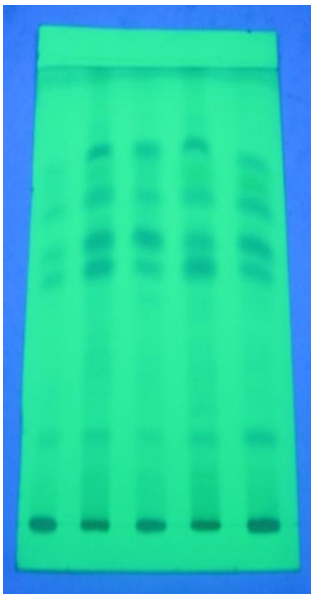
Sl.no.	Samples	Hardness*	Disintegration** time
1	Vv-A	7.78kg/cm^2	22min
2	Vv-B	3.8 kg/cm^2	47min
3	Vv-D	2.63 kg/cm^2	78min
4	Vv-E	2.1 kg/cm^2	15min

* Monsanto's Hardness Tester

**Tablet Disintegration Apparatus - Solution – Distilled water, Temperature – 39^{0}C, Oscillations – 30/min

TABLE 6: DETERMINATION OF TLC STUDY

S. No.	Samples	Wavelength	Rf. Values
1.	**Vv – A**	255nm	0.03, 0.07, 0.16, 0.37, 0.47, 0.52, 0.58, 0.62
		364nm	0.03, 0.07, 0.11, 0.37, 0.49, 0.55, 0.62, 0.69, 0.86, 0.96
2.	**Vv – B**	255nm	0.03, 0.05, 0.1, 0.16, 0.3, 0.34, 0.49, 0.56, 0.65
		364nm	0.02, 0.05, 0.1, 0.13, 0.24, 0.32, 0.39, 0.50, 0.54, 0.56, 0.61, 0.72, 0.77, 0.88, 0.92, 0.96
3.	**Vv – C**	255nm	0.03, 0.08, 0.1, 0.15, 0.34, 0.43,, 0.50, 0.57, 0.66
		364nm	0.03, 0.08, 0.13, 0.17, 0.20, 0.30, 0.37, 0.50, 0.54, 0.58, 0.63, 0.9
4.	**Vv – D**	255nm	0.01, 0.05, 0.09, 0.16, 0.21, 0.29, 0.43, 0.52, 0.56, 0.66
		364nm	0.01, 0.05, 0.09, 0.13, 0.21, 0.30, 0.37, 0.50, 0.53, 0.57, 0.63, 0.71, 0.8, 0.83, 0.90, 0.93, 0.96
5.	**Vv – E**	255nm	0.03, 0.06, 0.10, 0.16, 0.24, 0.33, 0.43, 0.50, 0.56, 0.64
		364nm	0.03, 0.07, 0.10, 0.13, 0.24, 0.32, 0.37, 0.50, 0.53, 0.56, 0.62, 0.70, 0.77, 0.83, 0.87, 0.9, 0.94, 0.96



UV – 254nm

Samples
Ethanol Extract of VyoshadiVati

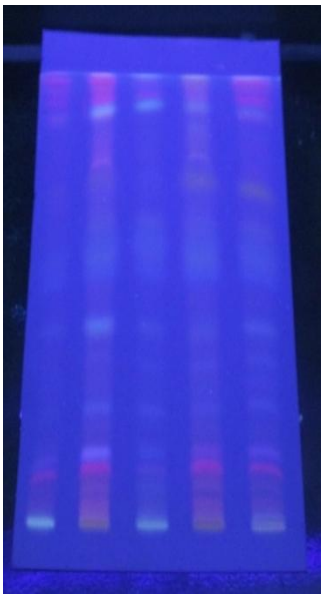
Solvent System

Mobile phase
Toulene : Ethyle Acetate[7 : 3]

Stationary phase
Pre-coated thin films of Silica plates of length 15mm

Development
Stepwise in Stahl chamber

Visualization
Under UV chamber
Short wave – 255nm
Long wave – 365nm



UV – 366nm

***0.24** - The standard Rf- value of purified Piperine[12]

TABLE 7: MICROBIAL LIMIT TEST

Sl.no	Microbial Organism	Limit as per IP	V.V -A	V.V -B	V.V -C	V.V -D	V.V –E
1	*Escherichia coli*	Absent	Absent	Absent	Absent	Absent	Absent
2	*Staphylococcus aureus*	Absent	Absent	Absent	Absent	Absent	Absent
3	*Pseudomonas aeruginosa*	Absent	Absent	Absent	Absent	Absent	Absent
4	*Salmonella ebony*	Absent	Absent	Absent	Absent	Absent	Absent

CONCLUSIONS: *Vyoshadivati* is a polyherbal formulation treated for the ailments *pinas*, *swarabheda*, *pratishyaya*, *kasa*, *shwasa*. Being the same formulation prepared by various manufacturers yet there is difference observed when markets samples and in house preparation are compared through standard quality control parameters as per Ayurvedic Pharmacopeia of India. Hence it is the need of hour that the Ayurvedic formulations are to be standardized in order to make them potent and therapeutically efficient.

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