Pharmacognostical Evaluation of Raw and Shodhita (Processed) Danti [Baliospermum montanum (Willd.) Muell.-Arg] Root

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ABSTRACT

Introduction: Danti, Baliospermum solanifolium (Burm.) Suresh [Syn. Baliospermum montanum (Willd.) Muell. Arg], of family Euphorbiaceae is an important herbal drug in the Ayurveda pharmacopoeia of India. In Ayurveda, Samskara (processing) has been shown to increase the efficacy of various drugs. Shodhana (purificatory measures/processing) is one of the steps involved in Samskara. The Charaka samhita describes the Shodhana (processing) of Danti by applying the fruit powder of Pippali (Piper longum L.) along with honey, wrapping it with Kusha (Desmostachya bipinnata Stapf.), and then fomenting it. The root thus obtained is dried under sunrays and then stored for further use. The exact pharmacognostical changes that transpire through Samskara (classical processing technique) remain to be explored scientifically. Hence, an attempt has been made to evaluate the pharmacognostical changes in Danti root, including its powder microscopy.

Materials and methods: Roots of raw Danti (RD) were collected from its natural habitat (Odisha) after proper botanical authentication. The roots were subjected to Shodhana and four groups of Danti root—RD, classically processed Danti root (CPDR), Kusha-processed Danti root (KPDR), and water-classically processed Danti root (WPDR)—were obtained. The raw and classically processed Danti roots were evaluated for their macroscopic and microscopic characters while RD, CPDR, KPDR, and WPDR were subjected to powder microscopy. The macroscopic powder images of the respective Danti samples was carried out by L*a*b* color-based image segmentation for identification.

Results: Transverse sections (TS) of CPDR show characteristic features with multilayered, ruptured reddish cork cells and presence of black debris of Pippali adhering to cork cells. Powder microscopy reveals Pippali with stone cells and dark-brownish oleoresin content in the CPDR group. WPDR reveals more swollen sclereids compared with the KPDR group. Macroscopic imaging showed distinct L*a*b* color-based segmentation.

Conclusion: Pharmacognostical findings of raw and shodhita Danti root will serve as a reference material for future scientific investigation.

Keywords: Baliospermum montanum, Baliospermum solanifolium, Danti, Image processing, Pharmacognosy, Shodhana.

INTRODUCTION

Baliospermum solanifolium (Burm.) Suresh. (Euphorbiaceae) is considered as the botanical source of Danti, a famous Ayurvedic drug used in many formulations of Ayurveda. It is a stout undershrub with the perennial rootstock running horizontally and sending out new shoots. It is known for its drastic purgative action and is used in the treatment of jaundice, constipation, piles, rheumatism, etc. The roots of Danti should be used after a series of Shodhana or Samskara (processing techniques). In Ayurveda, Shodhana has been advocated not only for purifying the drugs but also by altering their pharmacologic effects. It seems that Shodhana was carried out with the intention of not only purifying the drugs but also by altering their pharmacologic effects. The pharmacognostical characters of the root of Baliospermum solanifolium (Burm.) Suresh has been reported. But the pharmacognostical characters of the shodhita root remain unknown. Hence, this study aimed to evaluate the pharmacognostical changes transpiring in RD and processed Danti root.

MATERIALS AND METHODS

Collection and Authentication

Danti [Baliospermum solanifolium (Burm.) Suresh] was identified and collected from its natural habitat (Bolangir forest area of Odisha) and authenticated by comparing...
with the reported characters mentioned in the Flora of Orissa with the help of local taxonomists. The roots were collected in February 2016 (Fig. 1). The herbarium was preserved in the pharmacognosy laboratory of the Institute of Post Graduate Teaching & Research in Ayurveda (IPGT & RA) with voucher specimen no. PHM/6208/15-16 for future reference (Fig. 1). The collected root samples were shaken to remove adherent soil and dirt. The roots were separated from the stem and washed under running fresh water. Few pieces were stored in a solution of alcohol: Acetic acid: Formalin (ratio 90:5:5) for microscopic studies. The remaining roots were washed, shade-dried, and then subjected to Shodhana following the classical guidelines.

