Original Research Article

International Research Journal of Ayurveda & Yoga
Vol. 6 (6),11-20, June,2023
ISSN: 2581-785X: https://irjay.com/
DOI: **10.47223/IRJAY.2023.6602**

An Analytical Study for Validation and Standardization of *Vati.*

Jagriti Sharma,[1] Pooja Swami[2]

1. Associate Professor, Department of Saundarya Ayurveda, NIADU, Jaipur, Raj, India
2. Assistant Professor, Department of Agada tantra, Tantra Ayurveda College, Ganganagar, Raj, India

Article Info

Article history:
Received on: 01-05-2023
Accepted on: 22-06-2023
Available online: 30-06-2023

Corresponding author-
Jagriti Sharma, Associate Professor, Department of Saundarya Ayurveda, NIADU, Jaipur, Raj, India.

Email: drjagritisharma573@gmail.com

ABSTRACT:

Introduction-Traditional medicine has a long history of serving people all over the world. Today the world is looking towards the Ayurveda with great hope and faith. With these new challenges emerges in the form of queries of the modern man who would have a right to know about the drug he is consuming. To meet this new trust of inquisitiveness, standard of drugs of Indian System of Medicine is mandatory. In the process of assurance, to ascertain the prepared product is full proof and without any defects, these prepared products need to undergo several analytical parameters concerned to them and get clearance. This article is a step to standardized and validate the ayurvedic formulation through analytical study.

Material and methods- The drugs selected for study are one of the *mahakashaya Dasemani – Angamardha Prashaman Dasemani* described in *Charak Samhita Sutra Sthan* and *Bhagottar Gutika* a Herbo- mineral formulation is from *Bhaishajya Ratnavali*. The various parameters studies are or organoleptic characters, physicochemical parameters of *vati* like disintegration, friability, loss on drying, acid insoluble ash, water soluble ash etc. and microbial contamination.

Results – All the parameters studied are within the limits and hence validate the safely and effectively use of *vati*.

Conclusion Analytical study of a product provides standards to judge its quality. It is useful to decide future work plan and objective parameters to know the exact status of drug by conducting the comparative study of various samples during drug preparation.

Key words: Analytical study, *Angamardha Prashaman Dasemani, Bhagottar Gutika*, Validation, standardization

INTRODUCTION

The patients or consumers on one hand and the physician on the other are expected three main factors to have in the materials that they are going to use viz safety, quality and efficacy. All these three factors can be ascertained only by subjecting the prepared products to stringent quality measures. While discussing about quality two terms relating to quality are in practice i.e. quality control and quality assurance. The quality controls defined as the operational techniques and activities used to fulfill the requirements for quality, whereas the quality assurance is a complete system to assure the quality of the product. It is not only a process but a complete system including quality

control so as to assure the consumer with quality products. In the process of assurance, to ascertain the prepared product is full proof and without any defects, these prepared products need to undergo several analytical parameters concerned to them and get clearance.

Analytical study is the application of a process or a series of processes in order to identify and/or quantify a substance, the components of a solution or mixture, or determination of the structures of chemical compounds. For better utilization of *Ayurvedic* pharmaceutics, it is need of hour to analyze the drug through both classical and modern qualitative and quantitative parameters. Present paper is presented with aim and objectives to develop analytical profile of *Angamardprashman vati*[1] and *Bhagottar Gutika* by assessing it`s various parameters like organoleptic as well as physico-chemical parameters including hardness, disintegration time, pH, loss on drying & ash values.

MATERIAL AND METHODS

The samples subjected for analytical studies are -

1. ***Angamardprashman vati***[i]

The drug selected from ancient period belongs to one of the *mahakashaya Dasemani – Angamardha Prashaman Dasemani* described in Charak Samhita Sutra Sthan It has been named as **AMP.** The basic ingredients of this *vati are vidarikand, Prishparni, Brihati, Kantakari, Eranda, Kakoli, Raktachandan, Ushira,Ela and Madhuka*

2. ***Bhagottar Gutika***[2]

This herbo- mineral formulation is from Bhaishajya Ratnavali.The basic ingredients of this formulation are *Parada, Gandhaka, Pippali, Haritaki, Vibhitaki, Vasamoola, Bharangi, Babool Patra and Madhu* .

