Research Article

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ANALYTICAL PROFILE OF BILWADI AGADA

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ABSTRACT

Background: Analytical profile of the drug is essential to assure the drug's safety, quality and efficacy. Objective: The present study was conducted to develop an analytical profile of Bilwadi Agada. Material and Methods: Analytical profile of the Bilwadi Agada tablet was assessed by organoleptic characteristics, physicochemical parameters, pharmaceutical analysis 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. Also, pharmaceutical analysis was done by the parameters like hardness, friability, disintegration time, uniformity of weight etc. Results: PH value, Loss on drying, total ash, water-soluble ash, alcohol soluble extractive and water-soluble extractive were 3, 0.068%, 18.5%, 4.5%, 19.2%, and 44%, respectively. The hardness of the tablet and friability was 3.6 kg/cm^2 and 0.33%, respectively, whereas the disintegration time of the tablet was less than 15 minutes. Aflatoxins and heavy metals were absent, while no microbial growth was found in 24 hours in the Bilwadi Agada tablet. Discussion and Conclusion: Bilwadi Agada tablets pass all the parametric tests. These values of tests can be used as a reference value for further research on Bilwadi Agada.

Keywords: Bilwadi Agada, Analytical Profile, Vishaghna Yoga, Physicochemical evaluation

INTRODUCTION

Ayurveda is mainly divided into eight clinical branches called Ashtanga Ayurveda. Out of these eight clinical branches, Agadtantra deals with the study of poison with particular reference to their sources, properties, action, manifestations and management. Agadtantra describes the Ayurvedic perspective of toxicology. Various Vishaghna yogas (Antitoxic formulations) are mentioned in Agadtantra, which are valuable for the management of toxicity and can be indicated in various pathological conditions, including infectious diseases.

Ayurvedic drugs are mainly prepared from plants, minerals and some animal products. Ayurveda, an eternal science of life, is now evolving as an integrated part of the healthcare system through evidence-based scientific experimentation. These experiments undergo various phases: analytical, preclinical, and clinical studies. Nowadays, the commercialization of Ayurvedic drugs is going on, so assurance of herbal medicine's safety, quality and efficacy have become an important issue. Hence analytical profile or standardization of Ayurvedic formulations is essential to produce good quality, efficient and potent drugs.

Bilwadi Agada (BA) is a classical vishaghna yoga mentioned in Ashtanga Hridaya. It is prepared from thirteen medicinal plants (drugs) triturated in goat's urine. It is an antivenin formulation indicated for the snake, spider, rat, scorpion etc., venom intoxication along with diseased conditions like Cholera, indigestion, concocted/low potent poison, fever and psychological disorders. Bilwadi Agada is administered by various routes like oral, collyrium and nasal medication.[1] Bilwadi Agada is prepared by various pharmaceutical companies in tablet form. The Ayurvedic Pharmacopoeia of India and WHO Guidelines for Medicinal plants were referred to study the analytical profile of Billwadi Agada. In this study, Bilwadi Agada prepared by nine Bhavana (trituration) of Goat urine was tested for various standard parameters to confirm its quality, safety and efficacy. This analytical study of Bilwadi Agada will help settle standard parameters and can be used as a reference for further research.

MATERIALS AND METHODS

Authentication and Preparation of Bilwadi Agada (BA): All thirteen raw drugs required for the preparation of Bilwadi Agada were procured from the authorized vendor of Nagpur, Maharashtra, India. The goat urine required for preparation was freshly procured from the farmer. These 13 drugs were authenticated at Botany Department, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur, Maharashtra, India. Their authentication numbers are shown in Table 1.

All 13 drugs were dried and made into fine powder with a grinder mixer separately. These individual fine powders were sieved through 100-number mesh. Fine powders of all drugs were taken in the big steel container and mixed uniformly. This mixed Powder was taken in kharal (Mortar). Slowly goat urine was poured into a mortar, and bhavana (trituration) was started with the help of a pestle. Trituration is continued till the consistency of the mixture becomes susukshma pishtam (fine powder paste). A total of nine such bhavanas were given[2]; for each bhavana, fresh goat urine was used. On the ninth bhavana, when an excellent paste consistency appeared, then uniform tablets of 500 mg were prepared. These tablets were dried in the shade for 15 days and kept in an airtight bottle.

