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Comparative Pharmacognostical And Physicochemical Studies On Seeds Of *Holarrhena antidysenterica (L.) wall* And *Wrightia tinctoria (Roxb)* R.Br.

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Abstract: Kutaja in the name of *Holarrhena antidysenterica* Wall, has often been confused and adulterated with another member of the same family, that is *wrightia tictoria* R.Br. present study was taken to have brief knowledge of similarities and dis similarities between the seeds of both plants. The sample were subjected to macro and microscopic analysis, powder microscopy, physicochemical , phytochemical and HPTLC evaluation. A comparative study was carried out both the seeds included pharmacognostical evaluation. The determination of bitter- Value was carried out only on the seeds of *Holarrhena antidysenterica* Wall, since its seeds were found to be bitter compared to the seeds of *Wrightia tinctoria* R.Br. which are tasteless. The transverse section of *H. antidysenterica* seeds shows multi layered testa, a massive endosperm. Testa is composed of compressed, lignified sclerenchymatous cells and shows concentric or wavy layers. However in W. tinctoria testa is composed of thick-walled, compactly arranged polygonal to elongated and endosperm absent. HPTLC revealed at UV 366nm 4 band have similar Rf values.

Keywords: *Holarrhena antidysenterica*, *Wrightia tinctoria*, Kutaja, pharmacognostical evaluation, HPTLC, phytochemical analysis, , seed morphology, adulteration, Apocynaceae.

Introduction:

Holarrhena antidysenterica Wall and *Wrightia tinctoria R*.Br. seeds appear very similar and are often confused to be one and the same. The following species of Apocyanaceae are being used as a substitute of *Holarrhena antidysenterica* **(Wall).**, *Holarrhena mites* (R.Br), *Holarrhena africana* (A.Dc):- In West Africa, *Holarrhena wulfsbergii* (Stapf) In gold coast, *Hollarrhena congolensis* (Stapf) In the conga. The following Adulterants in different species of Apocynaceae family as follows: *Wrightia tinctoria*, *Wrightia tomentosa*, *Wrightia zeylanica*; *Strophanthus* and *Holerrhena antidysenterica* seeds (Pharmacognostically) are the same. Strophanthus seeds are sold in the market in the name of *Holarrhena antidysenterica.* This is also from Apocynaceae family.Even in API *Holarrhena antidysenterica* Wall is mentioned but not *wrightia tictoria R*.Br. plant. So it is much need to know the phytochemical properties of both the plants. The availability of both plants: The Bark and seed of *Holerrhena antidysenterica* is mentioned in API but the seed of *Wrightia tinctoria* is not mentioned in API. In Caraka kalpa sthana seeds are used. Hence a comparative study of the microscopy, macroscopy and phytochemical tests have been carried out to know the similarities and differences of both the seeds. The aim of this project was to carry out a comparative pharmacognostical evaluation of both verities Kutaja seeds.

Materials and Methods:

Both the plant materials are collected from Seshachalam Hill of Trumala, After authenticating them by the department of Dravyaguna and pharmacognosist, the voucher specimens were deposited in the department of Dravyaguna, S. V Ayurvedic College, Tirupati for further reference.

Fig: 1 Seeds of *Holarrhena antidysenterica*

Fig: 2 Seeds of *Wrightia tinctoria*

Pharmacognostical Evaluation:

The seeds of *Holarrhena antidysenterica* and *Wrightia tinctoria* were examined for their organoleptic characters (colour, taste, and size and nature of outer and inner surface). Permanent slides of the transverse section of both the seeds were prepared. The SOPs are followed based on the methods mentioned in API (Total ash Appendix 2.2.3, Acid insoluble ash Appendix 2.2.3, Water soluble ash Appendix 2.2.5, Water soluble Extractive Appendix 2.2.7)

1. Macroscopy:

The seeds of *Holarrhena antidysenterica* yellowish-brown to light brown; linear–lanceolate, narrow, elongated, flattened, tapering at both ends; smooth, glabrous, slightly shining surface with entire margin; one end shows a scar of detached coma; acute to acuminate apex; hard leathery texture; with characteristic odour and a starchy bitter taste.(Fig.1)

Microscopy

The TS of the seed is nearly oval in shape and shows a thick, wavy, multi layered testa, a massive endosperm, and a small embedded embryo. Testa is composed of compressed, lignified sclerenchymatous cells and shows concentric or wavy layers and a narrow compact crushed epidermis; followed by a parenchymatous tegmen made of 3 to 4 layers of compactly arranged parenchymatous cells; endosperm is well developed and occupies the major portion of seed; it is composed of compact polygonal thin walled parenchymatous cells rich in fixed oil and aleurone grains; few cluster crystals are seen randomly distributed in the endosperm; embryo is small and embedded within the endosperm and consists of two minute cotyledons containing parenchymatous palisade cells filled with aleurone grains; and a short parenchymatous radicle which is not prominently differentiated in transverse section; vascular elements are absent in the seed coat.