Parameters studied

The Test parameters were taken according to "Protocol for Testing of *Ayurvedic*, *Siddha* and *Unani* medicines", Govt. of India, Dept. of Ayush, Ministry of Health and Family Welfare, Pharmacopoeial Laboratory for Indian Medicines, Ghaziabad;[3] Ayurvedic Pharmacopoeia of India, 2008 Dept. of Ayush, Govt. of India[4], and "Laboratory Guide for the Analysis of Ayurvedic and Siddha Formulations", CCRAS, Dept. of Ayush, Govt. of India, 2010[5]

The Following tests were performed at Drug Testing Laboratory, Dept. of *Rasashastra* and *Bhaisajya Kalpana*, National Institute of Ayurveda, Deemed to be University, Jaipur and S. R. Labs, Jaipur, Rajasthan

The study has been carried out for the following parameters:-

A. Organoleptic characters[6]**:-**

1. Colour
2. Odour
3. Taste
4. Appearance/Texture

Pharmaceutical standardization of *Vati*:-

1. Hardness
2. Friability test
3. Disintegration Time (D.T.)

Physico-chemical parameters:-

1. pH value
2. Loss on drying
3. Total ash
4. Acid Insoluble ash
5. Alcohol soluble extractive
6. Water soluble extractive

B. Test for microbial contamination:-

1. Total bacterial count
2. Total fungal count

ORGANOLEPTIC CHARACTERS:-

Organoleptic characters of the samples are obtained by using sense organs, are very useful parameters to determine and compare the quality of samples. Here the parameters like colour, odour, taste, consistency, etc are considered.

Pharmaceutical Standardization Of *Vati:-*

Hardness of Tablet[7]**-**

This test is applicable to compressed *vati* and is intended to determine the physical strength of the *vati*. A small and portable hardness tester was used known as 'Monsanto' or 'stokes hardness tester' to test the hardness of the *vati*. The instrument measures the force required to break the *vati* when the force generated by a coil spring is applied diametrically. The force is measured in kilogram and when used in production, a minimum hardness of 4 kg/cm^2 is considered to be satisfactory.

Friability Test[8]**:-**

The resistance of the tablet to chipping, abrasion or breakage under condition of storage, transportation, and handling before usage.

Procedure:-

10 whole *vatis* were taken. De-dust the *vatis* carefully. Weigh accurately the required number of *vatis*. Place the

vatis in the drum and rotate it for 100 times at the 25 rotation/minute for 4 minutes. The *vatis* were removed and any loose dust was cleared from them and weighed accurately. To avoid irregular tumbling, the drum base was adjusted so that it formed an angle of about 10^0 with the horizontal. The *vatis* should not bind together when lying next to each other which prevent them from falling freely. Percentage of loss was calculated with the corresponding weight.

Disintegration Time[9]:-

Disintegration is defined as that state in which no residue of that unit under test remained on the screen of the apparatus or if a residue remains, it consists of fragments of disintegrated parts of the tablets, *vatis*, *gutika* and pills components parts such as insoluble coatings. This test determines whether dosage forms such as tablets, *vati*, *gutika* and pills etc. disintegrate within a prescribed time when placed in a liquid medium (water) under the prescribed experimental conditions.

Tank of the disintegration apparatus was filled with distilled water up to the mark. 750 ml of distilled water was taken in each of the 1000 ml beaker. Timer of the instrument was set for 30 minutes and the temperature of water in beaker to 37^0c ± 0.5^0c. One *vati* was introduced into each tube of apparatus. A disc was covered on each tube. The assembly was suspended in the beaker water and operation started. The time duration at which the *vati* disintegrate completely in the beaker was noted.

