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A COMPREHENSIVE ANALYTICAL STUDY ON *PADMAK AGAD*: A RESEARCH ARTICLE

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ABSTRACT

Background: Analytical profile of the drug is essential to assure the safety, quality and efficacy of a drug. **Objective:** The present study was conducted to develop an analytical profile of *Padmak Agad*. **Material and Methods:** Analytical profile of the *Padmak Agad Churn* was assessed by organoleptic characteristics, physicochemical parameters, phytochemical screening and TLC evaluation. The physicochemical evaluation was done by parameters viz. pH, loss on drying, total ash, water-soluble ash, alcohol-soluble extractive, and water-soluble extractives. Phytochemical screening was done for extraction, screening and identification of the medicinally active substances present in the drug. **Results:** PH value, Loss on drying, total ash, water-soluble ash, alcohol soluble extractive and water-soluble extractive were 5.9, 7.61%, 7.25%, 5.82%, 9.28%, and 22.45%, respectively. Phytochemical screening showed the presence of Carbohydrates, Alkaloids, Amino acids, Proteins, Saponins, Glycosides, Phenolic compounds, Flavonoids, Steroids and Tannins *in Padmak Agad*. **Discussion and Conclusion:** *Padmak Agad* passed all the parametric tests. These values of tests can be used as a reference value for further research on *Padmak Agad*.

KEYWORDS: *Padmak Agad*, Analytical study, Phytochemicals Screening, physiochemical study.

INTRODUCTION

Ayurveda is oldest medical science kenned to mankind and mainly aims at salubrious living and long life unlike other medical science which simply fixate on the treatment of ailments and diseases. *Agadtantra* is one of the main branches of *Ashtang Ayurveda* which deals with toxicological conditions and their management. There are lots of preparations mentioned in different *Samhitas* to combat effect of poison. Most of them are named as *AGADA*. *Padmak Agad* is one of them. *Padmak Agad* is described in *Ashtang Hridya Utter tantra* chapter 37. It is betokened in all types of *Luta and Keet Visha*. It has three main ingredients *Priyangu, Haridra*, and *Daruharidra*.

Every drug has unique and consequential properties, which avails to differentiate between the different drugs and among drugs of homogeneous species. Safety, quality and efficacy have always been a major concern related to public health. The analytical study avails us to reach the casual inferences about hypothesized relationship between risk factor and outcomes.

Table 1: *Raspanchak* of ingredient of *Padmakagad.*

S. No.	***Dravya***	***Rasa* (Taste)**	***Guna* (Property)**	***Veerya* (Potency)**	***Vipaka* (Bio-transformation)**	***Karma* (Action)**
1.	*Priyangu*	*Tikta, Kashaya*	*Guru, Ruksha*	*Sheeta*	*Katu*	*Dahaprashaman, Vednasthapan, Durgandhnashak Raktaprasadana*
2.	*Haridra*	*Tikta, Katu*	*Laghu, Ruksha*	*Ushna*	*Katu*	*Kaphapittahar, Varnya, TwakDoshhar*
3.	*Daruharidra*	*Tikta, Katu*	*Laghu, Ruksha*	*Ushna*	*Katu*	*Varnya, Twak Doshhar, jwarghna*

MATERIAL AND METHOD

The Methodology has been grouped in sections:

Collection of drug

- ***PRIYANGU-*** *Priyangu* was collected from Nepali Farm, Dheradun near Swami Narayan Temple in the month of December, 2021. Weight of the fruit was 116 gm. Fruits were dried in shades and the weight of dried fruit was 37 gms.
- ***HARIDRA-*** *Haridra* was collected from Rishikul Campus, Haridwar in the month of January, 2022. The weight of wet Haridra was 800 gms which was reduced to 185 gms on drying in sunlight.

- ***DARUHARIDRA-*** *Daruharidra* was collected from Betaal Ghat, Nainital in the month of February, 2022. The weight of collected stem and root was 350 gm. It was also dried in sunlight.