**Shodhana (Processing)**

The shade-dried RD was smeared with a thin-layered paste prepared from the fruits of Pippali (*Piper longum* L.) along with honey and then wrapped with dry leaves of Kusha (*Desmostachya bipinnata* Stapf.). The resultant was coated with mud and fomented with steam at 125°C for 3 hours. This process was adopted thrice. The resultant roots were dried under sunrays, powdered, assembled, and named as CPDR. Then it was kept in an airtight glass container for further study. In another batch, the raw drug was wrapped with dry Kusha and the resultant was coated with mud and fomented with steam at 125°C for 3 hours. This process was adopted thrice. The resultant roots were dried under sunrays, powdered, assembled, and named as KPDR group. In another batch, the RD was fomented with steam at 125°C for 3 hours. This process was adopted thrice. The resultant roots were dried under sunrays, powdered, assembled, and named as the WPDR group.

**Sample Preparation**

Root samples obtained after Shodhana were kept in hot water overnight and studied under a microscope. The remaining roots were powdered, passed through Mesh no. 80, and preserved in an airtight glass container and utilized for powder microscopy.

**Pharmacognostical Analysis**

**Macroscopic**

Morphological characters of the root of RD and classically processed Danti root were studied by observing under the dissecting microscope as well with naked eyes. Prior to microscopic observation, organoleptic characters of RD, CPDR, WPDR, KPDR were determined by studying color, odor, taste, texture, etc.
Powder Microscopy

For powder microscopy, slides were prepared using water, chloral hydrate as a clearing agent, stained with phloroglucinol and conc. HCl for lignified tissues, iodine for starch grains and glycerin as mountant. To locate the region for certain constituents of the drug, few histochemical tests were also performed. For the presence of lignified elements, the section was treated with phloroglucinol and conc. HCl.17

Histochemical Evaluation

Sample thick sections were subjected to histochemical tests to find starch grains, tannin, calcium, etc. by treating with various reagents.18

Preliminary Image Processing

A lab color space is a color component space with dimension L for lightness $a^*$ and $b^*$, based on nonlinearity compressed CIE XYZ color space coordinates. The original images of the respective samples captured in RGB color space. Only $a^*$ and $b^*$ component of $L. a. b$ were used for color feature extraction make the system more illumination independent. The image was acquired using the Image Acquisition Toolbox by Matlab 2017b. In brief $L. a. b$ represent the lightness of the color ($L^* = 0$, yield black and $L^* = 100$ indicates diffuse white, $a^*$ negative values indicate green while positive values indicate magenta, $b^*$ negative values indicate blue and positive values indicate yellow). The nonlinear relations for $L^*$, $a^*$ and $b^*$ are intended to mimic the nonlinear response of the eye. The uniform changes of components in the $L^*a^*b^*$ colour space is aiming in perceived color (Fig. 2).19,20

RESULTS AND DISCUSSION

Morphological

The morphological characters of both raw and classically processed Danti root sample differ in terms of color, odor, and taste. Raw Danti root has smooth fracture whereas the classically processed Danti root has more smooth fracture. This could be due to adherent unctuousness properties of honey. Presence of adhered Pippali powder and honey in classically processed Danti root did modify the color of its outer surface from brown to deep brownish with characteristic honey smell in classically processed Danti root. Fibers seen on RD root were found to be decreased in classically processed Danti root making it smoother. Detailed observations of the organoleptic characters of both RD and classically processed Danti root are presented in Table 1.

Microscopic

Transverse section of RD root and classically processed Danti root demonstrate similar characters as regards vascular bundles, phellogen layers, and medullary rays. The cork of classically processed Danti root shows visible ruptured character with the presence of black debris of Pippali while the cortex of classically processed Danti root was reddish in color in comparison to RD root (Figs 3 and 4). Details of comparative microscopic findings between TS of RD root and classically processed Danti root are presented in Table 2.
Pharmacognostical Evaluation of Shodhita and Baliospermum montanum (Willd.) Muell.-Arg Root

