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Pharm acognostic And Analytical Standardization Of Bacopa Monnieri Linn. And Coccinia Indica W& A - Ayurvedic Medicines For Shayyamutra W.S.R. To Enuresis In Children

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Abstract

Introduction: *Shayyamutra is an emerging problem now a day. It may lead to psychological burden for family. It can be managed with the help of Ayurvedic drugs. Bacopa monnieri Linn. And Coccinia indica W&A are good choice for the same. Proper identification and standardization of these drug is must for the era of globalization.*

Aim and objectives: *To study the macroscopic and microscopic characters of Brahmi Panchanga and Mool of Bimbi, To study the powder microscopy of both the drugs, To analyze the samples by using different physico-chemical parameters.*

Material and Method*: The identification was carried out based on the morphological features, organoleptic features and powder microscopy of the individual drugs. Loss on drying Ash value, Acid insoluble ash, Water soluble extractive, Alcohol soluble extractive, PH value, Tablet Hardness, Tablet disintegration time and Uniformity (Average Wt.) have been tested at modern pharmaceutical laboratory.*

Result*: Bacopa monnieri Linn powder showed xylem vessels with reticulate thickening, glandular hairs, simple, round and oval starch grains, measuring 4-14 μ in diameter. Coccinia indica W&A powder showed groups of round to polygonal parenchymatous cells, reticulate, spiral and pitted vessels, aseptate fibres, palisade cells, stone cells, simple and compound, round to oval starch grains. Weight variation was found more in Group A tablets i.e. 560 mg average weight of a single tablet while average weight of Group B tablet was found 480, which were within permissible variation.*

Conclusion*: All the parameters found almost equal to the given standard values for a Brahmi (Bacopa monnieri Linn) and Bimbi (Coccinia indica W& A) except uniformity of Brahmi tablets*

Key words: *Bacopa Monnieri Linn., Coccinia Indiaca, Shayyamutra, Enuresis, Pharmacognosy, physiochemical*

INTRODUCTION

Enuresis is defined as normal, nearly complete evacuation of the bladder at a wrong place and time at least twice a month after 5 years of age. Enuresis is usually functional while continuous or daytime enuresis is often organic[i]. A study conducted at southern Maharashtra found that the prevalence of nocturnal enuresis ranges from 7 to 12.6%.[ii] In rural area of Gujarat, prevalence of nocturnal enuresis is 11.13%.[iii] Despite of behavioral therapy, motivation therapy and alarm therapy; some patients need pharmacotherapy. Anticholinergic drugs are used for the same but relapse rate are high after stopping the medication.[iv] Ayurveda plays important role for management of the same with Medhya drugs.

In Ayurveda the description regarding the plants is available in the Nighantu wherein various synonyms are given to each plant while describing it. In this pattern, many drugs are available under one name and the same name is given to several drugs. Therefore, the crux of the entire problem virtually revolves around proper identification of the drug. The original and basic approach towards pharmacognosy includes study of morphological system, study of cell structure and organization and study of tissue systems, which still hold a key in identification of the correct species of the plant. The plants selected for the present study Brahmi also face the same problem as different plant is taken in the name of Brahmi all over the India. Therefore, it is very necessary for providing authenticity to any research work to have a clear-cut identification and description of the plant used in particular research work. Thus in order to establish the correct identity of the botanical source of the drugs, a detailed Pharmacognostical study was

carried out.At present, Ayurvedic science is spreading its wings all over the world, where the drug lore of this system has been the center of global interest. Ayurveda advocates that, as the Prakriti vary from person to person similarly every drug has got its own physical and chemical characteristics which help to separate it from other closely related drug. The Physico-chemical studies of these drugs done by making use of various parameters help in standardizing the drug and authenticate it. In this modern era it is an expected imminent need for a well-coordinated research plan touching physiochemical study of drug. It is essential to gratify the international standards and quality control of the drug used for convincing the drug regulatory authorities. The present study was carried out to evaluate the physico- chemical parameters of test drugs.