Authentification and preparation

All the selected plants were identified and verified by the eminent experts of Dravyaguna Dept. at Rishikul Campus, Haridwar (UAU) with reference no. DG/RC/UAU-82 dated on 01/07/22. Preparation of *Yavkuta churna* of all the three ingredients of *Padmakagad* was done at home.

Priyangu fruits, *Haridra* tuber and *Daruharidra* root and stem barks were crushed into coarse powder with the help of mixer grinder. Obtained powder was sifted through sieve no. 44 and kept in air-tight container for further use.

ANALYTICAL STUDY

The word analysis means detailed examination of something in order to gain better understanding of it. Analysis reveals even the minor aspects of the drug. Each drug has its unique properties which help to differentiate between the different drugs and among drugs of similar species. Quality, efficacy and safety has always been a major concern regarding public health. The drug before administering to human subject or experiment should be well understood interpreted in the light of modern chemistry to know its proper scientific background. Analytical study is essential to check drug quality and to standardize it. To explore the physicochemical characteristics and active principles it is necessary to do analytical research. The results are then compared to standard parameters. The analytical study helps us to reach the casual inferences about hypothesized relationship between risk factors and outcomes. The purpose is to know the safety, efficacy and chemical constituents of drug for standardisation.

PLACE OF WORK- Bilwal Medchem & Research Laboratory Pvt. Ltd. H-9 SKS Industerial Area, Reengus, Rajasthan.

ANALYSIS OF *PADMAKAGAD*

✓ **Macroscopic Study**

The collected sample was studied organoleptically with naked eye and magnifying lens. Pharmacognostical parameters i.e., appearance, shape, size, surface, colour, odour and taste were recorded.

✓ **Microscopic study**

Microscopy is a tool of sample identification.

Powder Microscopy

Powder microscopic inspection of medicinal plant materials is indispensable for the identification of broken or powdered materials; the specimen has to be treated with chemical reagents. An examination by microscopy alone cannot always provide complete identification, though when used in association with other analytical methods it can frequently supply invaluable supporting evidence. Comparison with a reference material will often reveal characteristics not described in the requirements which might otherwise have been attributed to foreign matter rather than normal constituents.

Procedure

For examining the characters of the powder take sufficient amount of powder in different chemical reagents on a slide and warm over a low flame for a short time. Put drop of glycerine on the slide, cover it with the cover slip and observe under the microscope.

Chemical reagents used for staining of the powder samples were as follows –

- Safranin
- Dilute Ferric chloride
- Eosine
- Methylene blue

✓ **Physicochemical Analysis**

➢ **Determination of Moisture Content**[1]

Moisture content is a water holding capacity of sample, higher moisture content in sample show that it may decrease stability. Moisture content was determined by placing weighed sample of 5gm of drug in oven at 105° for 5 hours, and calculated weight of sample for every 30 minute, until the weight of the sample came out to be constant, no variation of weight was recorded. This sample was allowed to cool at room temperature in a desiccator for 1 hour before weighing.

Weight of the empty petridish = W_1gm

Weight of the drug sample = X gm

Weight of the petridish with drug before drying= (W_3) (W_1 +X)

Weight of petridish after drying= W_2gm

Loss on drying in %-W_3-W_2x100/X

➢ **Determination of pH**

The pH value of an aqueous liquid may be defined as the common reciprocal of the hydrogen ion concentration expressed in gram per litre. It practically means the quantitative indication of the acidity or basic nature of a solution.

The pH of a given solution is measured by using digital pH meter.

- First Standardized the pH meter. Tablets of different pH were taken and each tablet was dissolved in 100 ml of distilled water to prepare solutions of different pH.
- The instrument was switched on and left for some time until required different pH solutions appeared
- Buffer solution was taken in the beaker and the electrode was dipped in it. Same procedure was repeated for the other buffer solution after washing the electrode thoroughly with distilled water.
- The sample was taken (10% aqueous solution) and electrode was dipped in it and the value of pH was noted.