Physico-Chemical Parameters:-

Determination of pH (10% Aqueous Solution)[10]:-

The pH value conventionally represents the acidity or alkalinity of an aqueous solution. pH as a measure of the hydrogen activity is important from the stand point of stability or physiological stability. The measurement of pH is generally done with a suitable potentiometric meter known as the pH meter, fitted with two electrodes, one constructed of glass and sensitive to hydrogenation activity and the other a calomel reference electrode. The determination is carried out at temperature of 254º±2ºc, unless otherwise specified in the monograph.

Procedure

A 10% w/v aqueous solution of the sample was prepared, filtered and the pH of the filtrate was noted in digital pH meter using combined glass electrode.

Loss on Drying[11]:-

This Parameter determine the amount of volatile matter (i.e., water drying off from the drug). For substances appearing to contain water as the only volatile constituent, this procedure is appropriately used.

Procedure:-

10 g. accurately weighed grind sample was placed (without preliminary drying) in a tared petridish. After placing the above said amount of the drug in the tared petri-dish, it was dried at 105^0c for 3 hours and weighed. The drying and weighing at one hour interval was continued until difference between two successive weighing corresponds to not more than 0.25%.Constant weight was reached when two consecutive weighing after drying and cooling for 30 minutes in a desiccator, showed not more than 0.001g. difference.

Total Ash:-

The ash value of the sample was determined by incinerated about 2 to 3g of accurately weighed drug in a tarred silica crucible at a temperature not exceeding 450°C until free from carbon. Then cooled and weighed. If a carbon free ash were not obtained in this way, then charred mass was exhausted with hot water and the residue was collected on an ash less filter paper. Incinerated the residue and the filter paper, the filtrate was added, evaporated to dryness and ignited at a temperature not exceeding 450°C. The percentage of ash was collected with reference to the air-dried sample.

Acid-Insoluble Ash:-

The test was carried out to evaluate the percentage of acid insoluble inorganic content of the sample i.e. sand, siliceous earth.

The ash obtained from the total ash content, was boiled for five minutes with 25 ml of dilute hydrochloric acid, the insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to air-dried sample.

Water Soluble Extractive Value:-

This test was carried out to evaluate the water soluble principle of the sample.

About 5g, accurately weighed sample was macerated with 100 ml of distilled water in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to

stand for eighteen hours. Filtered rapidly, taking precaution against loss of solvent and 25 ml of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish. First dried over water-bath and than at 105°C in hot air oven, to constant weight and weight was noted down. From the weight of the residue the percentage of water-soluble extractive was calculated with reference to air-dried sample.

Alcohol Soluble Extractive value:-

This test was carried out to evaluate the alcohol soluble principles of the sample. Alcohol soluble extractive the method followed as per the method described in Water soluble extract, but instead of 100 ml distilled water, 100 ml of methanol was used and the percentage was calculated.

Test For Microbial Contamination[12]:-

Total Bacterial Count:-

Total aerobic bacterial count is the most important test for evaluation of microbial contamination in *Ayurvedic* formulation and raw material.

Determination of total aerobic bacteria in raw materials and finish products ensures the evaluation of bacterial contamination, hygienic condition for handling and storage condition. Some toxic bacteria can cause severe diseases to the human.

Total Fungal Count:-

Total yeast and mould count is the most important test for evaluation of fungal contamination in herbal medicine and raw material. Determination of total yeast and mould analysis in raw materials and finish products ensures the evaluation of fungal contamination, hygienic condition for handling and storage condition. Some toxic fungus can cause severe diseases to the human.

Test for Aflatoxins[13]

Aflatoxins are closely related group of secondary metabolites shown to be mycotoxin. They are produced by fungus named *Aspergillus flavus*. There are four types of B1, B2, G1, G2.

RESULTS

The results procured from all the test done are presented in given tables.

Table 1 Showing the organoleptic characters of the Samples *Angamardprashman vati*

Table 2 Showing the organoleptic characters of the Samples *Bhagottar Gutika*.

Table 3 Showing the results of Hardness of *Angamardprashman vati*

Table 4 Showing the results of Hardness of *Bhagottar Gutika*.