MATERIAL AND METHODS

Pharmacognostical evaluation

The whole plant of Bacopa monnieri Linn. and root of Coccinia indica W& A were collected from the Sasoi region of Jamnagar district and periphery of Jamnagar district in the month of august – September 2008 by the scholar and authenticated by the experts of pharmacognosy department I.P.G.T and R.A., Gujarat Ayurved University , Jamnagar. A voucher specimen of both the species has been preserved in the herbarium attached to the Institute. Then the powders of both the drugs were used for powder microscopic study of drug. The whole plant of Bacopa monnieri Linn. and root of Coccinia indica W& A were used for present study. Photomicrographs of were taken by using Canon digital camera attached to Zeiss microscope[v] with the help of Pharmacognosy Department, I.P.G.T.& R.A., G.A.U., Jamnagar. The identification was carried out based on the morphological features, organoleptic features and powder microscopy of the individual drugs.[vi]The root powders of both the drugs were separately evaluated by organoleptic characters like taste, odour, color and touch. Macroscopic characters of the root, stolon and leaf of both the drugs were studied systematically as mentioned in the API. Transverse sections of root, stolon and leaf of Bacopa monnieri Linn and also transverse sections of root of Coccinia indica W& A were taken and photomicrography was done after proper mounting and staining. Powder of both the drugs was studied microscopically and microscopic characters of the powder were photographed by using Canon digital camera attached to Zeiss microscope.

Analytical evaluation

The separate tablet of both the drug were prepared at pharmacy, I.P.G.T and R.A., Gujarat Ayurved University, Jamnagar by following standard operating procedure for tablet making. these tablets were analyzed for physiochemical parameters at moderns analytical laboratory, I.P.G.T. & R.A., Jamnagar.

Loss on drying Ash value, Water soluble extractive, Alcohol soluble extractive, Ph Value, Tablet hardness, Tablet Disintegration time and Tablet Uniformity were studied for tablets Standardization. Standardization of tablets were done as per The British Pharmacopoeia 1932, Addendum VII 1945, introduced official standards for maintaining uniformity of the compressed tablets. shape, weight, percentage of medicament, rate of disintegration, rate of dissolution, mechanical strength / hardness and diameter were analysed for the same.

Observation and Results

Organoleptic characters of the whole plant powder of Bacopa monnieri Linn and Coccinia indica W&A have been tabulated below (Table 1)

Table 1: Organoleptic characters of Bacopa monnieri Linn. & Coccinia indica W&A

Sr No.	Parameters	Bacopa monnieri Linn	Coccinia indica W&A
1	Texture	Fine powder	Fine powder
2	Color	Light brown	Ash colored
3	Taste	Bitter, astringent	Bitter
4	Odor	Slight bitter	Pungent