➢ **Determination of Extractive value**[2]

It is a gravimetric analysis (Maceration Process), the extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituents. The composition of these phyto-constituents in that particular solvent depends upon the nature of the drug and Solvent used.

✓ **Determination of Alcohol Soluble Extractive**

5 g coarsely powdered air dried drug was macerated with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours. It was then continuously shaken for six hours using rotary shaker and allowed to stand for eighteen hours. The content was filtered using filter paper. The filtrate was transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. Then the dish was kept in oven at 105º, to constant weight and

weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

Calculations

Weight of the drug material = X gm

Weight of the empty petri dish = W1gm

Weight of the petri dish with dried extract = W2gm

Percentage of extractive value =W2-W1x100/X

The procedure was repeated three times and the mean value was calculated.

- **Determination of Water Soluble Extractive**

Procedure was same as that of alcohol soluble extractive value and it was proceeded using distilled water instead of alcohol.

- **Determination of Total Ash**[3]

Ash is a quantity analysis technique for determining siliceous material and inorganic substance in sample. Acid Insoluble Ash shows siliceous material and heavy metals. Water Soluble Ash shows quantity of water inorganic Substance.

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both physiological ash which is derived from the plant tissue itself and non-physiological ash which is the residue of the extraneous matter (e.g. sand and soil) adhering to plant surface.

Silica Crucible was cleaned, dried well, labelled with glass pencils and then weighed to constant weight. 5 gm of powdered drug sample was put in the Silica crucible. The drug was spread evenly in to a thin layer. This crucible was placed in a muffle furnace and ignited at a temperature of 450°C for about 6 hrs or more until the ash was totally free from Carbon. The crucible containing the ash was allowed to be cooled in desiccators and subsequently weighed to constant weight. The percentage of ash with reference to the air dried drug was calculated.

Calculation

Wt. of Empty Silica Crucible = A1 gm

Wt. of Sample (X) = X gm

Wt. of the Crucible with Ash = A_2 gm

Percentage of Total Ash = [A_2 - A_1 /X] x 100

➢ **Determination of Acid Insoluble Ash[4]**

Acid insoluble Ash value was determined as per Pharmacopoeia of India, 1996. Boiled the total ash with 25 ml of 2M hydrochloric acid for 5 minutes, collected the insoluble matter in a Gooch crucible or on an ash less filter paper, washed with hot water, ignite, cool in a desiccator and weighed. Calculate the percentage of acid - insoluble ash with reference to the air - dried drug.

Calculation

Wt. of drug sample - X gm

Wt. of Crucible = G1 gm

Wt. of Crucible with insoluble Ash = G2 gm

Wt. of insoluble ash (G3) = G2-G1

Percentage of acid insoluble ash = G3/X×100

✓ **Determination of Water soluble Ash[5]**

Water soluble ash value was determined as per Pharmacopoeia of India 1996. Boiled the total ashfor 5 minutes with 25 ml of water; collected the insoluble matter in a Gooch‘s Crucible or on an ash less filter paper, Washed with hot water and ignite for 15 minutes at a temperature not exceeding 450 C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represented the water – soluble ash. Calculate the percentage of water – soluble ash with reference to the air - dried drug.

Calculation

Wt. of drug sample - X gm

Wt. of total ash – A gm

Wt. of Crucible - G1 gm

Wt. of Crucible with insoluble Ash - G2 gm

Wt. of insoluble ash (G3) = G2-G1

Water soluble ash (G4) = Wt. of total ash gm- Wt. of insoluble ash (G3)

Percentage of water soluble ash = A – [(G3)/X] x 100

Phytochemical Screening[6]

Tests for Carbohydrates

- **Molisch's Test**

2 ml of test Solution was taken in a test tube and 2 ml of the Molisch's reagent was added and shaken carefully and then about 1ml. of conc. H_2SO_4 is poured from side of the test tube and allowed to stand for one 1 minute. A Purple colour ring at the junction of the two layers if formed indicated the presence of Carbohydrate.