Table 5 Showing the results of Friability test of *Angamardprashman vati*

Table 6 Showing the results of Friability test of *Bhagottar Gutika.*

Table 7 Showing the results of Disintegration Time of *Angamardprashman vati*

Table 8 Showing the results of Disintegration Time of *Bhagottar Gutika*

Table 9 Showing the results of pH of *Angamardprashman vati*

Table 10 Showing the results of pH of *Bhagottar Gutika.*

Table 11 Showing the results of Loss on Drying of *Angamardprashman vati*

Table 12 Showing the results of Loss on Drying of *Bhagottar Gutika.*

Table 13 Showing the results of Total Ash of *Angamardprashman vati*

Table 14 showing the results of Total Ash of *Bhagottar Gutika.*

Table 15 Showing the results of Acid-insoluble ash of *Angamardprashman vati*

Table 16 Showing the results of Acid-insoluble ash of *Bhagottar Gutika.*

Table 17 Showing the results of Water -soluble extractive Value of *Angamardprashman vati*

Table 18 Showing the results of Water-soluble extractive of *Bhagottar Gutika.*

Table 19 Showing the results of Alcohol -soluble extractive Value of *Angamardprashman vati*

Table 20 Showing the results of Alcohol-soluble extractive of *Bhagottar Gutika.*

Table 21 Showing Microbiological Analysis of the *Angamardprashman vati*

Table 22 Showing Microbiological Analysis of the *Bhagottar Gutika*.

Table 23 Showing result of tests for aflatoxins

Table 24 Showing result of tests for aflatoxins

DISCUSSION

Traditional medicine has a long history of serving people all over the world. Today the world is looking towards the Ayurveda with great hope and faith. With these new

challenges emerges in the form of queries of the modern man who would have a right to know about the drug he is consuming. To meet this new trust of inquisitiveness, standard of drugs of Indian System of Medicine is mandatory. In early days, vaidyas use to collect the drug personally from the places where they were growing in natural state. All care was taken by them for collection of drugs considering age of the plant, part of the plant to be collected, season of collection, time of collection, method of drying, storage etc. and preparation of formulation. Now days, An *Ayurvedic* preparation of medicine involves multi-step procedures and many plant and mineral drugs. The complex composition increases the difficulties of standardization and subsequently quality control of the finished product becomes more complex. It is therefore essential proper documentation and standardization of ingredients the botanical and chemical characterization of individual ingredient. Analytical study ensures not only chemical constituents but also tells us about standards of the preparation and indirectly gives suggestion for further advancement.

The drugs were analyzed for their organoleptic properties and physico-chemical parameters. Among the organoleptic properties the colour reflects the materials present in the drugs at acquiring the colour following levigating and the form of drug. The *ghan* always will be in black colour. Whereas the other drug because of the levigating with *babool patra swaras* might have acquired black colour. The odour is specific in preparations in *ghan vati* it is characteristic to cardamom as it has been added at the end stage. Where as in *Bhagottar gutika* it is specific to Bhawana *dravya*. The taste of *ghan vati* is bitter sweet and *Bhagottar gutika* is Kashaya due to *Babool Patra Swaras*. the *Yastimadhu* present in *ghan vati* and *elachi* might have given the bitter sweet taste. Among the physico-chemical parameters loss on drying determine the amount of volatile matter or water drying off from the drug. For substances appearing to contain water as the only volatile constituent, this procedure is appropriately used. Both selected drugs have exhibited different proportions of moisture present in the preparations. In *Bhagottar Gutika* more moisture might have retained while manufacturing whereas *ghan vati* might have absorbed after manufacturing. The total ash content is more in the AMP then *Bhagottar Gutika*. This indicates the presence of minerals/inorganic elements in the product. The acid insoluble value is the indicator for the presence of salicaceous material in the product and the results showed the presence of silica in very minute quantities in both products.