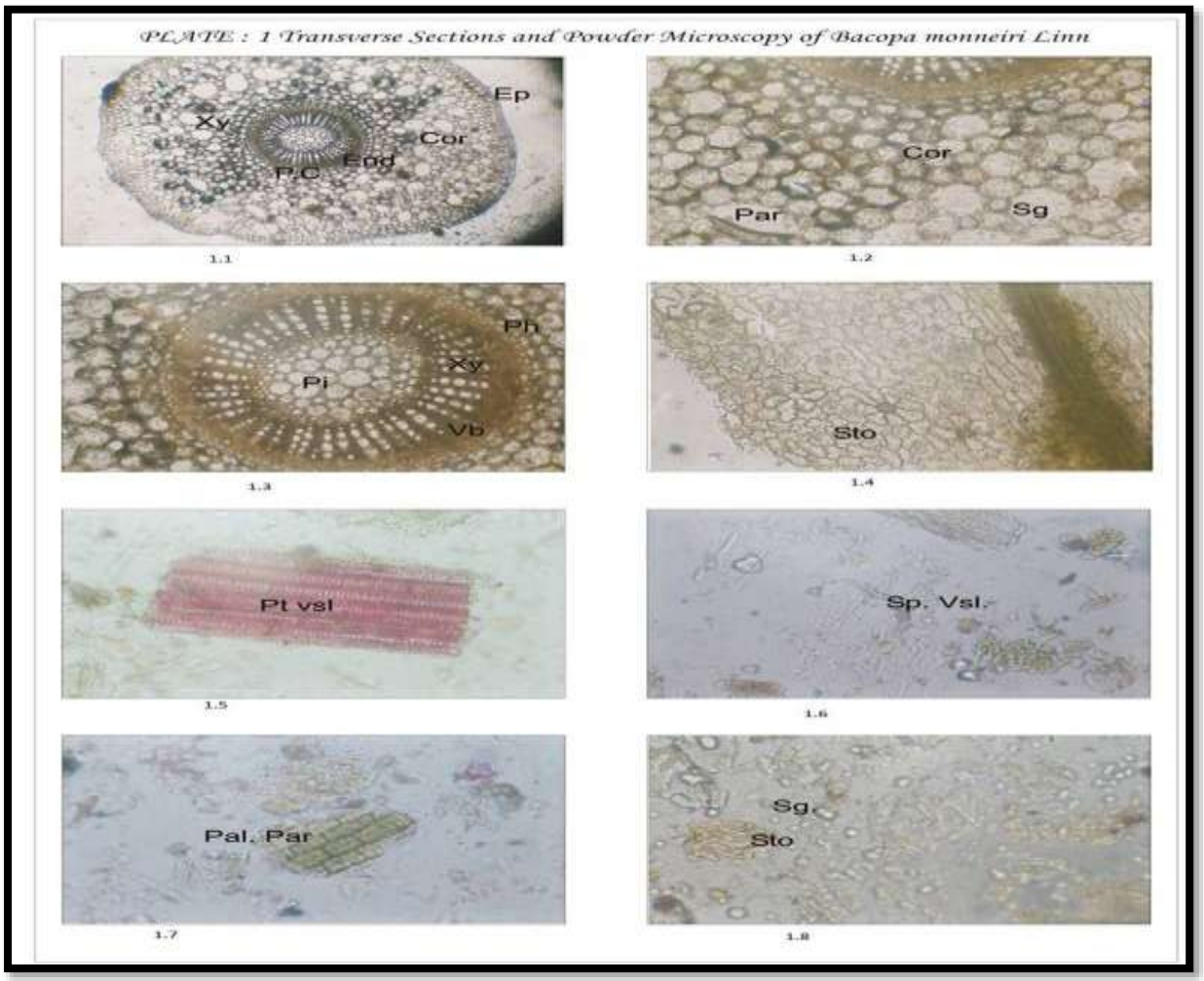
Macroscopic[vii] character of Bacopa monnieri Linn:

Root were thin, wiry, small, branched creamish-yellow. Stem were Thin, green or purplish green, about 1-2 mm thick, soft, nodes and internodes prominent, glabrous; taste, slightly bitter. Leaf were simple, opposite, decussate, green, sessile, 1-2 cm long, obovate-oblong; taste, slightly bitter.Flower were small,

axillary and solitary, pedicels 6-30 mm long, bracteoles shorter than pedicels.Fruit were capsules up to 5 mm long, ovoid and glabrous.

