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PHARMACOGNOSTIC STANDARDIZATION OF POLYHERBAL FORMULATION PALASHA BEEJADI CHOORNA

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Keywords:

Polyherbal formulation, PalashabeejadiChoorna, Pharmacognostic evaluation, herbal drug standardization

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ABSTRACT: Herbs are the main source of Ayurvedic formulations. Polyherbalism concept of Ayurveda enables fortified therapeutic effect when compared to single herb. The potency of polyherbal formulations depends on their ingredients. Plants tend to vary in their efficacy due to the influence of environmental factors, such as temperature, humidity, light, oxygen, moisture content. The quality of polyherbal formulations depends on factors like selecting the raw material, habitat, season, harvesting conditions, method of storage, and pharmaceutical processing. Hence the evaluation parameters for polyherbal formulations should be based on chemical, physical, microbiological, therapeutic and toxicological assessment. Standardization for each formulation is unique. The monograph thus prepared will be useful to ensure the quality of the pharmaceutical product. Palasha beejadi Choorna (PBC) is a formulation indicated and frequently prescribed as the best choice for krimiroga. It is mentioned in Rasoddaratantra and in the Ayurvedic Formulary of India (AFI). **Aims and objective:** The objective of the study is to standardize Palasha beejadi choorna of Rasoddaratantra, mentioned in the Ayurvedic Formulary of India. Methodology PBC was formulated by adding equal quantities of Palasha (*Butea monosperma* (Lamk.) Taub) seeds, Indrayava (*Holarrhena pubescens* (Buch.-Ham) Wall. ex G. Don) seeds, Vidanga (*Embelia ribes* Burm. F.) fruits, Nimba (*Azadirachta indica* A. Juss.) seed, Kiratatikta (*Swertia chirayita* (Roxb. ex Fleming) Karsten). PBC was subjected to Organoleptic, Macroscopic, Microscopic, Physicochemical, preliminary phytochemical analysis, and High-performance thin- layer chromatography (HPTLC). **Conclusion:** The results thus obtained from the pharmacognostic evaluation may be considered as standard values for Palasha beejadi Choorna.

INTRODUCTION: Pharmaceutical products with a combination of few herbs are commonly prescribed medicine by physicians. The therapeutic effect of the polyherbal formulation is better than single-drug therapy as in combination they produce a synergistic effect and also minimize the toxicity [1].

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The therapeutic efficacy of the formulation depends on the quality of each ingredient. Quality of a raw drug depends on habitat, cultivation method, season, collection, storage, transportation, and processing [2].

At present raw drug availability is also an important point of consideration as it has led to the usage of the different drugs in place of specified drugs. Variation in the quality of raw drugs will have a direct impact on the therapeutic efficacy of the formulation. The Ayurvedic Pharmacopeia of India (API) provides the standard value and assessment protocol for the raw drug.

This document enables to ensure the genuinity of raw drug. Chemical constituents in each herb itself is a complex structure; hence it is not easy to establish the standards for a formulation having more herbs. Therefore, the polyherbal formulation should have standards in terms of organoleptic, macroscopic, microscopic, physicochemical, phyto-chemical analysis and High- performance thin-layer chromatography (HPTLC) [1].

Pharmaco-gnostic standardization of polyherbal formulation is essential so that the product is ensured for its quality when tested and shows appropriate therapeutic efficacy. Presently the study is done with the objective to standardize Palasha beejadi choorna (PBC) of Rasoddaratantra, mentioned in Ayurvedic Formulary of India. PBC is a frequently prescribed medicine for krimiroga.

MATERIALS AND METHODS: Palasha beejadi choorna was prepared following the procedure as given in The Ayurvedic Formulary of India [3]. The ingredients *viz.,* Palasha (Butea monosperma (Lamk.) Taub) seeds, Indrayava (*Holarrhena pubescens* (Buch.-Ham) Wall. ex G. Don) seeds, Vidanga (*Embelia ribes* Burm.F.) fruits, Nimba (*Azadirachta indica*. Juss.) seed, Kiratatikta (*Swertia chirayita* (Roxb. ex Fleming) Karsten) whole plant were collected and authenticated from Department of Pharmacognosy, Sri Dharmasthala Manjunatheshwara Centre For Research In Ayurveda And Allied Sciences Kuthpady, Udupi and a voucher specimen deposited (Voucher No: Palashabeeja: SDMRC/947/17111703, Indrayava: SDMRC/947/17111705,Vidanga:SDMRC/947/17111704, Nimbabeeja: SDMRC/947/17111705, Chirayta: SDMRC/947/17111707. All the ingredients were powdered and passed through a number 80 sieve and then mixed together in specified proportions to get uniformly blended choorna.