Table 1: List of Raw Drugs of Bilwadi Agada with Authentication Number

Drug	Latin Name	Family	Part	Authentication Number
Bilwa	*Aegle marmelos* (L) Correa	Rutaceae	Root	10391
Tulsi	*Ocimum tenuiflorum* Linn. (*Ocimum sanctum* Linn.)	Laminaceae	Inflorescence	10392
Karanja	*Pongamia pinnata* (L.) Pierre	Fabaceae	Fruit	10393
Tagar	*Valeriana wallichii* D.C.	Valerianaceae	Root	10394
Surahawa	*Cedrus deodara* (Roxb.ex D.Don) G.Don	Pinaceae	Heart wood	10395
Amalaki	*Phyllanthus emblica* Linn. (*Embelica officinalis*)	Euphorbiaceae	Fruit	10396
Haritaki	*Terminalia chebula* Retz.	Combretaceae	Fruit	10397
Bibhitaka	*Terminalia bellirica* (Gaertn) Roxb.	Combretaceae	Fruit	10398
Sunthi	*Zingiber officinale* Rosc.	Zingiberaceae	Rhizome	10399
Maricha	*Piper nigrum* Linn.	Piperaceae	Fruit	10400
Pimpali	*Piper longum* Linn.	Piperaceae	Fruit	10401
Haridra	*Curcuma longa* Linn.	Zingiberaceae	Rhizome	10402
Daruharidra	*Berberis aristata* D.C.	Berberidaceae	Stem bark	10403

Analytical Profile of Bilwadi Agada

Organoleptic Characteristics: Organoleptic evaluation means studying drugs using the organs of the senses. It refers to analysis methods like colour, odour, taste, texture etc. Five tablets were randomly selected from the Bilwadi Agada (BA) tablets. These five tablets were powdered using a mortar and pestle. Organoleptic characteristics like colour, odour and taste were examined.
Colour - Brown
Odour - Characteristic (Goat urine)
Taste - Characteristic

Macroscopical Characteristics: Ten tablets of BA were powdered in mortar and pestle and spread on white paper. After observing it with the naked eye, dried parts of organized and unorganized crude drugs were found as fine particles.

Microscopical Characteristics: Microscopic examination was done by taking a pinch of coarsely powdered BA tablets on the glass slide. The slide was observed under the digital light microscope. Volatile oil globules, fibres, calcium oxalate crystals, lignified xylem vessels, starch grains, parenchyma and sclerenchyma were found. [3-6]

Physicochemical Evaluation

pH: pH value was determined using pH paper. A strip of pH paper was placed on a white tile surface. A drop of BA sample was poured on pH paper with the help of a dropper. The colour obtained on pH paper was compared with different shades of colour pH chart, and the pH value was noted down, which was 3 (Acidic).

Loss on drying: This method is used primarily to determine the moisture content in the sample.

Procedure: About 1.50 gm of coarse powder of BA was taken in the crucible. Initially, the weight of the empty crucible was taken. The sample evaporated to dryness for 4 hours in a hot air oven at 105 ^{0}C.

Observations noted were as follows-
Weight of empty crucible - 42.18 gm
Weight of sample taken - 1.5 gm
Weight of sample + weight of crucible - 43.68 gm
Weight of sample + crucible after 1 hour - 43.66 gm
Weight of sample + crucible after 2 hours - 43.65 gm
Weight of sample + crucible after 4 hours - 43.65 gm

Loss on drying = Initial weight – final weight × 100 / Initial weight = 43.68 - 43.65 × 100 = 43.68 = 0.068%

Total ash
Procedure: The weight of the empty porcelain dish was taken. About 2 gm of BA powder was taken in a dish. The dish was supported on a pipe clay triangle placed on the ring of the retort stand. Heated with flame till vapours almost cease to be evolved. The dish lowered and heated more strongly until all the carbon was burnt. Then it was cooled in the desiccator. Ash was weighed, and the percentage of total ash with reference to an air-dried sample of BA was calculated.