Figs 3A to I: Transverse sections of RD root: (A) Micrometric measurement of raw Danti root; (B) section upto central stellar portion; (C) cork with brown content & starch grain; (D) cortical cell with parenchyma; (E) cork with simple compound starch grain; (F) xylem with multiseriated medullary rays; (G) xylem with intra-xyllary pitting with xylem parenchyma with its fibers; (H) lignified pericyclic fibers; and (I) isolated pericyclic fiber

Figs 4A to L: Transverse sections of processed Danti root: (A) Cork with reddish dark brown content; (B) group of pericyclic fibers; (C) parenchyma cells with cluster crystals; (D) parenchyma with simple compound starch grain; (E) xylem with medullary rays; (F) xylem interxyllary pitting with xylem parenchyma; (G) medullary rays with simple-compound starch grain; (H) stained xylem; (I) stained xylem with parenchyma; (J) xylem parenchyma with its fibers; (K) stained cortex phloem xylem; and (L) cork cortex phloem xylem
Table 2: Comparative microscopic findings: TS of raw and classically processed Danti roots

<table>
<thead>
<tr>
<th>Characters</th>
<th>Transverse sections of RD root</th>
<th>Transverse sections of classically processed Danti root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cork</td>
<td>5–18-layered cork consisting of brown colored suberized or lignified brick-shaped cells and few cells containing tannin and red coloring matter</td>
<td>Circular, with ruptured outer bark and red coloring matter</td>
</tr>
<tr>
<td>Phellogen</td>
<td>Few layers of phellogen</td>
<td>Similar findings observed</td>
</tr>
<tr>
<td>Cortex</td>
<td>Outer cortex: Light brown with numerous lenticels</td>
<td>Reddish cortex</td>
</tr>
<tr>
<td></td>
<td>Inside: Creamish white, thin cork fractures smooth</td>
<td>Tannin and brown content change drastically to reddish</td>
</tr>
<tr>
<td>Phloem</td>
<td>2–7 layers of oval to elliptical, tangentially elongate usual elements, traversed by uniserrate to biserrate phloem rays</td>
<td>Similar findings observed as regards RD root</td>
</tr>
<tr>
<td>Xylem</td>
<td>Secondary xylem consists of vessels and tracheids, bordered pits; a few having reticulate thickening</td>
<td>Xylem separated by uniserrate to biserrate layers and heavily loaded with starch grains and crystals</td>
</tr>
<tr>
<td>Medullary</td>
<td>Often uniserrate or biserrate containing starch grains in their cells</td>
<td>Similar findings observed as regards RD root</td>
</tr>
<tr>
<td>rays</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extra feature</td>
<td>Slightly thick-walled fibers with narrow lumen and blunt tips</td>
<td>No prominent findings were observed</td>
</tr>
</tbody>
</table>

**Powder Microscopy**

**Organoleptic Characters**

Powder microscopy at different levels of Danti shodhana shows almost similar characteristics in terms of color, odor, taste, and touch between RD, KPDR, and WPDR groups whereas CPDR differs to a little extent. Detailed comparative characters are presented in Table 3.

**Microscopic Findings**

Microscopic findings of powder microscopy of CPDR demonstrate the presence of stone cells, black debris of Pippali, and dark-brownish oleoresin content indicating the presence of Pippali.21

Presence of saccharine content confirms the presence of honey. The KPDR group revealed abundant deposition of oil globules which is characteristic of Kusha along with wider, smooth and ruptured fibers. The brown content of cork becomes reddish. Presence of smoothed fibers with stretched sclereids with large lumen is evident in KPDR.22 This might be due to the reason that this group was wrapped with Kusha and subjected to water fomentation. In WPDR, the group is subjected to water fomentation; because of substantial absorption of water, the sclereids have turned out to be more swollen as compared with that in the KPDR group.

Comparative microscopic findings of powder microscopy at various levels of Danti root Shodhana are presented in Table 4 and Figs 5 to 8.

