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Comparative micro morphological and microscopic evaluation of *Croton tiglium* Linn. and *Baliospermum montanum* Muell.-Arg. seeds

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Abstract

The seeds of *Croton tiglium* Linn. and *Baliospermum montanum* Muell.-Arg., belongs to Euphorbiaceae family, are considered to be the botanical source of Ayurvedic raw drug *Dantibeeja*, for the management of various disorders. The similarities and dissimilarities between the two seed drugs can be well studied through pharmacognostical investigations. The present study was carried out to compare the micro-morphological characteristics of *C. tiglium* and *B. montanum* seed. The seeds were collected from the authenticated botanical sources and evaluated for their morphological, microscopical and quantitative microscopic characters following standard procedures. The transverse section of both the seed samples revealed the presence of aleurone grains, epidermis of cotyledon, epidermis of testa, rosette crystals of calcium oxalate, endosperm cells and oil globules. Micro morphological study results showed that the seeds of *C. tiglium* are greater than that of *B. montanum*. Microscopical characters depicted the presence of rosette crystals of calcium oxalate and secretory cavity in *C. tiglium* whereas prismatic crystals in *B. montanum*. The seeds of *C. tiglium* and *B. montanum* differ from each other both in morphological as well as microscopic characters.

Keywords: *Baliospermum montanum*, *Croton tiglium*, *Danti*, *Jayapala*, Pharmacognosy

Introduction

Family Euphorbiaceae includes nearly 280 genera and 8000 species which occur in tropical and temperate regions all over the world [1]. *Croton tiglium* Linn. (Family: Euphorbiaceae) commonly known as *Jayapala* is a shrub native to South East Asia. It is indigenous to India and widely distributed in North-Eastern part of India. Its seeds are well-known for its severe purgative action [2] and are used for the treatment of constipation, dyspepsia, dysentery, gastrointestinal disorders, intestinal inflammation, rheumatism, peptic ulcer, visceral pain, and headache [3]. *Baliospermum montanum* Muell.-Arg. (Family: Euphorbiaceae) commonly known as *Danti*, is a leafy, monoecious under shrub distributed throughout India, Burma and Malaya [4]. The seeds are used as a drastic purgative and seed oil as powerful hydragogue cathartic and applied externally in rheumatism [5]. *Dantibeeja* (seeds of *Danti*) is commonly used as the synonyms for *Jayapala* [6] but it is observed that *B. montanum* Muell.-Arg. is being used by tradition *Vaidyas* and also available in the market in the name of *Dantibeeja*. Therefore a thorough investigation is needed to find out similarities and dissimilarities between both the seed drugs. Micrometry is a measuring tool which aids in establishment an identity of crude herbal drugs at a microscopic level thereby proving essential in drug identification. Present available literature does not reveal any scientific data pertaining to the difference of the micromorphology and micrometry characters of the seed coat and kernel of *C. tiglium* Linn. and *B. montanum* Muell.-Arg.. Hence, the present study was undertaken to compare the micrometric & micro-morphological evaluation of both seeds by following standard parameters.

2. Materials and methods

2.1 Collection and authentication:

Mature seeds of *C. tiglium* were collected in fresh from Karnataka, in the month of November 2015 and *B. montanum* Muell.-Arg from Odisha in the month of February 2016. The collected samples were identified and authenticated by using various floras & texts with help of taxonomist and were subjected for shed drying and preserved in dry air tight container [7, 8]. The seeds sample of both the plant drug were submitted in the pharmacognosy laboratory with specimen number *C. tiglium* and *B. Montanum* Phm/15-16/6204, Phm/15-16/6205 respectively, for future reference.

2.2 Micro-morphological study

Morphological and organoleptic characters of the seeds were studied from the intact good quality of the seed, by the observation with naked eye and with the help of magnifying lens of 10x [9].

2.3 Microscopic study

Free hand thin sections of various parts i.e. seed coat and kernel of seed were taken and cleared with chloral hydrate. They were first observed in distilled water and then stained with phloroglucinol and Conc. HCL, iodine, Ruthenium Red. Microphotographs were taken by using Carl Zeiss Trinocular microscope, attached with camera. Fine powder was made of seed using mortar pestle. One to two drops of water were placed with chloral hydrate transverse section on a glass slide. The tip of the needle was moistened with water and dipped in to the powder. A small quantity of the material that adhered to needle tip was moved on the slide then cover glass applied and powder microscopy was carried out of the sample [10, 11].