Observations noted were as follows-
Weight of empty dish - 73.37 gm
Weight of drug is taken - 2 gm
Weight of dish + ash (after complete incineration) - 73.66 gm
Weight of ash - 0.37 gm
2 gm of the crude drug gives 0.37 gm of the ash
Total ash value of the sample = 0.37× 100/2 = 18.5%

Water soluble ash
Procedure: Total ash dissolved in 25 ml of water and boiled. It was filtered through ashless filter paper. Crucible was ignited in the flame, and after cooling, it was weighed. Filter paper and residue were put together into the crucible. It was heated gently until vapours ceased to be evolved and then more strongly until all carbon had been removed. It was cooled in the desiccator, and the weight of the residue was taken.

2 gm of the air-dried drug gives 0.09 gm of water-soluble ash.
Weight of the residue - 0.09 gm
Water soluble ash value of drug - 0.09×100/2= 4.5%

Alcohol soluble extractive
Procedure: 1.25 gm of BA was taken and kept in 25 ml alcohol for cold maceration.

25 ml of alcoholic extract gives 0.24 gm of residue.
Alcohol soluble extractive value of BA = 0.24 × 100 / 1.25 = 19.2%

Water soluble extractive
Procedure: 1.25 gm of BA boiled with 25 ml of water and proceeded for water-soluble extractive.

25 ml of water extract gives 0.55 gm of residue
Water soluble extractive value of the sample = 0.55 × 100 / 1.25 = 44%

Pharmaceutical Analysis of BA Tablets

Physical properties of the BA tablets, like hardness, friability, disintegration time, and uniformity of weight, were performed. [7]

Hardness: The force required for breaking a tablet in a diametric compression test is called the hardness of the tablet. This hardness testing value indicates the strength necessary to tolerate mechanical shocks at the time of handling during manufacturing, packaging and transporting. In this test, a few tablets' hardness was measured using a Monsanto hardness tester and the mean hardness was calculated.

Procedure: BA tablet was placed between the spindle and anvil, and pressure was applied by turning the screw knob to hold the tablet in position. The reading of the indicator on the scale was adjusted to zero. The pressure was applied until the tablet broke. Readings were noted and shown in Table 2.

Friability Test: Friability testing is important because some formulations, when compressed into tough tablets, tend to cap due to attritions resulting in loss of crown portions. Hence the friability test is essential to monitor tablets' resistance to such stress.

Procedure: 10 tablets of BA were taken and weighed. Tablets were placed in a chamber of a friabilator which rotates at 25 revolutions per minute and drops the tablets from a height of 6 inches with each revolution. The procedure is done for 100 revolutions in 4 minutes. The tablets were withdrawn and weighed. The percentage difference from their original weight was used to express friability.

Observations noted were as follows-
Initial weight - 5.98 gm
Final weight - 5.96 gm
% friability = initial weight – final weight × 100 / Initial weight = 5.98 – 5.96 ×100 / 5.98 = 0.33 %

Disintegration time: Disintegration time means the ability of a tablet to break down into small particles or granules to allow the active drug to be absorbed into the body.

Procedure: The disintegration time apparatus uses six glass tubes about 3 inches long open at the top and held against 10 mesh screens at the bottom. One tablet of BA was placed in each tube. The tablets were immersed in the liquid with the tubes by a cylindrical guide disc. The basket rack was placed in a 1-litre beaker of water at 37 ^{0}C ± 2 ^{0}C. The basket rack assembly was suspended so that the highest point was at least 2.5 cm below the surface of the liquid, and the lowest point was at least 2.5 cm above the bottom of the beaker. The assembly was raised and lowered 30 times in liquid.

Uniformity of weight
Uniformity of weight was essential to ensure that every tablet and capsule contained the same drug substance with a defined allowed variation within a batch. This helps in the quality control of tablets.

Limits for tablet weight variation mentioned in IP/BP are as follows-
1. 80 mg or less - ± 10%
2. More than 80 mg or less than 250 mg - ± 7.5%
3. 250 mg or more - ± 5%

Procedure: 20 BA tablets were weighed randomly and individually. The average weight was determined. Then for each tablet, the percentage of deviation of its weight was determined and shown in Table 3.

Microbial load determination
Procedure: Nutrient agar solution has been prepared in 3 gm in 100 ml of distilled water. Agar solution and petri plates were sterilized by autoclaving at a temperature of 121 ^{0}C and pressure of 15 psi for 15 minutes. Petri plates were prepared by transferring agar solution in the laminar airflow. BA sample was prepared and poured into one petri plate, and microbial growth was observed after 24 hours.