Following Pharmacognostic evaluation was performed on Palasha beejadi choorna.

A. Macroscopic and organoleptic evaluation
B. Powder microscopy
C. Physicochemical evaluation
D. Preliminary phytochemical evaluation
E. High-performance thin-layer chromatography (HPTLC)

Macroscopic details of PBC were observed with the naked eye and with the aid of a magnifying lens. The general conditions of the powder were noted. Organoleptic characters like colour, odour, taste, consistency were recorded.

Powder Microscopy: Pinch of powder previously sieved was put on the slide and mounted in glycerine, and powder characters were observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

Physico Chemical Evaluation: *viz.,* determination of moisture content, total ash, acid insoluble ash, water-soluble extractive, alcohol soluble extractive were evaluated as per API guidelines.

Determination of Moisture Content: 10 g of the drug (without preliminary drying) was accurately weighed in a tared evaporating dish. The dish containing the drug was dried at 105 ºC for 5 h in a hot air oven, cooled in a desiccator, and weighed.

The drying and weighing was continued until the difference between two successive weight corresponded to not more than 0.25%. Constant weight was reached when two consecutive weights after drying for 30 min and cooling for 30 min in a desiccator, showed not more than 0.01 g difference.

Determination of Total Ash: 2-3 g of accurately weighed ground drug was taken in a tared silica crucible and incinerated at a temperature not exceeding 450 ºC until free from carbon, cooled, and weighed. If carbon-free ash could not be obtained in this way, the charred mass was exhausted with hot water; the residue was collected on an ashless filter paper and incinerated the residue with filter paper. The filtrate was added, evaporated to dryness, and ignited at a temperature not exceeding 450 ºC. The percentage of ash with reference to the air-dried drug was calculated.

Determination of Acid Insoluble Ash: The obtained ash was boiled with 25 ml dilute Hydrochloric acid for 5 min, and the insoluble matter was collected on an ashless filter paper, washed with hot water, and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air-dried drug was calculated.

Determination of Alcohol Soluble Extractive: 5 g coarsely powdered air-dried drug was macerated with 100 ml of alcohol in a closed flask for twenty-four hours, frequently shaking for six hours and allowing to stand for eighteen hours.

It was filtered rapidly, taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish and dried at 105 ºC, to constant weight and weighed. The percentage of alcohol-soluble extractive with reference to the air-dried drug was calculated.

Determination of Water-Soluble Extractive: 5g coarsely powdered air-dried drug was macerated with 100 ml of water in a closed flask for twenty-four hours, frequently shaking for six hours and allowing to stand for eighteen hours. It was filtered rapidly, taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish and dried at 105 ºC, to constant weight and weighed. The percentage of water-soluble extractive with reference to the air-dried drug was calculated [4].

Preliminary Phytochemical Evaluation Test for Reducing Sugars: Fehling's test: Mix 1 ml Fehling's A and 1 ml Fehling's B solutions, boil for one minute. Add an equal volume of test solution. Heat in boiling water bath for 5-10 min.

First yellow, then brink red ppt. is observed. Benedict's test: Mix equal volume of Benedict's reagent and test solution in a test tube. Heat in boiling water bath for 5 min. The solution appears green, yellow, or red depending on reducing sugar present in the test solution.

Test for Non Reducing Polysaccharides (starch): Iodine test: Mix 3 ml test solution and few drops of dilute iodine solution. The blue colour appears; it disappears on boiling and reappears on cooling.

