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PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND PHARMACOGNOSTIC STUDY OF SEEDS OF *BUCHANANIA LANZAN* SPRENG

[1]MANOJ KUMAR TRIPATHI[1], ALOK KUMAR[2], MAYUR CHANDRANSHU MISHRA[3], AND RAVINDRA SINGH[4]

[1]Arogyadham (J.R.D. Tata Foundation for Research in Ayurveda & Yoga Sciences), Deendayal Research Institute, Chitrakoot, Dist Satna (M.P.)- 485 334

[2,3,4]Mahatma Gandhi Chitrakoot Gramodaya, Vishwavidyala, Chitrakoot, Satna (M.P.)

Email-trimanoj391@gmail.com,

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Buchanania Lanzan Spreng. Family-Anacardiaceae seeds are reported to have great medicinal value. It is known as priyal and used in ayurveda and the unani system of medicine. Priyala seed is commercially known as Chironji. All parts of the plant are used for the treatment of various disorders. The present communication provides a detailed account of the pharmacognostic evaluation carried out on Priyala seeds. The study includes macro and microscopic characters, preliminary phytochemical analysis, fluorescence study, physicochemical parameters and High Performance Thin Layer Chromatography (HPTLC) fingerprinting aspects. Established parameters can be used as standards for quality control and identification of the plants in compound formulations and also preparation of a monograph of the plant.

Keywords: Buchanania Lanzan, HPTLC fingerprinting, Phyto-chemical investigation, Pharmacognostic evaluation, Powder microscopy

Buchanania lanzan Spreng (Family-Anacardiaceae) commonly known as Priyala is a drug of the ayurveda and the unani system of medicine. Priyala is an almost evergreen, moderate sized tree, with straight, cylindrical trunk, up to 10-15 m height tomentose branches and found in the dry forests of Madhya Pradesh, Chattisgarh and Uttar Pradesh (Anonymous 1999). Chironji kernels contain about 52% oil which is used as a substitute for olive and almond oils, while the whole kernel is used in sweet- meats or as a substitute for almond kernels (Godbole *et al.* 1941, Banerjee 1998). It has great medicinal value, especially the kernel are used as expectorant and tonic. The oil extracted from kernels is used for treating skin diseases. Its nut has very good demand in foreign markets and thus, has become an important crop. Therefore, to earn foreign exchange the government and private agencies have evinced keen interest in developing this industry, both by the increasing its production and processing capacity. It bears fruits each containing a single seed, which is a popular edible nut, known as Chironji. All parts of the plant are used for the treatment of various ailments viz. skin disease, cardiotonic, stomach disorder, antiinflamation. The oil from the seeds is used to reduce granular swelling of the neck (Chopra *et al.* 1956 and 1969, Jain 1991). Ointment is made from the kernel which is used to relieve itch and prickly heat. The gum from the bark used for treating diarrhea and intercostals pains and leaves are used for promoting wound healing (Kirtikar and Basu 1935, Sikarwar *et al.* 2012, Tripathi and Sikarwar 2013, Verma *et al.* 1993).

Despite the numerous medicinal uses attributed to this plant, there are no systematic pharmacognostical studies on the seed of this plant have so far been carried out. Hence, the present work deals with the morphological, anatomical evaluation, physicochemical tests, preliminary phytochemical screening and HighPerformance Thin Layer Chromatography (HPTLC) fingerprint profile of *Buchanania lanzan* Spreng which could serve as a valuable source of information and provide suitable standards for further identification of this plant.

MATERIALS AND METHODS

Collection of specimens

The fresh plant seeds of Priyala were collected from the Bagdara ghati forest of, Chitrakoot of Satna district (M.P.) in the month of April. The plant was identified and authenticated. The voucher specimen (AD/AS/110/2016) maintained in the herbarium of Department of Pharmacognosy, Ayurveda Sadan, (Research Laboratory, Deendayal Research Institute

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Chitrakoot for further reference.

Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for physico-chemical tests, phytochemical screening and HPTLC studies.

Macroscopy

Macroscopic or organoleptic characters like appearance, colour, odour and taste were evaluated.

Microscopy

Fresh seed section was cut by free hand sectioning and numerous sections examined microscopically (Sholapur and Patil 2013). Photographs of the microscopical sections were captured with the help of Olympus Trinocular Research Microscope CX- 21l with Digi-eye camera using Caliper plus version 4.2 software.