Macroscopy

The dried seeds of *wrightia tinctoria R*.Br. are oblong–lanceolate to narrowly elliptic, flattened, light-weight; dark brown to grey in colour; smooth, glabrous, with a distinct tuft of silky white hairs at one end; measuring about 1.5 to 2 cm in length and up to 3 mm width ; firm but brittle on pressure with no characteristic odour and slightly bitter taste . (Fig.2)

Microscopy

The TS is oval to broadly elliptical in outline, showing a massive, folded embryo occupying almost the entire interior; endosperm absent. The seed coat consists of outer testa and inner tegmen; testa is composed of thick-walled, compactly arranged polygonal to elongated, darkly stained cells with numerous papillose outgrowths are seen; inner tegmen consists of thin-walled, compressed parenchymatous cells; few prismatic crystals are present in the testa region; embryo is well developed, large, and curved and occupies the entire seed cavity, endosperm absent; two cotyledons are present, appearing thick, fleshy, and highly convoluted made up of thin walled parenchyma cells rich in aleurone grains and fixed oils; numerous collateral vascular strands are visible within the cotyledons; embryonal axis present between the folded cotyledons and shows differentiation into plumule and radicle regions; small, scattered vascular bundles are embedded within cotyledonary tissue; numerous rosette crystals are seen scattered throughout the ground tissue.

2. Powder Microscopy of *Holarrhena antidysenterica* Wall. showed The powder is brown in colour, with a characteristic odour and aroma, and a starchy, bitter taste, and shows the surface view of the testa, the sectional view of the cotyledon, stone cells from the testa, endosperm cells, cluster crystals, aleurone grains and oil globules from the cotyledon while in *wrightia tictoria* R.Br., the powder is greyish brown in colour with no characteristic smell and slightly bitter taste and shows the presence of papilous outgrowth, epidermal fragments, cotyledonary fragments, vessels, oil globules and prismatic and rosette crystals.

3. Physicochemical Evaluation:

The loss on drying, Total ash values, extractive values and the results of both Varieties of Kutaja seeds are given in table 1. The evaluation is done based on the standard mentioned in API.

(Page. no.106 2.2.3 and 2.2.4)

Table: 1 shows the physicochemical result

Ash Values	Percentage w/w	
Drug	*Holarrhena antidysenterica*	*Wrightia tinctoria*
Loss on drying	8.4±0.00	10.8±0.00
Total ash	15.5%	8%
Acid insoluble ash	8%	4.5%
Water soluble ash	12%	8%
Water soluble extractive	21%	28%

Inferences:

1. Loss on drying: *Holarrhena antidysenterica* shows 8.4% ± 0.00 moisture, while *Wrightia tinctoria* has 10.8% ± 0.00, indicating higher water content in *Wrightia tinctoria*.

2. Total ash: *Holarrhena antidysenterica* has 15.5% total ash compared to 8% in *Wrightia tinctoria*, suggesting more inorganic material in *Holarrhena.*

3. Acid insoluble ash: *Holarrhena* has 8% versus 4.5% in *Wrightia*, implying more siliceous material in *Holarrhena*.

4. Water soluble ash: Holarrhena shows 12% and Wrightia 8%, indicating greater soluble inorganic content in Holarrhena.

5. Water soluble extractive: *Wrightia tinctoria* has a higher extractive value (28%) than *Holarrhena antidysenterica* (21%), showing better water-soluble compound yield in **Wrightia**.