Detection of aflatoxins
Stationary phase - silica gel G 62
Mobile phase - chloroform: acetone (88: 12 v/v)
No blue-coloured fluorescence at UV 365 nm. Therefore, aflatoxin was not present in the sample. [8]

Detection of Heavy Metals

Cobalt: Dissolve 20 mg of ash in 0.5 ml of distilled water and acidify with a few drops of dil. HCl. Add a few drops of dil. solution of NaOH. A blue ppt is formed, which turns pink on warming if cobalt is present. Here no blue ppt was observed; hence cobalt was absent.

Copper: Dissolve 20 mg of ash in 1 ml of distilled water. Add dil. ammonia solution until a clear solution is obtained. Heat to boiling. Add 2% alcoholic solution of the alpha-benzoin oxime dropwise. A green ppt is formed if copper is present. Here no green ppt was formed. Hence copper was absent.

Mercury: Dissolve 20 mg of ash in 1 ml of distilled water. Add 2 M NaOH until the solution becomes strongly alkaline. A dense yellow ppt is observed if mercury is present. Here no dense yellow ppt was observed; hence mercury was absent.

Nickel: Dissolve 20 mg of ash in 0.5 ml of distilled water and acidify with a few drops of dil. HCl. Then add drop by drop dil. Solution of NaOH. A blue ppt is formed, which turns green on warming if nickel is present. Here no blue ppt was formed; hence nickel was absent.

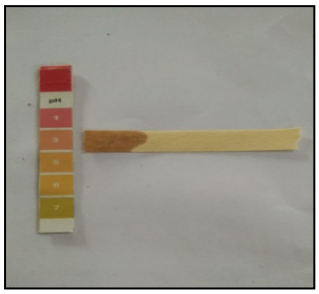
Silver: Dissolve 20 mg of ash in 3 ml of distilled water and add 0.2 ml of 7M HCl. A curdy ppt is formed, soluble in 3 ml of 6 M ammonia. A yellow ppt is developed by adding a few drops of 10% w/v potassium iodide solution. No curdy ppt was formed; hence silver was absent.

Zinc: Dissolve 20 mg of ash in 2 ml of distilled water and add 0.2 ml of NaOH. A white ppt is formed, which dissolves in 2 ml of 10 M NaOH. Add about 2 M ammonium chloride followed by 0.1 ml of sodium sulphide. A flocculent white ppt is formed. Here white ppt was not observed; hence zinc was absent. [9]

Thin Layer Chromatography (TLC)

Stationary phase: Silica plate G F_{254} and Mobile phase: Ethyl acetate: Methanol: Water (100: 13.5: 10) were used for TLC. TLC of 13 herbal ingredients along with Bilwadi Granules and two samples of Bilwadi Agada was done. A total of 16 points were used on TLC plates. Their Rf values, along with colours without derivatization, at UV 254 nm, at UV 365 nm, by spraying with anisaldehyde sulphuric acid reagent and in iodine chamber exposure, were shown in Table 4. [10,11]