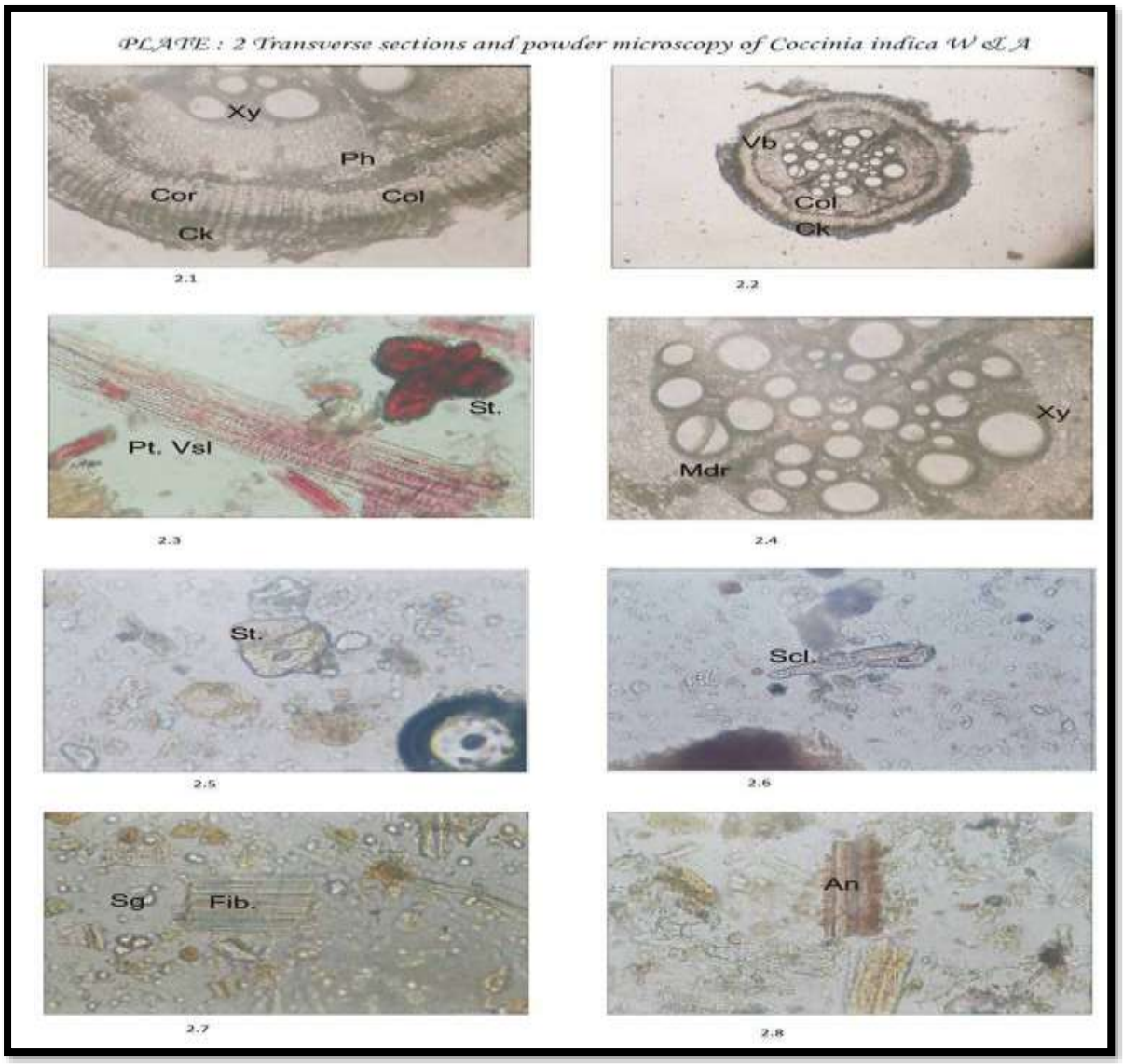
Microscopic of Bacopa monnieri Linn:

Microscopic characters of Bacopa monnieri Linn is shown in photo plate 1. Root were showed a single layer of epidermis, cortex having large air cavities; endodermis single layered; pericycle not distinct; stele consists of a thin layer of phloem with a few sieve elements and isolated material from xylem showed vessels with reticulate thickenings. Stem showed single layer of epidermis followed by a wide cortex of thin-walled cells with very large intercellular spaces; endodermis single layered; pericycle 3 consisting of 1-2 layers; vascular ring continuous, composed of a narrow zone of phloem towards periphery and a wide ring of xylem towards centre; centre occupied by a small pith with distinct intercellular spaces; starch grains simple, round to oval, present in a few cells of cortex and endodermis, measuring 4-14 μ in dia., and 8.0-14.0 x 2.5-9.0 μ in dia. Respectively. Leaf showed a single layer of upper and lower epidermis covered with thin cuticle; glandular hairs sessile, subsidiary cells present on both surfaces; a few prismatic crystals of calcium oxalate occasionally found distributed in mesophyll cells; mesophyll traversed by small veins surrounded by bundle sheath; no distinct midrib present.Powder microscopy of Bacopa monnieri Linn is shown in photo plate 1. Bacopa monnieri Linn powder showed xylem vessels with reticulate thickening, glandular hairs, simple, round and oval starch grains, measuring 4-14 μ in diameter. Coccinia indica W&A powder showed groups of round to polygonal parenchymatous cells, reticulate, spiral and pitted vessels, aseptate fibres, palisade cells, stone cells, simple and compound, round to oval starch grains, measuring 3-11 μ in diameter, fragments of epidermis with straight walled cells and anomocytic stomata.