Test for Proteins: Biuret test: To 3 ml test solution, add 4% NaOH and few drops of 1% $CuSO_4$ solution. Violet or pink colour appears. Precipitation test: The test solution gives white colloidal ppt. with following reagents: (a)absolute alcohol (b) 5% Mercuric chloride solution (c) 5% Copper sulphate solution (d) 5%lead acetate (e) 5% Ammonium sulphate

Test for Amino Acids: Ninhydrin test: Heat 3 ml test solution and 3 drops 5% Ninhydrin solution in boiling water bath for 10 min. The purple or bluish colour appears.

Test for Steroids: Salkowski reaction: To 2 ml of test solution, add 2 ml Chloroform and 2 ml conc. Sulphuric acid. Shake well. The chloroform layer appears red and acid layer shows greenish-yellow fluorescence. Libermann- Burchard reaction: Mix 2 ml of test solution with Chloroform. Add 1-2 ml acetic anhydride and 2 drops conc. Sulphuric acid from the side of the test tube. First red, then blue and finally green color appears. Libermann's reaction: Mix 3 ml extract with 3 ml acetic anhydride. Heat and cool. Add few drops of conc. Sulphuric acid. blue colour appears.

Test for Glycoside: Borntrager's test for Anthraquinone glycoside: To 3 ml of extract, add dilute sulphuric acid. Boil and filter. To the cold filtrate, add equal volume benzene/chloroform. Shake well. Separate the organic solvent. Add ammonia. The ammoniacal layer turns pink or red. Foam test for saponin glycoside: Shake the test solution vigorously with water. Persistent foam is observed.

Test for Flavonoids: Sulphuric acid: On addition of sulphuric acid (66% or 80%) flavones and flavonols dissolve into it and give a deep yellow solution. Chalcones and aurones give red or red-bluish solutions. Flavones give an orange to red colours.

Test for Alkaloids: Dragendorff's test: To 2 ml of the test solution, add few drops of Dragendorff's reagent. Orange-brown ppt. is formed. Wagner's test: To 2 ml of the test solution, add few drops of Wagner's reagent. Reddish-brown ppt. is formed. Mayer's test: To 2 ml of the test solution, add few drops of Mayer's reagent, ppt is formed.

Test for Tannins: Ferric chloride test: To 2 ml of the test solution, add few drops of 5% Ferric chloride solution, deep blue-black colour is formed. Lead acetate test: To 2 ml of the test solution, add few drops of Lead acetate solution, white ppt. is formed. Bromine water: To 2 ml of the test solution, add few drops of Bromine water, decoloration of bromine water occurs. Acetic acid

solution: To 2 ml of the test solution, add few drops acetic acid solution, red colour solution is formed [5].

High-Performance Thin Layer Chromatography (HPTLC): One gram of powdered samples of Palasha beeja (*Butea monosperma* (Lam.) Taub.), Indrayava (*Holarrhena antidysentrica* (L.)). Vidanga (*Embelia ribes* Burm.f.), Nimbabeeja (*Azadirachta indica* A. Juss.), Cirayata (Kiratatikta) (*Swertia chirayita* (Roxb.ex Flem.) were suspended in 10 ml ethanol and kept for cold percolation for 24 h and filtered. 5 µl of the above samples were applied on a pre-coated silica gel F254 on aluminum plates to a bandwidth of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (7.0: 1.0). The developed plates were visualized in short UV, long UV and then derivatized with vanillin sulphuric acid reagent and scanned under UV 254 nm, 366 nm and 620 nm following derivatization. R_f value and, colour of the spots and were recorded.

RESULTS AND DISCUSSION:

Macroscopic Characters: Brown colour, moderately fine powder; no characteristic odour; bitter and astringent taste.

Microscopic Evaluation: Powder microscopy of the palasha beejadi churna shows a group of sclereids **Fig. 1**, endosperm with aleurone grains **Fig. 2**, epicarp with striated **Fig. 3**, stone cells with sclereids **Fig. 4**, cells of epidermis of fruit **Fig. 5**, testa **Fig. 6**. These observations, when compared with the available reference of powder microscopy of ingredients following cells, were similar. Sclereids and fragments of testa are present in *Butea monosperma* (Lam.) Taub. [6] Sclereid cells present in *Azadirachta indica* A. Juss. [7] Endosperm with aleurone grains are the cells of *Embelia ribes* Burm. F. [8] and *Swertia chirayita* (Roxb. ex Fleming) Karsten [9] are found in PBC. Epicarp or epidermal cells of fruit and stone cells are found in *Embelia ribes* Burm. F [8].