2.4 Micrometric evaluation

Evaluation of the length and breadth of kernel, cotyledon individually including cellular constituents was followed by morphological study. Carl Zeiss Trinocular microscope attached with camera with preloaded micrometric analysis software was used for the purpose. Accurate readings of measurements of the length, breadth of the parts of seed etc. were recorded and noted down and the mean value was taken into consideration [12].

3. Result and discussion

3.1 Morphological characters

C. tiglium Linn

Seeds are oblong, oval to egg shaped, slightly trigonous, 1.3 cm in length, 0.7 cm in width and ventrally shows longitudinally running centrally located at the narrow end is the hilum, testa brittle, brownish black colour. Kernel - almost similar in shape like that of seed, but slightly smaller in size, appressed with scaly silvery white papery nucellus, faintly marked with longitudinally running vascular stands, a small projection located at its narrow end is radicle, kernel is white with oily, endosperm very wide, encircling a cavity in which thin embryo is located consisting of two leafy cotyledons and a small radicle at the top.

B. montanum Muell-Arg.

Seeds oval to ellipsoidal, somewhat laterally compressed, 5 to 8 mm in length, 4 to 6 mm. in width and 3 to 4 mm in thickness. Surface smooth, glossy, mottled with dark brownish patches. Hilum located at the apex on the ventral side at the base of the caruncle which is fleshy whitish, broadly diamond shaped ventrally, narrowly arrow shaped dorsally. Testa brittle, devoid of active chemical constituents and hence it is removed and discarded. Kernel white in colour, bitter in taste and with foul odour after crushing. A comparative organoleptic character of both the drugs are presented in table 1 and Distinguishing morphological characters of seeds are presented in table 2 and plate 1.

Table 1: Comparative organoleptic character of seeds

Organoleptic characters	<i>C. tiglium</i> Linn.	<i>B. montanum</i> Muell.- Arg.
Colour	Brownish black	Dark Brownish black
Odour	Characteristic	Foul
Taste	Pungent and bitter	Bitter
Texture	Velvety touch	Smooth to touch

Table 2: Distinguishing morphological characters of *C. tiglium* Linn. and *B. montanum* Muell.-Arg. seeds.

<i>C. tiglium</i> Linn	<i>B. montanum</i> Muell.-Arg.
The seeds are 1-1.25 cm long, 0.8cm (maximum) wide, rounded in extremities with the dorsal side more convex than the ventral.	The matured seeds are small, oval, 0.5-0.8 cm long and 0.4-0.6 cm wide, smooth and generally mottled on outer surface.
The outer seed coat of the shell is yellowish brown and inner surface being grey and smooth. On removing the outer hard coat, an inner thin and membranous covering is distinctly seen surrounding the endosperm.	Externally brownish or greyish brown and internally buff coloured. The immature and dried seeds are shrunken, light pale in colour without mottling and with papery testa. At the one of the seed coat, there is a distinct white outgrowth called caruncle, nearby which hilum is seen as a small scar.
The endosperm is reddish brown lying embedded in endosperm and consists of two cotyledons, more or less distinctly marked by veins and a short axis.	The endosperm is light yellow and full of oil.
The axis consists of a radicle as a small protuberance and a minute plumule in between the two cotyledons.	Embryo is dicotyledonous with a small radicle pointing towards the caruncle and a minute undifferentiated plumule within cotyledons.



Fig 1a: Natural habitat of *Croton tiglium* Linn.



Fig.1.b: Natural habitat of *Baliospermum montanum* Muell-Arg



Fig 2a: Micro-measurement of Seeds



Fig 2 b: Micro-measurement of Seeds

Plate 1: Morphological characters of seeds of *C. tiglium* Linn. and *B. montanum* Muell.-Arg.