1.1 - T. S. of Bacopa stem showing epidermis, endodermis, cortex, pericycle and xylem**, 1.2 -** T. S. of Bacopa stem showing parenchyma starch grain, cortex.**, 1.3 -** T. S. of Bacopa stem showing pith,

xylem and phloem.1.4 –Surface view of Bacopa leaf showing stomata. 1.5 - Powder microscopy Bacopa showing pitted vessel. 1.6 - Powder microscopy Bacopa showing spiral vessel. 1.7 - Powder microscopy Bacopa showing palisade parenchyma. 1.8 - Powder microscopy Bacopa showing simple and compound starch grain and stomata.



2.1 - T. S. of Coccinia root showing cork cell, cortex, collenchyma, xylem and phloem. 2.2 - T. S. of Coccinia root showing collenchymas, cork cell and vascular bundle. 2.3 - Powder microscopy of Coccinia root showing pitted vessel and stomata. 2.4 - T. S. of Coccinia root showing medullary ray and xylem. 2.5 - Powder microscopy of Coccinia root showing stone cell. 2.6 - Powder microscopy of Coccinia root showing scleride cell. 2.7 - Powder microscopy of Coccinia root showing starch grain and septate fiber. 2.8 - Powder microscopy of Coccinia root showing annular fiber.

Physico-chemical parameters

Loss On Drying (LOD)[viii] is an important parameter for determining the moisture content of the drug. The moisture content of the drug should be minimized in order to prevent decomposition of the crude drugs either due to chemical change or microbial contamination. 1 gram of drug sample was taken in a pre-weighed dried petri dish. It was dried in an oven at 105°c until reaching a constant weight. The petri dish was taken out, self-cooled and weighed immediately. The weight loss i.e. Loss on drying was calculated and expressed as % w/w. Ash Value [ix](AV) was conducted to evaluate the percentage of inorganic salts, naturally occurring in the drug or adhering to it or deliberately added as a form of adulteration.[x] One gram accurately weighed sample was taken in a pre-weighed dried crucible. It was incinerated in a muffle furnace up to 450°c. The crucible was taken out, self cooled and weighed

immediately. From the weight of the ash, the ash value was derived with reference to the air dried drug. It was calculated and expressed as % w/w. Obtained ash boiled for 5 minutes with 25 ml of dilute hydrochloric acid; the insoluble matter was collected in a Gooch crucible or on an ash less filter paper, washed with hot water and ignited to constant weight. Calculated the percentage of acid-insoluble ash with reference to the air dried drug. Water Soluble Extractive [xi](WSE) test was carried out to determine the water soluble extractive and approximate measures of their chemical constituents of the test drug. Water soluble extractive value shows the content of polar compounds such as Flavonoids, Glycosides, Tannins and Saponins which are soluble in water. 5 gram of the sample was weighed accurately. 50 ml of distilled water was added and kept covered overnight. It was stirred intermittently in the initial period. Next day, it was filtered. 20 ml of the filtrate was accurately measured with a pipette and transferred to the already weighed evaporating dish. The evaporating dish was placed on a water bath for evaporation of the water. After evaporation of the water it was dried in an oven, allowed cooling and weighed immediately. From the weight of the residue obtained, the percentage of water soluble extractive was calculated and expressed as % w/w. Alcohol Soluble Extractive (ASE) test was carried out to determine the alcohol soluble extractive of the test drug. Alcohol soluble extractive values show the presence of non-polar compounds such as Alkaloids, Glycosides, etc. Present in the sample.[xii] The method adopted for this experiment was same as that of water-soluble extract but by using methanol instead of water. Percentage of methanol soluble extract was calculated and expressed as % w/w. P^H Value test was carried out to determine the P^H of the test drug with the help of P^H meter.[xiii] 10gram of test drug sample was weighted and taken in a conical flask. Then added 50 ml accurately measured water and stirred well for few minutes; kept this solution for some time and then filtered it through filter paper & the filtered solution was taken in a beaker. Standardized by P^H meter and electrodes with buffer solution of known P^H i.e. 7 ph. Rinsed electrodes with distilled water was introduce into the test solution contained in a small beaker & the P^H value of solution was read.[xiv] For standardization of tablets, Tablet disintegration time[xv] was tested to determine whether tablets disintegrate with in a prescribed time, when placed in liquid medium under the prescribed experimental condition.[xvi] For this test apparatus basket and peddle apparatus were used. One tablet into each tube introduced and a disc to each tube were added, after that assembly was suspended in the beaker containing the liquid at specified temperature for dispersible tablets for specified time. Assembly was removed then from the liquid. When all of them have disintegrated, then time taken for total disintegration was noted. It is desirable that every tablet in a batch should be uniform in weight. Small variation in weight of individual tablets is inevitable and is admissible. The weight variation, if any, should be within the permissible limits. For this test, twenty tablets from prepared drugs were weighed from each group on an electronic weighing machine and individual weight of all the 20 tablets were done & they were added to get total weight then divided by total number of tablets to get average weight of a single tablet. Hardness is a property which is dependent on density and porosity of the material on one hand and pressure of the compression on the others at cold, in the ultimate analysis, means resistance to attrition, abrasion, bending, breaking etc. Hardness of the tablets is measured in terms of pressure required to crush it when placed on its edge. Monsanto hardness tester and Pfizer tester etc. can be used for this. Hardness of 5 kg is considered as minimum for uncoated tablet for mechanical stability. The hardness has influence on disintegration and dissolution times and is as such a factor that may affect bio-availability. Physio chemical parameters of both the samples are as describes in table 2