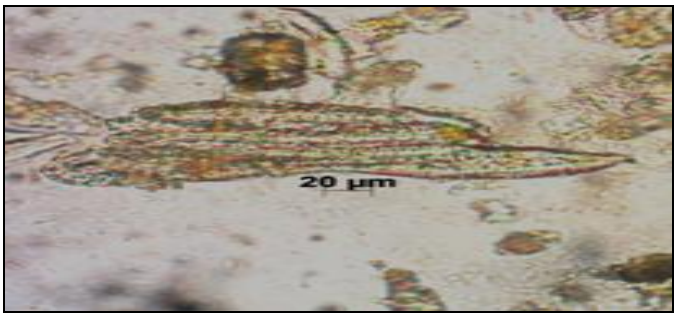

FIG. 1: GROUP OF SCLEREIDS

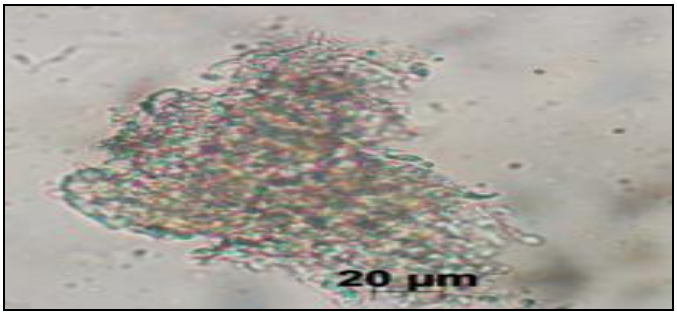

FIG. 2: ENDOSPERM WITH ALEURONE GRAINS PASSING THROUGH

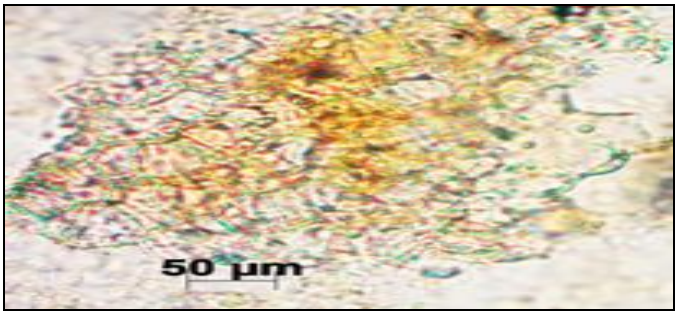

FIG. 3: EPICARP WITH STRIATED CUTICLE

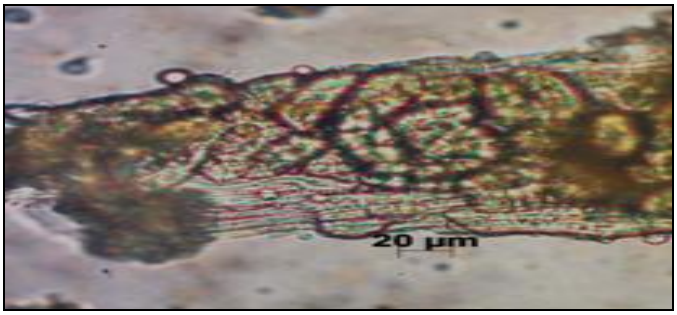

FIG. 4: STONE CELLS AND SCLEREIDS

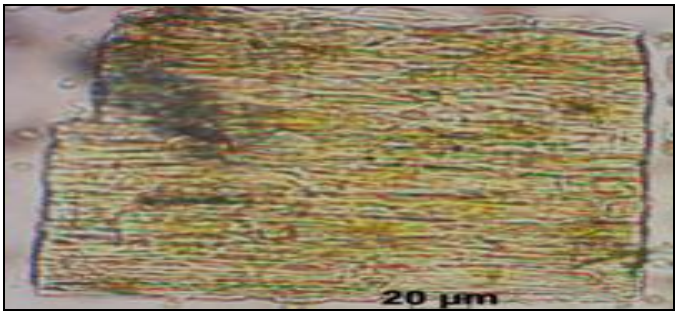

FIG. 5: CELLS OF EPIDERMIS OF FRUIT

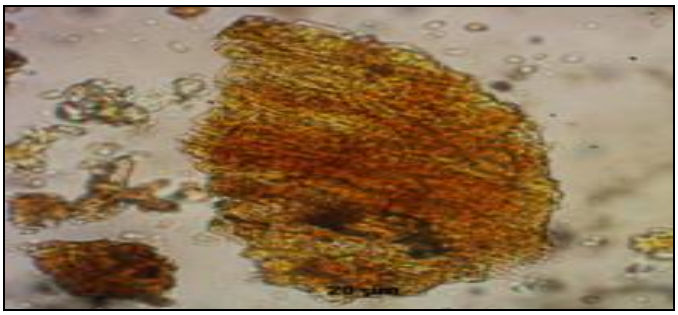

FIG. 6: TESTA

Physico Chemical Evaluation of Palasha Beejadi Churna: The results of the physicochemical evaluation are tabulated in **Table 1**.

TABLE 1: OBSERVATIONS OF PHYSICO CHEMICAL EVALUATION OF PBC

Parameter	Result n = 3 (% w/w) Mean ± SD
Loss on drying at 105 ^{o}C	8.73
Total ash	6.32
Acid insoluble ash	0.6
Water-soluble extractive	19.00 ± 1.00
Alcohol soluble extractive	14.67 ± 3.51

Preliminary Phytochemical evaluation of aqueous and alcoholic extract of Palasha beejadi churna showed the presence of alkaloids, carbohydrates, proteins, and tannins.

Chromatography: The chromatographic result of Palasha beejadi choorna showed the presence of various compounds as shown in the densitometric scan at different wavelengths are shown in **Tables 2**, **3,** and **4** and **Figures 7**, **8,** and **9**.

TABLE 2: R_f VALUE AT 620 nm