Powder microscopy

The dried seeds were powdered and completely passel through 355 µm IS Sieve (old sieve number 44) and not less than 50% passel on through 180 µm IS Sieve (old sieve number 85). About 2 g of powder washed thoroughly with potable water, poured out the water without loss of material. Mounted a small portion in glycerin were used to all characters of the Priyala seeds, small quantity of sample cleared by heating with chloral hydrate solution, wash and mounted in glycerin, were used to identify diagnostic characters viz. elongated thick-walled sclereids, spiral, reticulate and annular vessels, epidermis of testa in surface view etc., treat a few mg with iodine solution and mount in glycerin, were used to examine the starch grains and aleurone grains, another small quantity of sample stained with Sudan red solution and mounted with glycerin was used to examine fixed oil, a few mg of powder stained with ruthenium red solution and mounted in glycerin to examine the mucilage, all mounted slide were seen under microscope at 40 x 10x magnification of the Trinocular Research Microscope (Anonymous 2007, Kokate 1994).

Physico-chemical parameters

Physico-chemical parameters such as moisture content (loss on drying at 105^0C), water soluble extractive value, alcohol soluble extractive value, total ash value, acid insoluble ash value and water soluble ash were calculated (Mukherjee 2002, Anonymous 2010).

Determination of Moisture Content (Loss on drying at 105^0C)

2gm of sample powder was transferred to weighed thin porcelain dish and kept in oven at 105°C for 5 hours. Dish was cooled in desiccators and weighed. The dish again kept in oven for 30 minute. Dish again cooled in desiccator and take weight. The loss in weight is calculated as percentage.

Determination of alcohol soluble extractive

Weighed accurate 5g powder sample and transfer in 250 ml Iodine flask. Add 100ml alcohol solvent. Provide continuous shaking for 6 hrs and leave for 18 hrs (maceration). After this extract was filtered by using what man filter paper no. 1 and weighted in thin porcelain dish, solvent was evaporated on water bath and residue was weighed. Percentage of extractive value (w/w) was determined.

Determination of water soluble extractive

Weighed accurate 5g powder sample and transferred in 250 ml Iodine flask. Add 100ml water solvent. Provide continuous shaking for 6 hrs and leave for 18 hrs (maceration). After this extract was filtered by using what man filter paper no. 1 and weighted in thin porcelain dish, solvent was evaporated on water bath and residue was weighed. Percentage of extractive value (w/w) was determined.

Determination of Ash values

The ash value is useful to determine the quality and purity of the drug. Ash contains inorganic radicals like phosphate, carbonates and silicates of sodium, potassium, magnesium, calcium, etc. Different ash values such as total ash value and

acid insoluble ash value was determined.

Determination of total ash

2 gm accurately weighed of the ground drug in a tared platinum or silica dish (previously weighed). Material was incinerated with the help of the muffle furnace at 450^{O}C, until vapors almost cease to evolve. Dish was heated until all carbon was burnt off. Dish was cooled, and calculated the percentage of ash value with reference to the air dried drug.

Determination of Acid-insoluble ash

Ash was boiled with 25 ml 5% hydrochloric acid for 5 minutes. Insoluble matter was collected in the ash less filter paper no. 42. It was washed with the hot water until neutralize and ignite to constant weight, cooled in a desiccators and weighted. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

Preliminary phyto-chemical investigation

Preliminary phyto-chemical tests were carried out on ethanolic and water extract for the presence\absence of phyto-constituents like alkaloids, flavonoids, tannins, resins, carbohydrates, proteins and saponins (Mitra et al.1980, Tripathi and Sikarwar 2014, Venugopal et al. 2015).

Test for Alkaloids

Mayer's test: Add few drops of Mayer's reagents to 1 ml of the acidic, aqueous extract of the drug. White or pale yellow colour is formed.

Wagner's Test: Acidify 1 ml of the alcoholic extract with 1.5% v/v of HCl and few drops of Wagner's reagent. A yellow or brown ppt. is formed.

Test for Carbohydrate

Anthrone's test: To 2 ml of anthrone's test solution, add 0.5 ml of aqueous extract of drug. A green or blue colour indicates the presence of carbohydrates.