Organoleptic Evaluation

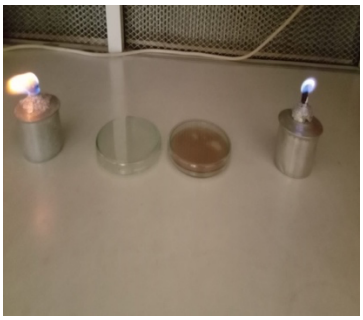


pH

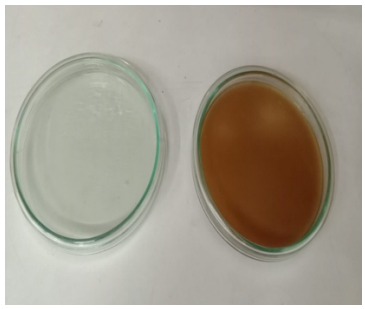
Total Ash

Alcohol Soluble Extractive

Water Soluble Extractive

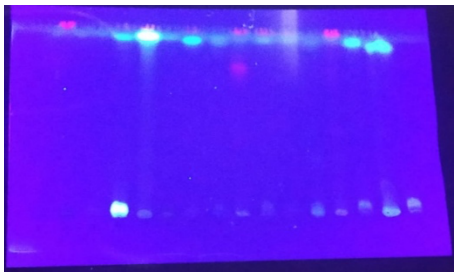


Before 24 hours

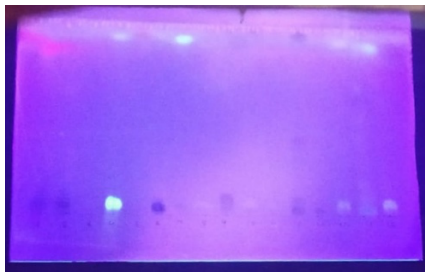


After 24 hours

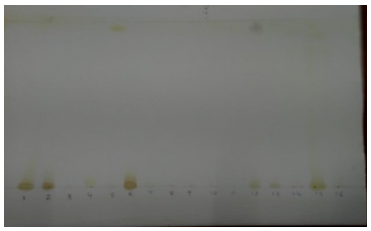
Microbial Load Determination



TLC at 254 nm



TLC at 365 nm



Spraying with anisaldehyde sulphuric acid reagent

Iodine chamber exposure

Detection of aflatoxins

Table 2: BA tablet hardness readings

Tablet	Hardness in Kg/cm^2
1	3.6
2	3.8
3	3.4

Table 3: BA tablets Weight and Percentage of deviation of its weight

Tablet	Weight (mg)	Percentage of deviation (%)	Pass/ Fail
1	595.6	4.40	Pass
2	595.6	4.40	Pass
3	553.6	-2.96	Pass
4	601.5	5.43	Fail
5	557.4	-2.29	Pass
6	595.6	4.40	Pass
7	578.9	1.47	Pass
8	566.7	-0.66	Pass
9	597.3	4.69	Pass
10	597.3	4.69	Pass
11	588.7	3.19	Pass
12	570.3	-0.03	Pass
13	569	-0.26	Pass
14	595	4.29	Pass
15	595.6	4.40	Pass
16	595.6	4.40	Pass
17	559.3	-1.96	Pass
18	595.6	4.40	Pass
19	549.3	-3.71	Pass
20	609.4	6.82	Fail

Table 4: TLC Rf values of Bilwadi Agada along with its ingredients

Drugs	Without derivatization	UV 254 nm	UV 365 nm	Anisaldehyde	Iodine
Amalaki	-	-	Red 0.87	Red 0.86	Light yellow 0.93
Bibhitak	-	-	Red 0.86	Red 0.85	Light yellow 0.94
Bilwa	-	Pink 0.93	Red 0.97	Light blue 0.87	-
Daruharidra	Yellow	Yellow 0.97	yellow 0.95	Violet 0.95	-
Haridra	-	Yellow 0.94	yellow 0.97	-	-
Haritaki	-	-	Light blue 0.97	Violet 0.96	Yellow 0.93
Karanj beej	-	Blue 0.89	Blue 0.94	Blue 0.94	-
Marich	Yellow 0.52	Pink 0.83	Blue 0.97	Orange 0.57	Yellow 0.74
Pimpali	Blue 0.97	Blue 0.97	Green 0.98	Reddish orange 0.95	Yellow 0.93
Suravaha	Blue 0.65	Blue 0.64	Violet 0.62	Blue 0.59	Yellow 0.68
Sunthi	-	-	Light green 0.97	-	-
Tagar	-	-	Light green o.96	-	-
Tulsi	Dark green 0.93	Red 0.98	Red 0.98	Blue 0.83	Yellow 0.83
BA granules	-	-	Blue 0.94	Light blue 0.93	-
BA tablet 1	-	-	Blue 0.91	Blue o.92	-
BA tablet 2	-	-	Blue 0.91	Blue 0.93	-

Table 5: Analytical Study of Bilwadi Agada

Parameter	Observation	Inference
Organoleptic Evaluation	Colour: Brown Odour: Characteristic Taste: Characteristic	Passes the test
Macroscopical Characteristics	Dried parts of organized and unorganized crude drugs are found present in the form of fine particles.	Passes the test
Microscopical Characteristics	Volatile oil globules, fibres, calcium oxalate crystals, lignified xylem vessels, starch grains, parenchyma, and sclerenchyma, were present.	Passes the test
Physicochemical Evaluation		
pH	3	Acidic
Loss on drying	0.068%	-
Total ash	18.5%	-
Water soluble ash	4.5%	-
Alcohol soluble extractive	19.2%	-
Water soluble extractive	44%	-
Pharmaceutical Analysis of BA Tablets		
Hardness	3.6 kg/cm^2	Passes the test
Friability	0.33%	Passes the test
Disintegration test	All 6 tablets were disintegrated within 15 minutes	Passes the test