4. Phytochemical study:

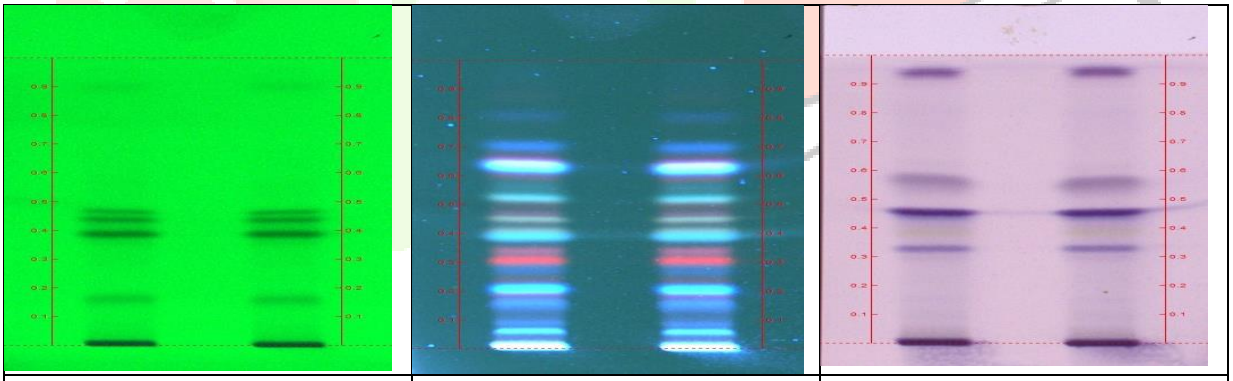
Phytochemical	Test name	Changes observed	H.A	W.T
Saponin	Foam test	Stable froth formation	+	+
Alkaloids	Wagner's test	Dull Reddish brown precipitate formed	+	+
Test for carbohydrates	Benedicts's test	Red precipitate	+	+
Test for tannins	Dilute HNO3	Formation of reddish to yellow colour	+	-
Test for flavonoids	Sulphuric acid test	Deep yellow solution	+	+
Test for phenolic compounds	lead acetate test	Formation of white precipitation	+	+
Test for Triterpenoids	Salkowski test	Reddish-brown colouration	+	+
Test for Coumarins	NaOH Test	Yellow colour solution	+	+

1. Both plant samples contain saponins, carbohydrates, flavonoids, triterpenoids, coumarins, phenolic compounds, and alkaloids.

2. Tannins are present only in Holarrhena antidysenterica (L.) Wall, differentiating its phytochemical profile from Wrightia tinctoria.

5. HPTLC:

TLC Photographs of *Wrightia tinctoria* seed

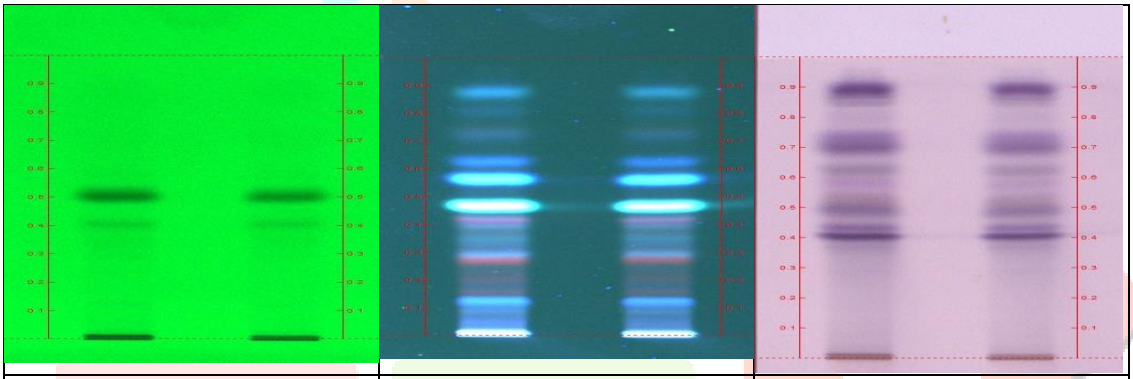


UV 254 nm	UV 366nm	White light after dipping in Vanillin-sulphuric acid

Rf and colour of spots of *Wrightia tinctoria* seed

Rf	Color	Rf	Color	Rf	Color
0.16	Green	0.06	Sky blue	0.29	Violet
0.38	Green	0.16	Blue	0.33	Violet
0.44	Green	0.20	Sky blue	0.39	Violet
0.47	Green	0.27	Blue	0.46	Violet
		0.34	Pink	0.57	Brown
		0.39	Sky blue	0.94	Violet
		0.45	White		
		0.52	Sky blue		
		0.56	Sky blue		
		0.63	Blue		
		0.70	Blue		
		0.80	Blue		