Palasha beeja	*Vidanga*	*Indrayava*	*Nimbabeeja*	*Kiratathikta*	*Palasha beeja dichurna*
-	-	-	0.06 (Purple)	-	0.06 (Purple)
-	-	-	0.23 (Yellow)	-	-
0.26 (Purple)	0.26 (Purple)	0.26 (Purple)	-	0.26 (Purple)	-
-	0.34 (Purple)	-	0.34 (Purple)	-	-
-	-	-	0.37 (Purple)	-	-
0.40 (Purple)	-	0.40 (Purple)	-	0.40 (Purple)	-
-	-	0.50 (Purple)	0.50 (Purple)	-	-
0.54 (Purple)	-	-	-	-	-
-	-	0.55 (Purple)	-	0.55 (Purple)	0.55 (Purple)
-	-	-	0.65 (Purple)	-	0.65 (Purple)
-	-	-	0.73 (Purple)	-	-
-	-	-	-	0.76 (Purple)	-
-	-	0.78 (Purple)	-	-	-
-	-	0.84 (Purple)	-	-	-

TABLE 3: R_f VALUES AT 366 nm

Palashabeeja	*Vidanga*	*Indrayava*	*Nimbabeeja*	*Kiratathikta*	*Palashabeejadichurna*
-	-	0.06 (F. red)	0.06 (F. red)	-	-
-	-	0.09 (F. urple)	-	0.09 (F. purple)	-
-	-	-	0.12 (F. blue)	0.12 (F. purple)	0.12 (F. blue)
-	-	-	-	0.28 (F. purple)	-
-	-	-	-	0.44 (F. red)	-
-	0.48 (F. blue)	-	-	-	-
-	-	-	-	0.50 (F. red)	0.50 (F. blue)
0.53 (F. green)	0.53 (F. green)	0.53 (F. green)	0.53 (F.green)	0.53 (F aqua. blue)	0.53 (F. red)
-	-	-	-	-	0.56 (F aqua. blue)
-	-	0.60 (F. red)	-	0.60 (F. red)	
-	-	-	-	-	0.62 (F. purple)
-	0.65 (F. red)	0.65 (F. red)	0.65 (F. red)	0.65 (F. red)	0.65 (F. red)
-	-	-	-	0.69 (F. red)	-
0.88 (F. blue)	0.88 (F. blue)	0.88 (F. blue)	0.88 (F. blue)	0.88 (F. blue)	0.88 (F. blue)