Weight uniformity test	Less than ± 5%	Passes the test
Microbial load determination	No microbial growth in 24 hours	No microbial growth
Detection of Aflatoxins	No blue-coloured fluorescence at UV 365 nm	Aflatoxins were absent in the sample.
Detection of heavy metal		
Cobalt	No blue ppt was formed	Cobalt was not present in the sample.
Copper	No green ppt was formed	Copper was not present in the sample
Mercury	No dense yellow ppt was formed	Mercury was not present in the sample
Nickel	No blue ppt was formed.	Nickel was not present in the sample.
Silver	No curdy ppt was formed	Silver was not present in the sample
Zinc	No white ppt was formed	Zinc was not present in the sample
TLC Chromatography	Well-separated spots of all phytochemical ingredients found to be present in TLC chromatogram	Ingredient phytoconstituents were found to be present in the final formulation of a sample.

OBSERVATIONS AND RESULTS

The hardness of three tablets of Bilwadi Agada was noted and shown in Table 2. The mean of three values was calculated and considered as the hardness of Bilwadi Agada. The mean hardness of the BA tablet was 3.6 Kg/cm^2.

The weight and percentage deviation of the BA tablet are shown in Table 3. The weight of 20 BA tablets was noted, and the average weight was derived. After that percentage of deviation of weight was calculated by using the following formula-
Average weight = weight of 20 tablets / 20 = 11581.4 / 20 = 579.07
Percentage of deviation = weight of tablet – average weight × 100 / Average weight
Less than 2 tablets deviate from ± 5%

Rf values of BA tablets and their ingredients are shown in Table 4.

All the observations of the analytical study of Bilwadi Agada were summarized in Table 5.

DISCUSSION

BA was prepared from 13 raw herbal drugs by triturating it 9 times with goat urine. Its organoleptic characters show brown colour, characteristic odour (goat urine) and characteristic taste. Macroscopic examination with the naked eye shows organized and unorganized crude drugs in the form of fine particles. Microscopic examination shows various parts, volatile oil globules, fibres, calcium oxalate crystals, lignified xylem vessels, starch grains, and parenchyma.

pH values determine the acidic nature (3) of BA tablets. The loss on drying value for BA was 0.68%, indicating the absence of moisture. The residue remaining after incineration is the total ash content of the drug, which was 18.5% for BA. The total ash content, which is soluble in water, is water soluble ash of the drug, which was 4.5% for BA. Alcohol-soluble extractive represents the alcohol-soluble constituents in the drug, while water-soluble extractive indicates water-soluble constituents in the drug. The water-soluble extractive value (44%) was more than the alcohol-soluble extractive (19.2%) in BA.

The hardness of BA was 3.6 kg/cm^2, indicating good mechanical resistance for the tablets while handling or shipping. Friability also measures the strength of the tablet, which was 0.33% in BA. BA tablets were disintegrated within 15 minutes, indicating how rapidly the solid de-aggregates to physiological solution and the drug get absorbed. For tablets weighing 250 mg or more deviation of weight allowed is ±5%. Out of 20 tablets, only 2 tablets show a deviation of more than ±5%, which proves uniformity of weight in prepared BA Tablets.

No microbial growth was expected in the drugs. Aflatoxins are naturally occurring mycotoxins and should be absent from the drug. Also, Heavy metals should be absent in a standard drug. The absence of aflatoxins, heavy metals, and no microbial growth in 24 hours indicate that BA tablets were safe. TLC of BA with its ingredients shows well-separated spots, and ingredient phytoconstituents were found to be present in the final formulation of BA.

CONCLUSION

The Analytical profile of Bilwadi Agada was studied as per the Ayurvedic Pharmacopoeia of India and WHO guidelines for medicinal plants. Organoleptic characteristics, physicochemical parameters and physical properties of BA pass all the tests, which proves its quality. TLC of BA shows phytoconstituents of raw drugs. The absence of aflatoxins, heavy metals and no microbial contamination in BA prove the drug's safety. These parameters can be used as a reference value for further research on BA.

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