3.2 Microscopy and Micrometry:

***C. tiglium* Linn.**

Detailed transverse section (TS) of seedcoat shows unicellular trichomes followed by two layers spool shaped palisade cells; upper and lower somewhat collapsed epidermal cells. Outer trichomes to inner collapsed parenchyma 2.9mm in length; trichomes are 0.8mm in length, 0.1 mm in width and Pallisade spool cells are 1.6mm in length. Detailed TS of kernel shows outermost tissue of nucellus consisting of narrow, compressed thin walled parenchymatous epidermal cells with vascular stands and thin cuticle, underneath of this lies 1 to 2 rows of thin walled tangentially elongated parenchyma cells, followed by a layer of small sized rectangular, tangentially running epidermal cells of endosperm which is very wide and composed of spherical, thin walled parenchyma cells embedded with secretory cavity, cluster and rosette crystals of calcium oxalate, oil globules and aleurone grains; underneath of this lies 4 to 5 rows of tangentially running compressed cells of parenchyma, cotyledon embedded in an elliptic cavity, consists of a layer of upper and lower epidermis of squarish shaped cells enclosing 8 to 10 rows of mesophyll cells containing oil globules, aleurone grains, cavities and simple starch grains, a layer of palisade is located underneath the lower epidermis.

***B. montanum* Muell.- Arg.**

Detailed TS of seed coat shows two layers of spool cells and one layer of epicarp cells. Detailed TS of kernel shows tangentially running, sinuous walled compressed parenchyma cells of nucellus embedded with well-developed vascular strands under each of the ridge, the innermost one to two rows of parenchyma cells being somewhat obliterated; underneath this lies very wide endosperm of thin walled parenchyma cells of various sizes and shapes embedded with aleurone grains, oil globules, prismatic crystal and at places large rosette

crystals of calcium oxalate often getting concealed under the lumps of protein masses; encircled by one to two rows of tangentially elongated cells of outer and inner epidermis, embedded with aleurone grains; followed by a narrow band of obliterated cells of cotyledon of embryo is composed of upper and lower epidermis of squarish shaped cells enclosing 8 to 10 rows of mesophyll cells, embedded with cluster crystals of calcium oxalate, oil globules and aleurone grains, one to two layers adjoining the inner epidermis being of palisade cells. A comparative statement of the drugs are presented in Plate 2.