TABLE 4: R_f VALUES AT 254 nm

Palashabeeja	*Vidanga*	*Indrayava*	*Nimbabeeja*	*Kiratathikta*	*Palashabeejadichurna*
-	-	-	-	-	0.06 (Green)
-	-	-	-	0.09 (Green)	0.09 (Green)
-	-	-	0.16 (Green)	-	0.16 (Green)
-	-	-	-	-	0.22 (Green)
-	-	-	0.24 (Green)	-	-
-	-	-	0.29 (Green)	-	-
-	0.34 (Green)	-	-	-	-
-	-	-	0.38 (Green)	-	-
-	-	-	-	0.40 (Green)	-
-	-	-	-	-	0.42 (Green)

-	-	-	0.50 (Green)	-	-
-	-	-	-	-	0.52 (Green)
-	-	-	-	0.59 (Green)	-
-	-	-	0.64 (Green)	-	0.64 (Green)
-	-	-	-	0.67 (Green)	-

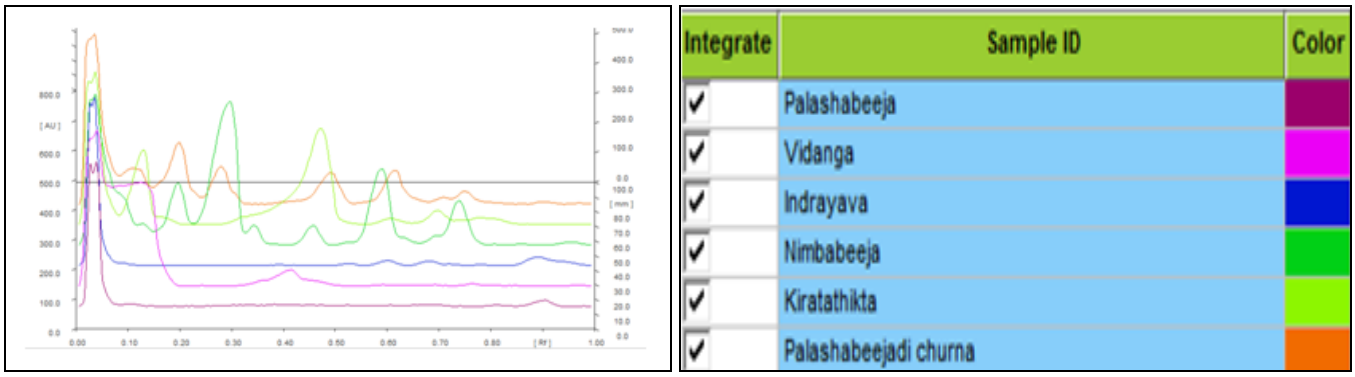

FIG. 7: AT 254 nm

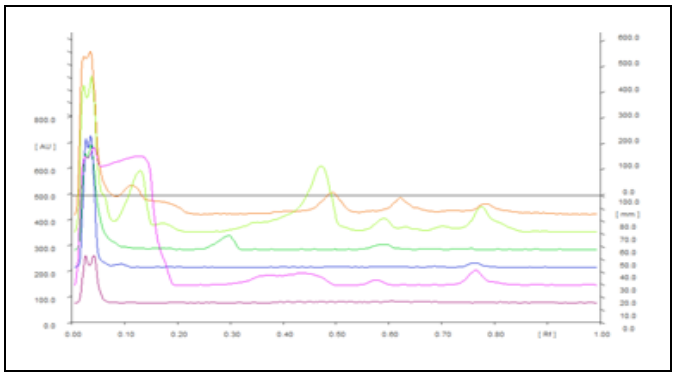

FIG. 8: AT 366 nm

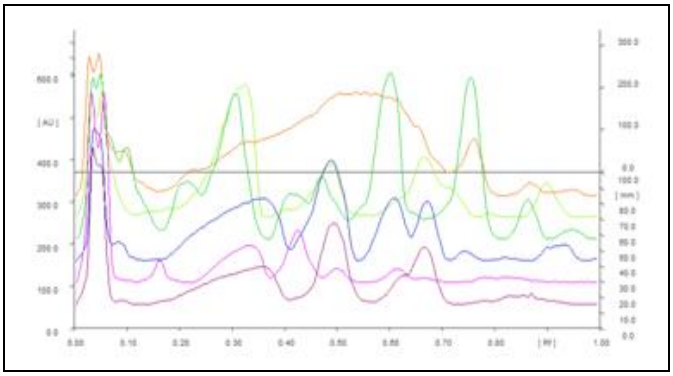

FIG. 9: AT 620 nm

At 620 nm wavelength, four purple colour spots with 0.06, 0.55, 0.65 Rf values were observed. In these three spots 0.06 and 0.65 are found in *Azadirachta indica* A. Juss. 0.55 spot found in *Swertia chirayita* (Roxb.ex Fleming) Karsten. At 366 nm wavelength, seven fluorescent spots with the R_f values were observed. 0.12, 0.50, 0.88 were blue in colour. The spots 0.53 and 0.65 were red, 0.56 was aqua blue and 0.62 was purple in colour.

Among these spots 0.12, 0.50, 0.53 were found in *Swertia chirayita* (Roxb.ex Fleming) Karsten, 0.65 was found in all the ingredients except *Butea monosperma* (Lamk.) Taub. The spot 0.88 was observed all the ingredients. Rf value 0.06, 0.09, 0.16, 0.22, 0.42, 0.52, 0.64 corresponding green colour spot was observed at 254 nm wavelength. 0.09 R_f value was observed in *Swertia chirayita* (Roxb. ex Fleming) Karsten. 0.16, 0.64 were observed in *Azadirachta indica* A. Juss. 0.56, 0.62 spots observed at 366nm and 0.06, 0.42, 0.52, 0.22 at 254 nm are found only in PBC only that has to be evaluated with further tests.

CONCLUSION: The study reveals that the polyherbal formulation Palasha beejadi choorna (PBC) is moderately fine powder with brown colour having bitter and astringent taste. PBC has no characteristic odour. Powder microscopy with particular cells of its ingredients is characteristic to the PBC.