The water-soluble extract values indicate the presence of the active elements that are soluble in water and all the samples showed higher values with AMP *vati* superseding the other as the AMP *vati* is manufactured by extracting the water-soluble portion hence the values are very high in *ghan vati.* The pH of the 10% suspension of all the three drugs showed acidic in nature. The disintegration time was also recorded for both the *vati* . Both of them is within the normal limits. Disintegration is defined as that state in which no residue of that unit under test remained in the apparatus or if a residue remains, it consists of fragments of disintegrated parts of the tablets, *vatis*, *gutika* and pills components parts such as insoluble coatings. Disintegration Time is very important test for vati and both the vati has time appropriate for usage. The hardness test is applicable to compressed *vati* and is intended to determine the physical strength of the *vati*. The minimum value should be 4 kg/cm^2 and both the *vati* has passed this test. Similarly, friability test determines the resistance of the tablet to chipping, abrasion or breakage under condition of storage, transportation, and handling before usage. The three samples are prepared and assessed analytically of both *vati* for standardization and validation purpose.

CONCLUSION

The term 'analysis' means the detailed examination, which reveals the minor, but important aspects regarding the drug. So without analytical study Standardization and validation is incomplete. Ayurvedic texts has its own methodology of explaining the nature of a plant and its properties. In Ayurveda the analytical techniques have always been mentioned in classical texts to understand the quality of the end product e.g. *"Gatarasatva" of Kwatha dravyas* indicates the completion of Paka. Analytical study of a product provides standards to judge its quality. It is useful to decide future work plan and objective parameters to know the exact status of drug by conducting the comparative study of various samples during drug preparation.

Acknowledgment

Authors are highly acknowledged to National Institute of Ayurveda, deemed to be University, Jaipur for all the needful support and guidance.

Conflicts Of Interest- Nil

Source of finance & support – Nil

ORCID

Jagriti Sharma , https://orcid.org/ 0009-0009-4054-599X

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How to cite this article: Sharma J, Swami P "An Analytical Study for Validation and Standardization of *Vati"* IRJAY. [online] 2023;6(6);11-20.
Available from: https://irjay.com.
DOI link- https://doi.org/**10.47223/IRJAY.2023.6602**

Table 1 Showing the organoleptic characters of the Samples *Angamardprashman vati*

Sample	Appearance	Colour	Taste	Odour
AMP-1	Round shaped uncoated *vati*	Black	*Tikta, Madhur*	Characteristic
AMP-2	Round shaped uncoated *vati*	Black	*Tikta, Madhur*	Characteristic
AMP-3	Round shaped uncoated *vati*	Black	*Tikta, Madhur*	Characteristic

Table 2 Showing the organoleptic characters of the Samples *Bhagottar Gutika*.

Sample	Appearance	Colour	Taste	Odour
BG-1	Round shaped uncoated *vati*	Black	*Kashaya, Amla, Katu*	Characteristic
BG-2	Round shaped uncoated *vati*	Black	*Kashaya, Amla, Katu*	Characteristic
BG-3	Round shaped uncoated *vati*	Black	*Kashaya, Amla, Katu*	Characteristic

Table 3 Showing the results of Hardness of *Angamardprashman vati*

Parameter	AMP-1	AMP-2	AMP-3
Hardness (kg/cm^2)	6	6.2	6.5

Table 4 Showing the results of Hardness of *Bhagottar Gutika*.

Parameter	BG-1	BG-2	BG-3
Hardness (kg/cm^2)	4.5	4.7	4.0

Table 5 Showing the results of Friability test of *Angamardprashman vati*

Parameter	AMP-1	AMP-2	AMP-3
Friability	0%	0.1%	0%

Table 6 Showing the results of Friability test of *Bhagottar Gutika.*

Parameter	BG-1	BG-2	BG-3
Friability	0.1%	0.1%	0%

Table 7 Showing the results of Disintegration Time of *Angamardprashman vati*

Parameter	AMP-1	AMP-2	AMP-3
Disintegration Time	35 min.	32 min.	31 min.