Fig 1a: Mesocarp with cotyledone



Fig 1b: Mesocarp with cotyledone



Fig 2a: Seedcoat with trichomes and spool shaped cells

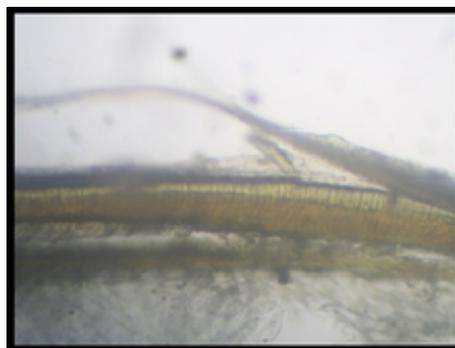


Fig 2b: Seed coat without trichomes and spool shaped cells

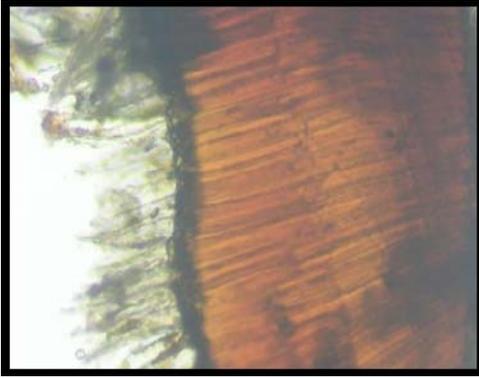


Fig 3a: Collapsed epidermal cells with two layers of palisade cells

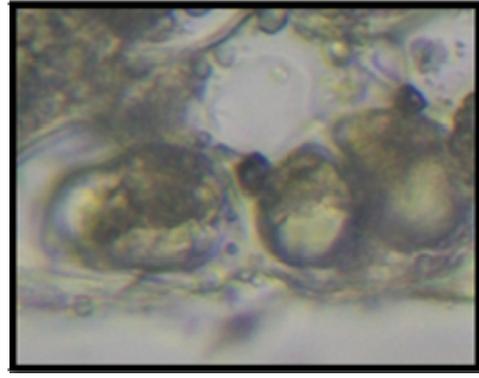


Fig 5a: Endosperm cells, oil globules and aleurone grains

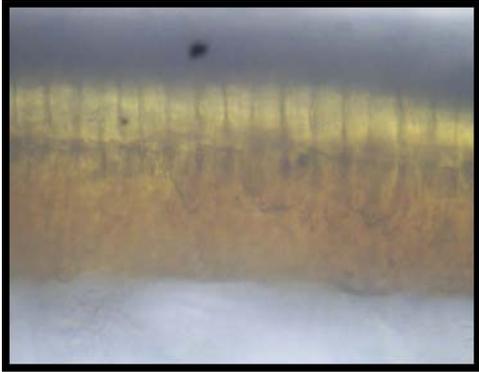


Fig 3b: Collapsed epidermal cells with two layers of smaller palisade cells



Fig 5b: Endosperm cells, oil globules and aleurone grains

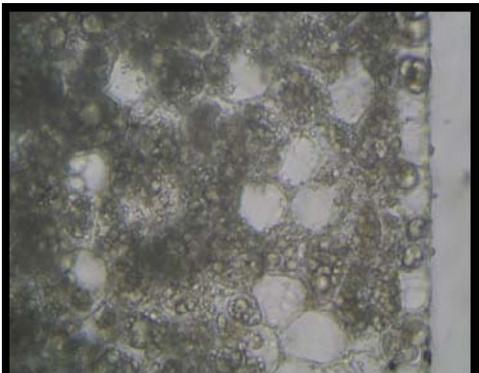


Fig 4a: Aleurone layer followed by endosperm cells

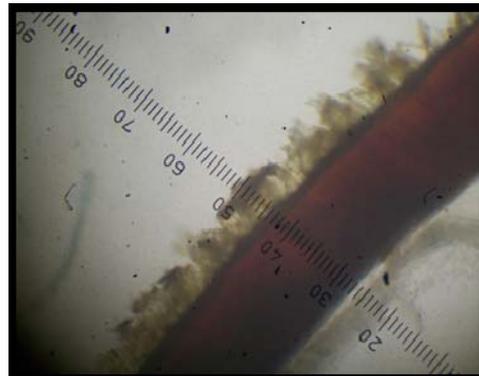


Fig 6a: Micromerements of seed coat with trichomes and spool shaped cells

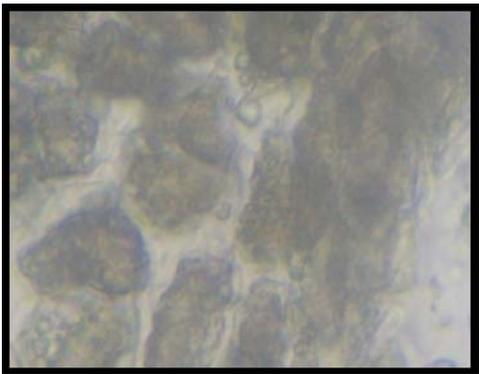


Fig 4b: Aleurone layer followed by mass of endosperm cells

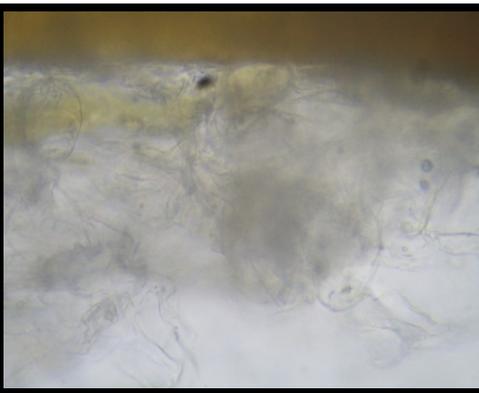


Fig 6b: Micromerements of seedcoat with trichomes and spool shaped cells

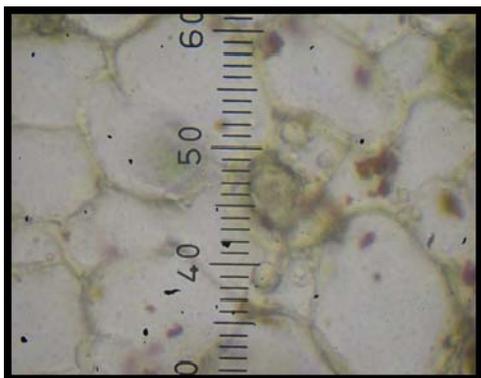


Fig7a: Cluster crystal embedded in parenchyma cells

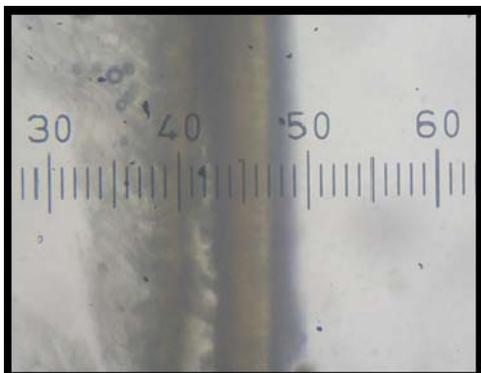


Fig 7b: Prismatic crystal embedded in parenchyma cells

Plate 2: Transverse section of both the seeds

3.3 Powder microscopy