Benedict's test: To 0.5 ml of aqueous extract of drug, add 5 ml of Benedict's solution and boil for 5 minutes. Formation of coloured ppt. is due to presence of carbohydrates.

Fehling's test: To 2 ml of aqueous extract of drug, add 1 ml of mix. Of equal parts of Fehling's solution A and Fehling's solution B and boil the content of the test tube for few minutes. A red or brick red ppt. is formed.

Test for Proteins

Millon's test: Dissolve small quantity of aqueous extract of drug in 1 ml of distilled water and add 5 - 6 drops of millon's reagent. A white ppt. is formed which turns red on heating.

Ninhydrin test: 3 ml test solution and 3 drops 5% Ninhydrin solution were heated on water bath for 10 min. for development of purple or bluish color.

Test for resins

Dissolve the 1 ml of extract in 1 ml of acetone and pour the solution into 5 ml distil water. Turbidity indicates the presence of resins.

Test for saponins

In test tube containing about 5 ml of an aq. extract of drug, add drops of sodium bicarbonate. Shake it vigorously and left for few minutes. Honey comb – like structure is formed.

Test for flavonoids

In the test tube containing 0.5 ml of lcoholic extract of drug, add 5-10 drops of dil. HCl followed by small piece of 'Mg'. In the presence of flavonoids, pink, reddish pink or brown colour is produced.

Test for Phyto steroids

Small quantity of extract is dissolved in 5 ml of chloroform. The chloroform solution was subjected to Salkowski and liebermann-Burchard tests.

Salkowski test: One ml of Concentrated. sulphuric acid was added to the above solution and allowed to stand for 5 min. After gentle

shaking, lower layer turning into golden yellow color indicates the presence of steroids.

Liebermann Buchard Test: 1 ml of sample extract was treated with chloroform, a few drops of acetic anhydride. To this, 1 ml of Concentrated H2SO4 was added from the sides of the test tube and allowed to stand for 5 min. Formation of brown ring at the junction of the two layers and the upper layer turning green indicates the presence of steroids.

Test for tannins

To 1 – 2 ml of extract of drug, add few drops of 5% $FeCl_3$ solutions. A greenish colour indicate the presence of gallacto tannins while brown colour tannins.

High Performance Thin Layer Chromatography (HPTLC) fingerprint profile

For High Performance Thin Layer Chromatography (HPTLC) fingerprint profile, the powdered seeds 2 gm of sample was extracted with 50 ml of ethanol overnight, filtered and concentrated. It was applied by spotting extracted sample 8 µl on pre-coated silica-gel aluminium plate 60 F_{254} (5x10 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The application rate was 5 µl/s. The samples, in the form of bands of length 6 mm, distance between bands 8 mm were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates were developed using mobile phase consisting of Toluene: Ethyl acetate: (7:3 v\v). Linear ascending development was carried out in 10x10cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min. at room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, TLC plates was dried with the help of Hot Air Oven. The peak area for samples and standard were recorded with Camera photo documentation system Camag Reprostar 3. Visualization of spots were made before and after derivatization (with 5% Methanolic- sulphuric acid reagent) at 254nm and 366nm with Win cat software and R_f values noted (Tripathi and Sikarwar 2015, Tiwari et al. 2015, Tripathi et al. 2015).

Table 1- Loss on drying of *Buchanania lanzan* Spreng, seeds powder

S. No.	Weight of empty Petri plates	Sample weight + Weight of Petri plates (Sample taken 2gm)	Ist reading (after 5 hours)	IInd reading (after 1/2 hours)	Difference (Ist reading-IInd reading)	Result
1	17.63	19.63	19.50	19.49	0.14	6.50%
2	16.57	18.57	18.44	18.43	0.13	
3	16.01	18.01	17.89	17.88	0.14	
Average weight					0.14	

Table 2- Water soluble extractive value of *Buchanania lanzan* Spreng, seeds powder

S. No.	Weight of Empty Petri plates (M1)	Weight of Petri plates after drying (Sample extract taken 10 ml in each Petri plates) (M2)	Difference (M2-M1)	Result

1	47.65	47.67	0.026	13%
2	49.76	49.79	0.026	
3	36.90	36.93	0.026	
Average weight			0.026	