The observed value of determination of moisture content, water and alcohol extractive value and ash value are standard values for PBC. HPTLC presents the R_f values with specific spots of the ingredients. The spots at 0.56, 0.62 at 366 nm, 0.06, 0.42, 0.52, 0.22 at 254nm are observed only in PBC that may be considered as standard values for PBC.

Monograph: Palasha beejadi choorna is brownish coarse powder. It has characteristic odour with bitter and astringent taste.

Identification: Microscopic study shows the following results:

- **Palashabeeja:** sclereids, fragments of testa

- **Nimbabeeja:** sclereids
- **Vidanga:** Endosperm with aleurone grains, Stone cells
- **Kiratatikta:** Endosperm with aleurone grains.

High-Performance Thin-Layer Chromatography: At 620 nm wavelength, four spots with 0.06, 0.55, 0.65 R_f values were observed. (All were purple colour) At 366 nm wavelength seven spots with following 0.12, 0.50, 0.53, 0.56, 0.62, 0.65, 0.88 R_f values were observed. (three were fluorescent blue, two were fluorescent red, one fluorescent purple, and one was fluorescent aqua)

At 254 nm wavelength seven spots with following R_f value 0.06, 0.09, 0.16, 0.22, 0.42, 0.52, 0.64 corresponding spot were observed. (all green)

PHYSICOCHEMICAL PARAMETER

Parameter	Result n = 3 (% w/w) Mean±SD
Foreign matter	0±0
Loss on drying at 105^{o}C	8.73±0.06
Total ash	6.32±0.06
Acid insoluble ash	0.6±0.06
Water soluble extractive	19.00±1.00
Alcohol soluble extractive	14.67±3.51

Preliminary Phytochemical evaluation of aqueous and alcoholic extract of Palash abeejadi churna showed the presence of Alkaloids, Carbohydrates, proteins, and tannins.

Adulterants and Substitutes: Nil

Storage: It should be stored in a cool, dry place in tightly closed containers, protected from light and moisture.

Therapeutic uses: PBC is used in the management of Krimiroga.

Dose: 3-6g

Anupana: Honey

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CONFLICTS OF INTEREST: Nil

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