C. tiglium Linn. : Powder of seed is light white yellowish in colour, pungent and bitter in taste, rough in touch and having nauseous odour. Diagnostic characteristic of dried powder showed oil globules, starch grains with hilum, simple fibres. Fragment of collapsed parenchyma cells along with spiral and annular vessels and fragment of nucellus in surface view embedded with vascular stands were observed. Rosette and cluster crystals of calcium oxalate, aleurone grains and yellowish brown content were present. Transversely cut fragments of cotyledon showing upper and lower epidermis.

B. montanum Muell. - Arg.: The diagnostic characters of the powder are fragment of nucellus in surface view embedded with vascular strands, transversely cut fragment of endosperm embedded with aleurone grains and fixed oil globules, fragment of endosperm in surface view embedded with aleurone grains and fixed oil globules and prismatic crystals of calcium oxalate, transversely cut fragment of cotyledon showing inner epidermis and a row of palisade underneath it, annular and spiral vessels, epidermal cells of cotyledons in surface view embedded with undeveloped stomata, lumps of protein masses, clusters and oil globules scattered.(table 3) (plate 3)

Table 3: Comparative organoleptic characters of *C. tiglium* Linn. and *B. montanum* Muell.- Arg powder

Organoleptic characters	<i>C. tiglium</i> Linn.	<i>B. montanum</i> Muell.- Arg.
Colour	Light white yellowish	Dull brown to pale yellowish brown
Odour	Nauseous	Foul
Taste	Pungent and bitter	Bitter
Texture	Rough	Oily



Fig 1a: Powder of *Croton tiglium* Linn.



Fig 1b: Powder of *Baliospermum Montanum* Muell.- Arg.