Table 8 Showing the results of Disintegration Time of *Bhagottar Gutika*

Parameter	BG-1	BG-2	BG-3
Disintegration Time	21 min.	23 min.	20 min.

Table 9 Showing the results of pH of *Angamardprashman vati*

Parameter	AMP-1	AMP-2	AMP-3
pH(10%w/v suspension)	5.41 @ 27 ^{0}C	5.12 @ 27 ^{0}C	5.41 @ 27 ^{0}C

Table 10 Showing the results of pH of *Bhagottar Gutika.*

Parameter	BG-1	BG-2	BG-3
pH(10%w/v suspension)	5.0@ 27 ^{0}C	4.8@ 27 ^{0}C	4.8@ 27 ^{0}C

Table 11 Showing the results of Loss on Drying of *Angamardprashman vati*

Parameter	AMP-1	AMP-2	AMP-3
Loss on Drying	13.56%w/w	12.87%w/w	13.34 % w/w

Table 12 Showing the results of Loss on Drying of *Bhagottar Gutika*

Parameter	BG-1	BG-2	BG-3
Loss on Drying	8.65%w/w	9.35%w/w	7.95%w/w

Table 13 Showing the results of Total Ash of *Angamardprashman vati*

Parameter	AMP-1	AMP-2	AMP-3
Total Ash	14.37%w/w	14.65%w/w	13.56 % w/w

Table 14 showing the results of Total Ash of *Bhagottar Gutika.*

Parameter	BG-1	BG-2	BG-3
Total Ash	7.17% w/w	8.45% w/w	7.50% w/w

Table 15 Showing the results of Acid-insoluble ash of *Angamardprashman vati*

Parameter	AMP-1	AMP-2	AMP-3
Acid-insoluble ash	0.89%w/w	1.23%w/w	0.98 % w/w

Table 16 Showing the results of Acid-insoluble ash of *Bhagottar Gutika.*

Parameter	BG-1	BG-2	BG-3
Acid-insoluble ash	2.47 % w/w	3.12 %w/w	2.64 % w/w

Table 17 Showing the results of Water -soluble extractive Value of *Angamardprashman vati*

Parameter	AMP-1	AMP-2	AMP-3
Water -soluble extractive Value	45.76%w/w	46.65%w/w	44.32 % w/w

Table 18 Showing the results of Water-soluble extractive of *Bhagottar Gutika.*

Parameter	BG-1	BG-2	BG-3
Water-soluble extractive Value	34.40% w/w	37.40 % w/w	33.20 % w/w

Table 19 Showing the results of Alcohol -soluble extractive Value of *Angamardprashman vati*

Parameter	AMP-1	AMP-2	AMP-3
Alcohol -soluble extractive Value	6.22%w/w	6.54%w/w	5.98 % w/w

Table 20 Showing the results of Alcohol-soluble extractive of *Bhagottar Gutika.*

Parameter	BG-1	BG-2	BG-3
Alcohol-soluble extractive	8.42 % w/w	7.92% w/w	7.86% w/w

Table 21 Showing Microbiological Analysis of the *Angamardprashman vati*

Serial No.	Analysis	AMP-1 (cfu/ g.)	AMP-2 (cfu/ g.)	AMP-3 (cfu/ g.)
1	Total Aerobic Microbial count	70	80	90
2	Total Fungal count	<10	<10	<10

Table 22 Showing Microbiological Analysis of the *Bhagottar Gutika*.

Serial No.	Analysis	BG-1 (cfu/ g.)	BG-2 (cfu/ g.)	BG-3 (cfu/ g.)
1	Total Aerobic Microbial count	80	90	110
2	Total Fungal count	<10	<10	<10

Table 23 Showing result of tests for aflatoxins

Sample	B1	B2	G1	G2
AMP1	ND	ND	ND	ND
AMP2	ND	ND	ND	ND
AMP3	ND	ND	ND	ND

Table 24 Showing result of tests for aflatoxins

Sample	B1	B2	G1	G2
BG1	ND	ND	ND	ND
BG2	ND	ND	ND	ND
BG3	ND	ND	ND	ND