Table 3- Alcohol soluble extractive value of *Buchanania lanzan* Spreng, seeds powder

S. No.	***Weight of Empty Petri plates (M1)***	***Weight of Petri plates after drying (Sample extract taken 10 ml in each Petri plates) (M2)***	***Difference (M2-M1)***	***Result***
1	*39.37*	*39.40*	*0.034*	*17%*
2	*39.31*	*39.34*	*0.033*	
3	*43.65*	*43.69*	*0.035*	
Average weight			*0.034*	

Table 4- Total ash value of *Buchanania lanzan* Spreng, seeds powder

S. No.	**Weight of Empty Crucible (M)**	**Sample taken in each Crucible**	**Weight after Incineration (M2-M1)**	**Difference (M-M1)**	**Result**
1	16.66	2gm	16.76	0.0997	5.00%
2	14.83	2gm	14.84	0.1045	
3	16.51	2gm	16.60	0.0960	
Average weight				0.1000	

Table 5- Acid insoluble ash value of *Buchanania lanzan* Spreng, seeds powder

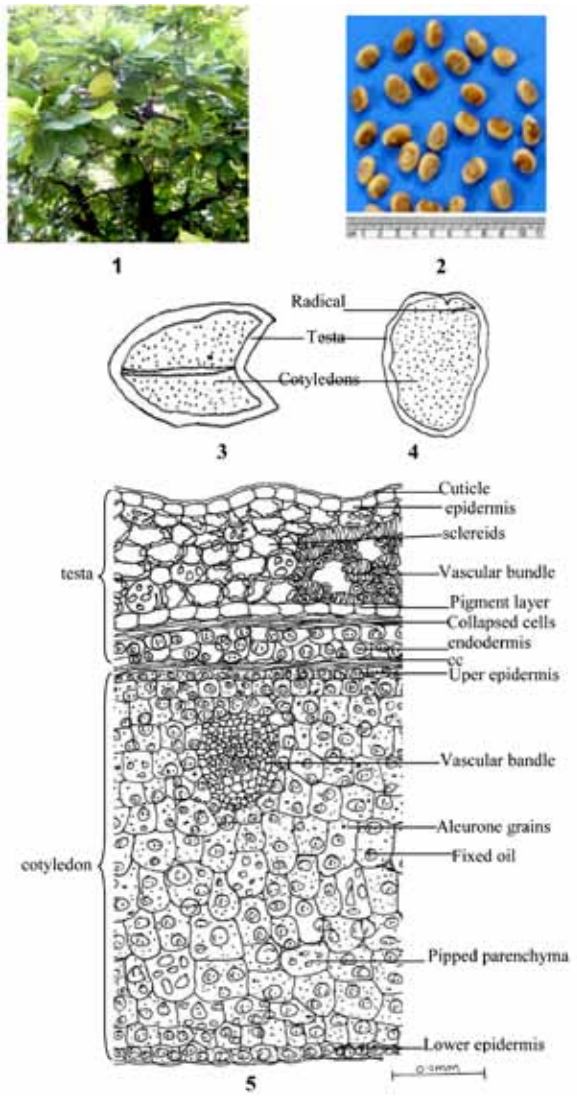
S. No.	Initial weight	Final weight	Difference	Result
1	16.76	16.74	0.0214	90%
2	14.84	14.82	0.0149	
3	16.61	16.59	0.0208	
Average weight			0.0.0190	

Table 6- Preliminary phytochemical screening of *Buchanania lanzan* Spreng, seeds powder extracts