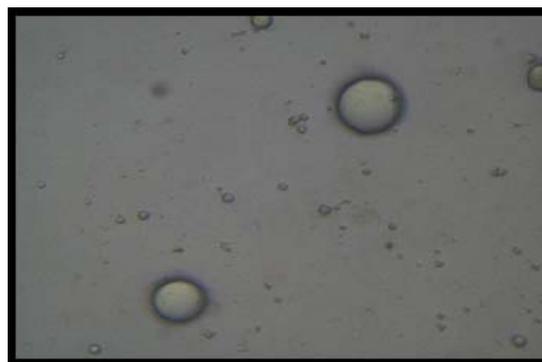


Fig 2a: Oil globules

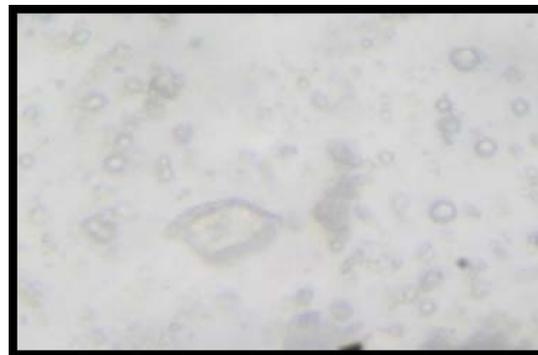


Fig 2b: Oil globules with prismatic Crystal



Fig 3a: Endosperm cells with oil Globules



Fig 5a: Aleurone grains

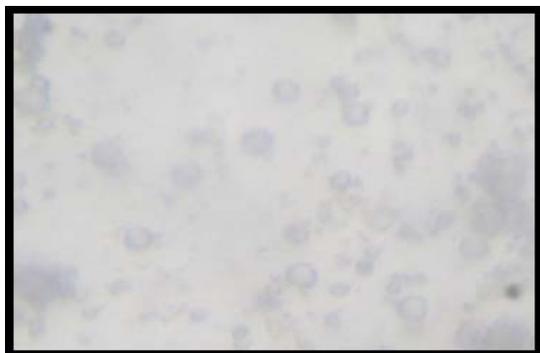


Fig 3b: Aleurone grains



Fig 5b: Oil globules



Fig 4a: Fragments of fibers

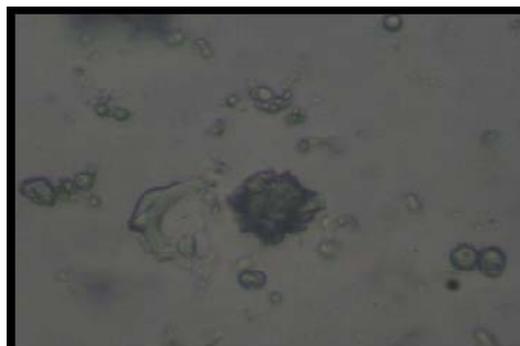


Fig 6a: Rosette crystal



Fig 4b: Fibers with wide lumen



Fig 6b: Endosperm cells with oil Globules

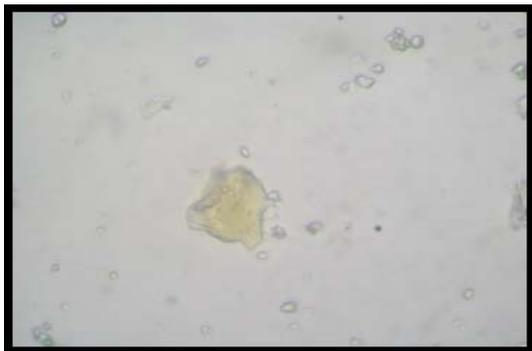


Fig 7a: Brown content

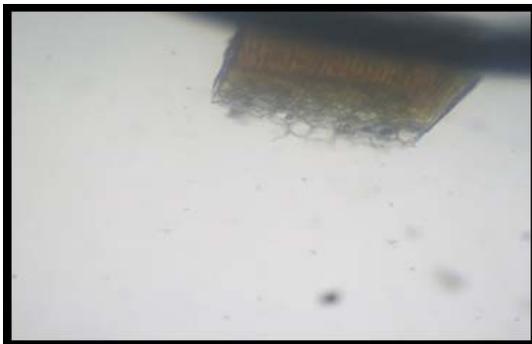


Fig 7b: Palisade cells with oil globules

Plate 3: Powder microscopy

3.4 Micrometric measurement of typical characters

***C. tiglium* Linn:** Outer epidermal cells of *Croton tiglium* Linn. seeds are 0.9 mm in length and 0.4 mm in width while endosperm cells are 1.6 mm in length and 1 mm in width. The rosette crystal of 0.6 mm length and 0.5 mm width are found in seeds.

***B. montanum* Muell. Arg.:** Prismatic crystals are 0.3mm in length and 0.2 mm in width and oil globules are 0.5 mm and aleurone grains of seeds are 0.1 mm.

Organoleptic study revealed presence of characteristic odour and velvety touch in the *C. tiglium* whereas its foul odour and smooth touch and shiny appearance were presence in *B. montanum*. This suggested preliminary difference between the samples. Common characters found in *C. tiglium* and *B. montanum* are spool shaped palisade cells, fixed oil globules, parenchymatous cells, aleurone grains, simple starch grains. In case of *C. tiglium*, TS of seed coat showed two layers of unicellular trichomes which are responsible for velvety touch. Whereas it was absent in *B. montanum*. TS of kernel of *C. Tiglium* revealed the presence of rosette crystals, whereas *B. Montanum* showed presence of prismatic crystals and cluster crystals were found in both the samples. Secretory cavity in kernels and elliptic cavity in cotyledon was only observed in *C. tiglium* which is the key distinguishing character among both the samples.

3.5 Histochemical Tests

When powder was treated with iodine, starch grains became extremely blue colour in *C. tiglium*. While in *B. montanum* very rarely found starch grains and powder was treated with Ruthenium Red, oil globules get red in colour in both seeds. When powders of both samples were treated with phloroglucinol and hydrochloric acid, vessels appeared pinkish red in colour due to presence of lignin.

4. Conclusion

Microscopic characters of seeds of *C. tiglium* and *B. montanum* of conspicuously vary from each other. Micro morphological study results indicated that, the seeds of *C. tiglium* are bigger than that of *B. montanum* Muell.-Arg. Diagnostic powder microscopical characters showed that rosette crystals of calcium oxalate and secretory cavity, trichomes are noticed in *C. tiglium* whereas prismatic crystals are observed in *B. montanum* Muell.-Arg. Histochemical test also showed more starch grains found in *C. tiglium*. These are the key identification characters at microscopy levels, which will help in distinguishing the samples.

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