TLC Photographs of *Holarrhena antidysenterica* Seed



UV 254 nm	UV 366nm	White light after dipping in Vanillin-sulphuric acid

Rf and colour of spots of *Holarrhena antidysenterica* Seed

Rf	Color	Rf	Color	Rf	Color
0.29	Green	0.09	Blue	0.40	Purple
0.40	Green	0.13	Red	0.44	Purple
0.51	Green	0.20	Red	0.53	Purple
		0.27	Red	0.58	Purple
		0.34	Blue	0.63	Purple
		0.42	Red	0.70	Purple
		0.47	Red	0.74	Purple
		0.56	Aqua	0.85	Purple
		0.62	Blue	0.90	Purple
		0.71	Blue		
		0.81	Blue		
		0.88	Blue		

HPTLC analysis at UV 366 nm shows 4 bands with similar Rf values for both samples, indicating comparable chromatographic profiles for certain constituents.

Discussion:

The comparative pharmacognostical evaluation of *Holarrhena antidysenterica* and *Wrightia tinctoria* seeds shows both their similarities and differences. Although both belong to the Apocynaceae family and share certain phytochemical constituents like saponins, alkaloids, flavonoids, coumarins, triterpenoids, phenolics, and carbohydrates, they differ significantly in morphology, microscopy, physicochemical parameters, and specific phytochemical presence.

H. antidysenterica seeds are bitter, with a multi-layered lignified testa and a massive endosperm, while *W. tinctoria* seeds are tasteless, with a compact testa and absent endosperm. The embryo in *W. tinctoria* is large and folded, occupying the entire seed cavity, unlike the small embedded embryo of *H. antidysenterica*. Both show oil globules and crystals, but *H. antidysenterica* exhibits cluster crystals and endosperm cells, whereas *W. tinctoria* shows papillose outgrowths and vascular elements.

H. antidysenterica has higher ash values (total, acid-insoluble, and water-soluble), indicating more inorganic and siliceous content. In contrast, *W. tinctoria* shows higher water-soluble extractives, suggesting richer yield of soluble compounds.

The presence of tannins exclusively in *H. antidysenterica* provides a clear differentiating marker.Both seeds share some bands with similar Rf values at UV 366 nm, indicating overlapping chemical constituents, but their overall chromatographic profiles remain distinct.

Conclusion:

The comparison ensures that the correct, more potent variety is used in formulation. While provide a distinction for safety and quality control.

Acknowledgement:

We are thankful to the S.V Ayurvedic college, Tirupati, for providing the necessary laboratory and library facilities.

References:

1. **Macroscopy** (SOP No. PCOG-004-SOP): External feature of the test sample was documented using Nikon D-5600 Digital camera.
2. **Microscopy** (PCOG-005-SOP): Sample was preserved in fixative FAA for more than 48 h. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with 0.8% Safranin and 0.5% Astra blue. Transverse sections were photographed using Axiolab5 trinocular microscope attached with Zeiss Axiocam208 color digital camera under bright field light. Magnifications were indicated by scale bar.
3. **Powder microscopy** (PCOG-006-SOP): A pinch of the powdered sample was mounted on a microscopic slide with a drop of 50% glycerol after clearing with saturated solution of chloral hydrate. Sample was treated with iodine solution to confirm the presence of starch grains. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERc5s digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented.
4. **Sample Preparation for TLC**

Sample (1 g) was dissolved in 10 ml of ethanol and then sonicated for 15 minutes and filtered. This solution was used for TLC.

5. TLC/HPTLC Methodology

Applied sample extract on TLC plate using Camag's ATS4 applicator and developed by the Mobile phase: Toluene: Ethyl acetate: Acetic acid (9:1:0.5, v/v/v) for *Wrightia tinctoria* seed; Toluene: Ethyl acetate: Acetic acid (9.5:1:0.5, v/v/v) *Holarrhena antidysenterica* Seed up to 8.5 cm distance. After development, the plate was photo documented using Camag's TLC Visualizer under UV 254 nm and UV 366 nm. Scanned the plate using Camag's Scanner 4 at UV 254 nm (D_2 lamp/Absorption mode) finger print profiles of the extract were documented. Then the plate was dipped in 5% vanillin-sulphuric acid reagent followed by heating at 105^{O}C till development of coloured spots. The plate was then photo documented in white light and scanned at 520nm (W lamp /Absorption mode)