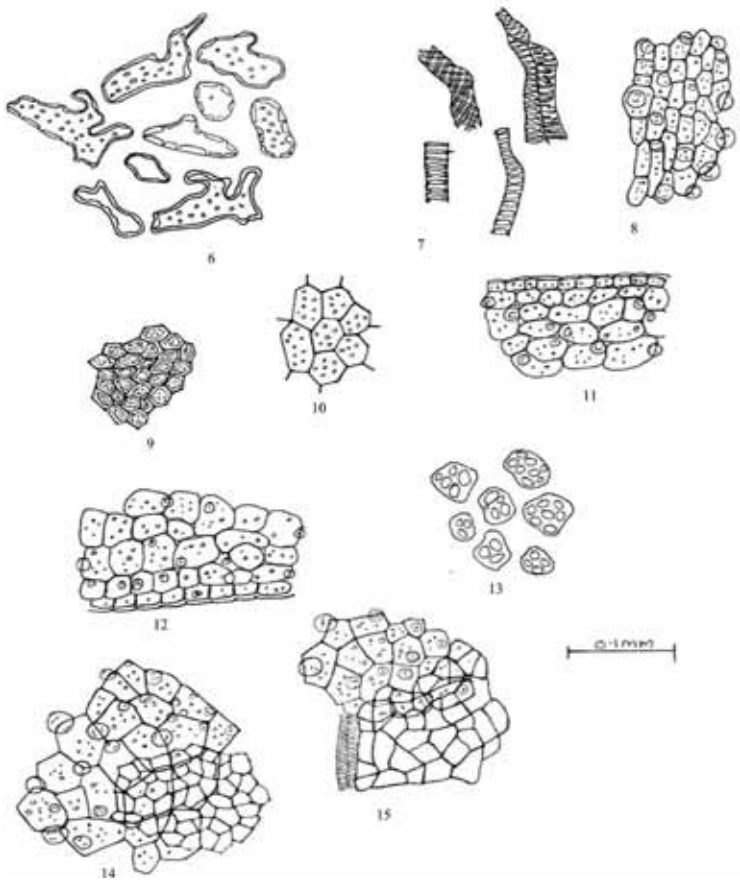
S. No	**Name of tests**	**Result**
1	Alkaloid	Present
2	Carbohydrate	Present
3	Protein	Present
4	Resins	Present
5	Saponin	Present
6	Phytosteroids	Present
7	Tannin	Absent
8	Flavonoids	Absent

Table 7- R_f values of High performance Thin Layer Chromatography of *Buchanania lanzan* Spreng, seeds powder extracts

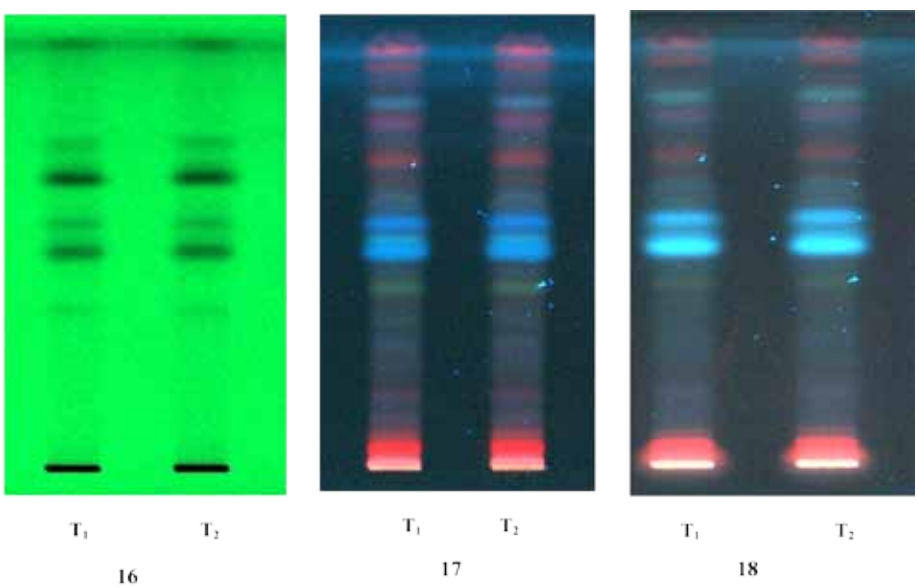
R_f values	Test solution of Buchanania lanzan Spreng.		
	254 nm (before derivati zation)	366nm (after derivati-zation)	366nm (after derivati-zation)
R_f1	0.52(black)	0. 06 (red)	0.04(red)
R_f2	0.60 (black)	0.54 (light yellow)	0.54 (fluor-escence blue)
R_f3	0.64(black)	0.60 (sky blue)	0.60 (fluorescence blue)
R_f4	0.70 (black)	0. 64 (sky blue)	0.80 (blue
R_f5	0.74(black)	0.70(red)	0.80(dark brown)
R_f6	0.90(black)	0.76(red)	0.90 (red)
R_{f7}	-	0.80(blue)	-
R_f8	-	0.90(red)	-