Table 2: Physico-chemical parameters of bacopa monnieri linn **and coccinia indica** w&a

Sr.	**Parameters**	**bacopa monnieri** linn **Sample A**	**coccinia indica** w&a **Sample B**
1	Loss on drying	12.03 % w/w	6.42 % w/w
2	Ash value	18.9 % w/w [18%]	24.31%w/w [21%]
3	Acid insoluble ash	2.05 % w/w [6%]	3.98 % w/w [2%]
4	Water soluble extractive	30 % w/w [15%]	20 % w/w [14%]
5	Alcohol soluble extractive	14.8 % w/w [6%]	2.9 % w/w [3%]
6	P^H value	6.23	7.26
7	Tablet Hardness	2.162 kg/cm^2	1.975kg/cm^2

8	Tablet disintegration time	20min	10min
9	Uniformity (Average Wt.)	560 mg	480mg

DISCUSSION:

Authentication of drug is essential for better treatment.in present study, Bacopa monnieri contents alkaloids[xvii], which might be responsible for its slightly bitter odor. Taste is bitter might be because of Tikta Rasa of Brahmi. Bitter taste of Coccinia indica might be because of its Tikta Rasa. It contents saponins and fixed oil[xviii], which might be responsible for its pungent odor. Organoleptic and microscopic characters of both the drugs were similar to the API standard, which proves the authenticity of the drugs. There were not much variation in the loss of drying of samples, as it is 12.3% w/w and 6.42% w/w in Sample A and Sample B respectively. The ash value indicates the inorganic load of a drug. There is variation in the ash value of both the samples. Ash value of Sample A is 18.9% w/w which is almost equal to the standard given for it i.e. Not more than 18% & slightly higher in Sample B which is 24.31% w/w than standard i.e. Not more than 21% which indicates slightly presence of impurity . The water soluble extractive value of Sample A (30% w/w) is higher than Sample B (20 %w/w) which indicates that the load of polar components is higher in Sample A. Both samples have higher WSE than Standard. The alcohol soluble extractive value which is indicative of the load of non-polar components is higher in Sample A (14.8% w/w) than Standard while for Sample B (2.9% w/w) nearly equal to standard value. The Ph of Sample A and B is 6.23 and 7.26 respectively, which shows that Sample A is slightly acidic while the Sample B is slightly alkaline. The acid insoluble ash suggest the amount of silica in the drug which can be present due to a number of reasons such as improper washing being one of them. The acid insoluble value of Sample B is more i.e. 3.98% w/w which is more than Standard while Sample A i.e.2.05 % w/w which far less than Standard given for it, Suggests purity of sample A. Tablet disintegration time was found with in prescribed standard for tablets by British Pharmacopeia, though no such standard is given for Ayurvedic tablets. Weight variation was found more in Group A tablets i.e. 560 mg average weight of a single tablet while average weight of Group B tablet was found 480, which were within permissible variation.

CONCLUSION:

All the parameters found almost equal to the given standard values for a Brahmi (Bacopa monnieri Linn) and Bimbi (Coccinia indica W& A) except uniformity of Brahmi tablets that found beyond permissible limit of variation applicable for allopathic tablets . No such standard was found to check the standard variation of tablets for Ayurvedic tablets.

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