- **Benedict's test**

It is used for reducing sugars and composed of mainly Copper sulphate and sodium hydroxide. To the 4 ml of aqueous solution of drug, 1 ml of Benedict's solution was added and heated almost to boiling. Formation of green, yellow, orange, red or brown color in order of increasing concentrations of simple sugar in the test solution, due to formation of cuprous oxide.

- **Barfoed's test**

The test sample was dissolved in water and heated with a little of the Barfoed's reagent. Formation of red precipitate of cuprous oxide within two minutes indicates the presence of monosaccharides.

- **Fehling solution test**

It is generally used for reducing sugars and composed of two solutions, which are mixed in situ. Fehling solution A composed of 0.5% of copper sulphate whereas Fehling solution B composed of Sodium Potassium Tartarate.

Equal volumes of Fehling A and Fehling B solutions were mixed (1 ml each) and 2 ml of aqueous solution of drug was added followed by boiling for 5-10 minutes on water bath.

Tests for Alkaloids

- **Mayer's reagent test**

2 ml of test Solution was taken in a test tube to which 2 ml of the Mayer's reagent (Potassium Mercury Iodide solution) was added. A White or Pale Yellow precipitate if formedindicated presence of Alkaloids except with Alkaloids of the Purine groups and few others.

- **Dragendroff's reagent test**

2 ml of test Solution was taken in a test tube in which 2 ml of the Dragon Droff's reagent (Mixture of Potassium Iodide and Bismuth sub nitrate solution) was added. An orange precipitate if formed indicated presence of Alkaloids.

- **Wagner's Test**

Drug solution + few drops of Wagner's reagent (dilute Iodine solution), formulation of reddish-brown precipitate indicates the presence of alkaloids.

- **Hager's Test**

A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with this reagent, an orange yellow precipitate was obtained which indicates the presence of alkaloids.

Test for Amino acids

- **Ninhydrin test**

The Ninhydrin test is used to detect the presence of alpha-amino acids and proteins containing free amino groups. Protein solution when heated with ninhydrin molecules, it gives characteristic deep blue or pale yellow colour due to the formation of complex between two ninhydrin molecule and nitrogen of free amino acid.

Tests for Proteins

- **Biuret test**

A few mg of the residue was taken in water and 1 ml of 4% sodium hydroxide solution was added to it, followed by a drop of 1% solution of copper sulphate. Development of violet or pink colour indicates the presence of proteins.

- **Xanthoproteic test**

A small quantity of test sample was taken with 2 ml of water and 0.5 ml of concentrated nitricacid was added to it. Development of yellow colour indicates the presence of proteins.

- **Millon's test**

A small quantity of test sample was taken and 2 to 3 ml of millons reagent was added. The white precipitate slowly turning to pink, indicate the presence of proteins.

Test for saponin

- **Foam test**

A small quantity of the test sample was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. A stable, characteristic honeycomb like froth indicates the presence of saponins.

Test for Glycosides

- **Borntrager's Test**

1 ml of Benzene and 0.5 ml of dilute ammonia solution was added to the ethanolic extract and was observed for the formation of reddish pink colour.

Test for Phenolic Compound

The extract was taken in water and warmed; to this 2 ml of ferric chloride solution was added and observed for the formation of green and blue colour.

Test for Flavonoids

- **Shinoda test**

A small quantity of test sample was dissolved in 5 ml ethanol (95%v/v) and reacted with few drops of concentrated hydrochloric acid and 0.5 gm of magnesium metal. Appearance of pink, crimson or magenta color within a minute or two indicates the presence of flavonoids.

Test for Steroids

- **Salkowski reaction**

Few mg of extract was taken in 2 ml of chloroform and 2 ml of concentrated sulphuric acid was added from the side of test tube. The test tube was shaken for few minutes. The development of red colour indicates the presence of steroids.