Plates 1-5: 1: Plant; 2 Dried seeds; 3 TS Seed diagrammatic; 4 LS seed; 5 TS seed (detailed)



Plates 6-15: 6 Sclereids; 7 Spiral, reticulate and annular vessels; 8 Sclereids; 9 Pigment cells of testa in surface view; 10 Epidermis of testa in surface view; 11 Cotyledon with layer of upper epidermis; 12 Cotyledon with layer of lower epidermis; 13 Pitted cells of raphe; 14 Pigment layer of testa overlapping with endosperm in surface view; 15 Upper epidermis of cotyledon overlapping with collapsed celled layer and vessels in surface view



Plates 16-18: 16 HPTLC finger print profile at 254nm; 17 HPTLC finger print profile at 366nm; 18 HPTLC finger print profile at 366nm after derivatization

RESULTS AND DISCUSSION

Macroscopic characters

The Priyala seeds colour is brownish, odour pleasant and sweetish, oily taste. Seed is oblong to rectangular, 4 to 9 mm in length, 5 to 7 mm in width, dorsiventral convex. Oval to circular fan shaped brown patch with white streaks. A ridge runs throughout the edge (Plates 1-2).

Microscopic characters

Diagrammatic LS of the seed shows outer brown thin lignified testa encircling the narrow endosperm, wide two thick white cotyledons almost of the same size and shape as that of seed and a stout cylindrical short radical, embedded with fixed oil globules and aleurone grains (Plates 3-4).

Seed transverse section shows a layer of epidermis of the testa, usually getting peeled off from the inner 2 to 5 rows of lignified pitted parenchymatous tissue traversed with long spiral xylem vessels specially when the section is through raphe and few tangentially running narrow small sclereids. A pigment layer lies underneath this followed by collapsed parenchymatous celled layer. Endosperm is narrow, consisting of 3 to 6 rows of thick-walled parenchymatous cells, Cells lying underneath this are collapsed, cotyledons exhibit usual structure, its few outermost cell lying underneath the epidermis being palisade like, other cells are loaded with fixed oil, protein and starch grains (Plate5).

Powder microscopic characters

Under microscope examined powder shows elongated thick-walled sclereids, spiral, reticulate and annular vessels, small fragments of pigment cells in surface view, Epidermis of testa in surface view, Cotyledon with layer of upper epidermis, Cotyledon with layer of lower epidermis, Pitted cells of raphe, Pigment layer of testa overlapping with endosperm in surface view and Upper epidermis of cotyledon overlapping with collapsed celled layer and vessels in surface view (Plate 6- 15)

Physico-chemical analysis

The physico-chemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug; ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Physico-chemical results of the drug are given in (Table 1-5).

Preliminary phyto-chemical investigation

Qualitative phyto-constituents were screened in the extracts taken in water and ethyl alcohol. The result are given in (Table 6)

HPTLC finger print profile

High performance thin layer chromatography (HPTLC) study of the ethanolic extract two spots of the sample extracts applied in the TLC plate. Major spots R_f values with colour were recorded under, 254nm, 366nm, and after derivatization 366nm. Chromatogram profile and R_f values are given (Plates 16-18 & Table 7).

The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify *Buchanania lanzan* Spreng seed. Several researchers have been reported macroscopic and microscopic distinguished characters on seeds of *Buchanania lanzan* Spreng viz. Mitra *et al.* 1980, Anonymous 1999 and Anonymous 2010). The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity of this drug. High Performance Thin Layer Chromatography (HPTLC) finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The HPTLC profile also helps to identify and isolate's important phyto-constituents. These finding could be helpful in identification and authentication.

CONCLUSION

Buchanania lanzan Spreng has numerous uses

in traditional medicine to treat several ailment viz. skin disease, cardiotonic, stomach disorder, antiinflamation, swelling of the neck, ointment is made from the kernel which is used to relieve itch and prickly heat, gum from the bark used for treating diarrhea and intercostals pains and leaves are used for promoting wound healing. Due to its wide therapeutic importance it is worthwhile to standardize it for use as drug. The present study reveals pharmacognostical standardization of drug Buchanania Lanzan Spreng, which would be of immense value in botanical identification and authentication of plant drug may help us in preventing its adulteration.

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