**Image Processing L* a* b* Scale**

Image segmentation is a set of segments that collectively cover the entire image and set of contours extracted from the raw image.23 Each of the pixels in a region is similar with respect to some characteristic or computed property, such as color, intensity, and texture. The most important attribute of the L*a*b* model is the image of RD powder along with that of various groups of processed Danti samples, i.e., CPDR, KPDR, and WPDR (Fig. 9). Clouding of characteristic violet, yellow, green, and black color was observed in the CPDR, KPDR, WPDR, and RD samples respectively. In this way, the real-time color was stored, which can serve as a database in future for samples obtained through various purification levels of B. solanifolium (Burm.) Suresh (Table 5).

**CONCLUSION**

Raw and processed Danti roots possess certain similar and dissimilar pharmacognostical characters. Classical
Table 4: Comparative powder microscopic findings of various groups of Danti root before and after Shodhana

<table>
<thead>
<tr>
<th>Diagnostic characters</th>
<th>RD</th>
<th>CPDR</th>
<th>KPDR</th>
<th>WPDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cork cells</td>
<td></td>
<td>Stone cells</td>
<td>Deposition of oil globules in abundance</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black debris of <em>Pippali</em> found</td>
<td>Brown content of cork has become reddish</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark-brownish oleoresin content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibers</td>
<td>Group of fibers</td>
<td>Fibers of <em>Kusha</em></td>
<td>Fibers more smoothened</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fibers wider, smooth, and ruptured</td>
<td>Fibers swollen</td>
<td></td>
</tr>
<tr>
<td>Sclereids</td>
<td></td>
<td></td>
<td>Sclereids with large lumen and stretched</td>
<td>Sclereids swollen</td>
</tr>
<tr>
<td>Xylem vessels</td>
<td>Fragment of border pitted vessels</td>
<td>Fragment of border pitted vessels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crystals</td>
<td>Acicular crystals of calcium oxalate</td>
<td>Drastic reduction in acicular crystals</td>
<td>Reduction in cluster and rosette crystals</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rosette crystals centrally placed</td>
<td></td>
<td>Acicular crystals drastically deformed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cluster crystals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch grains</td>
<td>Simple–compound starch grains</td>
<td>Simple–Compound starch grains</td>
<td>Decolorization of dark-brown tannin content</td>
<td></td>
</tr>
<tr>
<td>Tannin content</td>
<td>Fragment of tannin content</td>
<td>Simple starch grains with hilum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saccharine content</td>
<td>Present</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figs 5A to L: Powder microscopy of RD: (A) Powder of RD; (B) simple, compound starch grain; (C) acicular crystals; (D) rosette crystal; (E) tannin; (F) cluster crystals; (G) group of fibers; (H) border pitted vessels; (I) lignified border pitted vessels; (J) lignified fibers; (K) cork in surface view; and (L) iodine stained starch grain.
Figs 6A to L: Powder microscopy of CPDR: (A) Stone cells of Pippali; (B) black debris of Pippali; (C) prismatic crystals of Danti; (D) rosette crystals of Danti; (E) border pitted vessels; (F) starch grains of Danti; (G) simple starch grain with hilum; (H) sacharine content of honey; (I) fibers of Kusha; (J) lignified cork surface; (K) lignified border pitted vessels; and (L) iodine stained starch grain.

Figs 7A to H: Powder microscopy of KPDR: (A) Powder; (B) group of fibers of Kusha; (C) fibers with oil globules of Kusha; (D) cork of Danti; (E) sclereiform vessels of Kusha; (F) tannin content of Danti; (G) lignified fibers of Danti; and (H) iodine stain starch grain.
Processed *Danti* root can be differentiated from RD root with characteristic characters along with additional characters of multilayered, ruptured reddish cork cells with the presence of black debris of *Pippali* adhering to cork cells. Presence of black debris of *Pippali* with stone cells and dark-brownish oleoresin content can differentiate CPDR powder from RD root powder. The WPDR powder can be differentiated from the KPDR group by the presence of more swollen sclereids indicating more fomentation. The results of the pharmacognostical findings can be considered as reference standards for further studies and will throw new light toward standardization of raw and processed *Danti* roots.

**ACKNOWLEDGMENT**

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Figs 9A to H: Representative different image of CPDR, KPDR, RD, WPDR using image acquisition tool box
(1) A, C, E, G raw powder images and (2) B, D, F, H as L*a*b* color space converted images.