Test for Tannins

- **Ferric chloride solution**

A 5 percent solution of ferric chloride in 90 % alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Appearance of dark green or deed blue color indicate the presence of tennin.

- **Lead acetate**

A 10 percent w/v solution of basic lead acetate in distilled water was added to the test filtrate. Development of precipitate indicates the presence of tannins.

- **Pot. Dichromate**

A solution of potassium dichromate was added to the filtrate. Appearance of dark colour indicates the presence of tannins.

- **Chromatograph[7]**

Chromatography plates

T.L.C. plate coated with 0.25 mm layer of silica gel 60 F_{254} with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width)

Activation of pre-coated Silica gel 60 F_{254}

Plates were dried in hot oven at 105^0 C for one and half hour.

Test solution: Alcoholic Extract

Preparation of mobile solution: Toluene: Ethyl Acetate: Formic acid (6:3: 1)

Visualization: Vanillin sulphuric acid Spray.

Rf Value

Measured and recorded the distance of each spot from the point of its application and calculated Rf. value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase**.**

Calculation of Rf Value

$$Rf = \frac{\text{Distance travelled by solute from origin line}}{\text{Distance travelled by solvent from origin line}}$$

OBSERVATIONS AND RESULTS

Organoleptic characters, physicochemical analysis, phytochemical screening and Chromatography was done and finding were summarised in Table2, Table 3, and Table 4.

Table 2: Result of Macroscopic Study of Padmak Agad.

S. No	Organoleptic	Observation
1	**Colour**	Light Ochre
2	**Odour**	Characteristics
3	**Appearance**	Coarse Powder

Table 2: Results of Physicochemical Analysis of Padmak Agad.

S. No	Tests	Value	Test method
1	Loss on drying (%)	7.61	A.P.I, Part II, Vol-I, Appendices- 2.2.10
2	Aqueous Extractive Value(%)	22.45	A.P.I, Part II, Vol-I, Appendices- 2.2.8
3	Alcoholic Extractive Value (%)	9.28	A.P.I, Part II, Vol-I, Appendices- 2.2.7
4	Total Ash (%)	7.25	A.P.I, Part II, Vol-I, Appendices- 2.2.3
5	Acid Insoluble Ash (%)	2.16	A.P.I, Part II, Vol-I, Appendices- 2.2.4
6	Water Soluble Ash (%)	5.82	A.P.I, Part II, Vol-I, Appendices- 2.2.5
7	pH	5.9	A.P.I, Part II, Vol-II, Appendix-3.3

Table 3: Result of Phytochemical screening of Padmak agad.

Name of Test	Aqueous Extract	Ethanolic Extract
Carbohydrate		
Molish test	+ *ve*	- *ve*
Benedict test	+ *ve*	+ *ve*
Fehling test	+ *ve*	+ *ve*
	Alkaloids	
Dragendorff test	- *ve*	+ *ve*
Wagner's test	- *ve*	- *ve*
Hager's test	+ *ve*	+ *ve*
	Amino acids	
Ninhydrine	+ *ve*	- *ve*
	Protein	
Biuret test	- *ve*	- *ve*
Xenthoprotic test	+ *ve*	- *ve*
	Saponin	
Foam test	+ *ve*	- *ve*
	Glycosides	
Borntrager's test	- *ve*	+ *ve*
	Phenolic compound	
Phenolic test	+ *ve*	+ *ve*
	Steroids	
Salkowaski	- *ve*	+ *ve*
	Tannins	
$Fecl_3$	+ *ve*	+ *ve*
Lead acetate	+ *ve*	+ *ve*
Pot. Dichromate	- *ve*	- *ve*

Aflatoxin

S.No	Aflatoxin	Observation	Reference	Test method
1	**Aflatoxin B1**	Not Detected	**0.5 PPB**	**A.P.I, Part II, Vol-I, Appendix - 2.7**
2	**Aflatoxin B2**	Not Detected	**0.1 PPB**	
3	**Aflatoxin G1**	Not Detected	**0.5 PPB**	
4	**Aflatoxin G2**	Not Detected	**0.1 PPB**	

1. Thin Layer Chromatography

2. TLC image	R_f Value	Test method
	0.52, 0.62, 0.64, 0.83, 0.96	**Stationary Phase:** Silica gelG60F254 **Mobile solution** Toluene : Ethyl Acetate (6:4) **Visualization:** Iodine Vapours

DISCUSSION

Organoleptic study was done by using the sense organs. During organoleptic study, colour of Padmak Agad was golden ochre, odour was characteristic and appearance was coarse powder. Moisture content is a water holding capacity of sample, higher moisture content in sample show that it may decrease stability. Loss on drying in *Padmak Agad* was 7.61%. Ash value is the method to know the adulteration of the inorganic materials and it has greater importance in quality control and standardization. Total ash value of Padmak Agad was 7.25%. Acid insoluble ash represents presence of silica and silicate impurities. *Padmak Agad* contained 2.16% of siliceous content. While water soluble ash percentage of *Padmak Agad* was 5.82%. Alcoholic extractive value and aqueous extractive value of Padmak Agad was 9.28% and 22.45% respectively. Extractive value indicates the presence different constituents and TLC fingerprinting is used for identification and semi-quantitative analysis of these extracts. Rf values 0.52, 0.62, 0.64, 0.83, 0.96 were found after thin layer chromatography. Phytochemical screening was done for extraction, screening and identification of the medicinally active substances present in the drug. This study was done in both alcoholic and aqueous extract. Qualitative analysis for the presence of various functional group showed the presence of Carbohydrates, Alkaloids, Amino acids, Proteins, Saponins, Glycosides, Phenolic

compounds, Flavonoids, Steroids and Tannins. At high level, Aflatoxins can cause illness, liver damage. There was absence of any kind of Aflatoxins in *Padmak Agad.*

CONCLUSION

The Analytical profile of *Padmak Agad* was studied as per the Ayurvedic Pharmacopoeia of India and WHO guidelines for medicinal plants. Organoleptic characteristics, physicochemical parameters and physical properties of *Padmak Agad* passed all the tests, which prove its quality. TLC of *Padmak Agad* shows phytoconstituents of raw drugs. The absence of Aflatoxins and microbial contamination in *Padmak Agad* prove the drug's safety. These parameters can be used as a reference value for further research on *Padmak Agad*.

REFERENCES

1. The Ayurvedic Pharmacopeia of India, Part 2. Reprinted Appendix 2, (2.2.10). Department of AYUSH, New Delhi, 2007: Government of India: Ministry of Health and Family Welfare; 1, 1: 141.
2. The Ayurvedic Pharmacopeia of India, Part 2. Reprinted Appendix 2, (2.2.7). Department of AYUSH, New Delhi, 2007: Government of India: Ministry of Health and Family Welfare; 1, 1: 141.
3. The Ayurvedic Pharmacopeia of India, Part 2. Reprinted Appendix 2, (2.2.3). Department of AYUSH, New Delhi, 2007: Government of India: Ministry of Health and Family Welfare; 1, 1: 140.
4. The Ayurvedic Pharmacopeia of India, Part 2. Reprinted Appendix 2, (2.2.4). Department of AYUSH, New Delhi, 2007: Government of India: Ministry of Health and Family Welfare; 1, 1: 140.
5. The Ayurvedic Pharmacopeia of India, Part 2. Reprinted Appendix 2, (2.2.8). Department of AYUSH, New Delhi, 2007: Government of India: Ministry of Health and Family Welfare; 1, 1: 141.
6. Laboratory guide for the analysis of Ayurveda and siddha formulations, CCRAS, Dept. Of Ayush, ministry of health and family welfare, govt. of India New Delhi, P. 83-87.
7. Laboratory guide for the analysis of Ayurveda and siddha formulations, CCRAS, Dept. Of Ayush, ministry of health and family welfare, govt. of India New Delhi, P. 89-92.