हिंदी सारांश
शोधित तथा सूचीन्त्री दाती (बालीस्पर्म मोटेनम वाइल्ड) मूल अर्ग, मूल का फार्माकोनोमिस्टिकल मूल्यांकन

"सिवा पी राहुत, बालीस्पर्म अर्ग हरिया, वेदितारण आचार्य"

रिकॉर्ड: दाती, Baliospermum solanifolium (Burm) Suresh -एवं मूल का एक महत्वपूर्ण औषधि है जो आयुर्वेद फार्माकोनोमिस्टिकल में उल्लेखित है। इसकी मूल तीव्र रंगक क्रिया के लिए जानी जाती है जिसका विभिन्न भीषणताओं में संकेत दिया है। आयुर्वेद में विभिन्न औषधियों की प्रमाणकारकता की बढ़ाने के लिए संस्कार का उपलब्ध है। चर्चा केन्द्रों में बताया गया है कि दाती के शोधन के लिए पिपली फल दूध को मूल के साथ दत्ती मूल पर लेखित कर कुरा में वास्तव में वर्णन है जिसकी नामित करते है। उसके पश्चात प्राप्त मूल को दूध की रेखाएँ ने मुख्य तथा आगे के उपयोग के लिए रख दिया। संस्कार के द्वारा दत्ती मूल के जो फार्माकोनोमिस्टिकल परिवर्तन आयो के वैज्ञानिक रूप से उपलब्ध नहीं है। इसलिए दत्ती मूल ने जो फार्माकोनोमिस्टिकल परिवर्तन हुए उसका मूल्यांकन करने के लिए एक प्रयास किया गया है।

सामग्री और तरीके: कच्चे दत्ती मूल को अर्ग रूप में एवं मूल का अर्ग तथा प्राप्त वातावरण उद्धरण के साथ वातावरिक प्रमाणकारक के बाद एकत्र किया गया। संगठित मूल को शारीरिक शोधनधिक के द्वारा शोधित किया गया तथा दत्ती के विभिन्न स्तरों पर शोधन के द्वारा 3 समूह प्राप्त हुए थे। यादों विविध द्वारा प्राप्त दत्ती, दूध द्वारा शोधित दत्ती तथा पानी द्वारा शोधित दत्ती। वातावरण तथा शोधित दत्ती का MICROSCOPIC तथा MICROSCOPY तथा रात्रि पर मूल्यांकन किया गया जबकि पात्र समूह की POWDER MICROSCOPY किया गया। Microscopic छवि पूर्ण संचित विकल्प नमूने की L द्वारा बाहर किये गए *A*, *B* रेंग आयुर्वेदिक छवि विचारण की पहचान के लिए किया गया।

परिणाम: कच्चे दत्ती मूल की अनुसूचक कार्य से उसको वातावरिक स्थिर हो प्राप्त हुआ जबकि शोधित दत्ती मूल के अनुसूचक कार्य से वातावरिक स्थिर को साथ REDDISH CORK CELLS (माल कार्य कोशिकाओं) की खोज तथा प्राप्त कार्य कोशिकाओं के साथ पिपली का कार्य अवशेष विचार दिखाई दिया। पाउडर MICROSCOPY से दाता हुआ कि पिपली का कार्य अवशेष पत्तर (STONE) कोशिकाओं के साथ तथा OLEORESIN CONTENT शालकों निर्देश द्वारा प्राप्त दत्ती समूह में उपस्थित है। पानी द्वारा शोधित दत्ती, दूध द्वारा शोधित दत्ती पानी द्वारा मालकोशिकाओं समूह की हत्या में सफल हुए तथा Oléoreasenes शोधित देखा गया। माइक्रोस्कोपी छवि के अलावा लग L* a* b* रेंग आयुर्वेदिक विश्लेषण नमूने की पहचान करने के लिए देखा गया।

निर्णय: कच्चे के Pharmacognostical निर्णय और शोधित दत्ती मूल भारी वैज्ञानिक जांच के लिए एक संदर्भ सामग्री के रूप में काम करेगा।

संदर्भ: फार्माकोनोमिस्टिकल, बालीस्पर्म, शोधित दत्ती।