ABSTRACT

Objective: Ayurveda recommends the use of Danti root after Shodhana (Processing/Purification) where the powder Pippali (Piper longum Linn.) fruit, honey and Kusha (Desmostachya bipinnata Stapf) leaves are being used. But the additive effect of all these drugs on Danti root are yet to be explored scientifically. Principal component analysis (PCA), a multivariate data analysis technique targeting to assess the discrimination effect of psychic nut, for evaluating the additive effect, can be used to assess the effect of Shodhana on preliminary physicochemical, phytochemical parameters upon four levels of Danti (Baliospermum montanum Willd.) root.

Methods: Roots of raw Danti, after proper botanical authentication, were subjected for classically recommended Shodhana procedure and four groups of Danti root like raw Danti (RD), Classical processed Danti root (CPDR), Kusha processed Danti root (KPDR), water processed Danti root (WPDR) were obtained at various levels of Danti Shodhana. Methanolic macerated extracts of all four Danti root groups were subjected for preliminary physicochemical, phytochemical and chromatographic screening. The obtained data were analyzed with the help of the Un-scrambler Camo Software for multivariate data analysis.

Results: The methanolic and water extractive value of CPDR group is more than remaining sections holding lower ash value and high-intensity colour reaction during phytochemical screenings of steroid, flavonoid etc.

Conclusion: Analysis of PCA technique suggests a similar trend in between RD and KPDR group while CPDR and WPDR on a different in score plot.

Keywords: Danti, Shodhana, PCA, Classical processed Danti root, Baliospermum montanum

INTRODUCTION

Physicochemical analysis provides the objective parameters to fix up the standards for quality of raw drugs as well as finished products. Analytical study of a drug helps to interpret the pharmacokinetics and pharmacodynamics of the same [1]. Ayurveda has described numerous herbal, mineral, herbomineral drugs, including poisonous and semi-poisonous drugs, in all its treaties and advocated to use poisonous plants after passing through Shodhana procedures. The concept of Shodhana (detoxification technique/processing) in Ayurveda is not only a process of purification/detoxification, but also a process to enhance the potency and efficacy of the drugs [2]. More to say a process where different procedures have been recommended to pass through specific Shodhana process before administration for the medicinal purpose [7]. According to Charaka Danti root should be used after being delivered through certain Samskara (processing techniques). In the light of the above background, the present study was designed and undertaken to study of Danti root sample to assess the effect of Shoshana on preliminary physicochemical, phytochemical and chromatographical fingerprinting.

MATERIALS AND METHODS

Collection and authentication

Danti (Baliospermum montanum Willd.), basing upon its morpho-logical characters were identified 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 taxonomist [8] and the roots were collected in the month of February 2016. The herbarium was preserved in pharmacognosy laboratory of IPGT and RA with voucher specimen (No. PHM/6208/15-16) for future reference.

Sample preparation

Collected raw Danti (R. D) root being washed properly was smeared with a thin layer of paste prepared from a powder of Pippali (Piper longum Linn.) and honey then wrapped with leaves of Kusha (Desmostachya bipinnata Stapf.). The resultant was coated with mud and fomented with steam at temperature 125 °C for 3 h [9]. This process was adopted for three times obtaining 3 batches of Classical processed Danti root (CPDR). Then the obtained three batches of roots were dried in sun rays, then grinded with a mechanical grinder, assembled, finally, the coarse powders were separated by sieving using 80 meshes and stored in an airtight container for further use. Individual powders at different levels of Danti Shodhana like Kusha processed Danti root (KPDR) group and Water processed Danti root (WPDR) group was also obtained in the same way.

Preparation of extract

Different groups of Danti root were grinded into coarse powders and then subjected to macerated with methanol (Merk, Germany) in a ratio of 1:5. The extracts were filtered and the solvents were evaporated under temperature controlled water bath. The dried extracts were stored in a refrigerator at 4 °C until further analysis [10].

Physicochemical and phytochemical study

The loss on drying, moisture, ash, extractive value and other physico-chemical constant were determined by using the association of Official Analytical Chemist [11]. Preliminary phytochemical investigations such as Molisch's test, Salkowski test,
dispensed into a test tube, followed by 1.5 ml of methanol, 0.1 ml of
Total flavonoid content (TFC) was determined using aluminium
absorbance mode at 254 nm and 366 nm under the control of
was performed with a Camag T. L. C. scanner III in reflectance
Wincats software. Further spectral comparison was also performed.
Documentation
With the TLC Visualizer under short UV 254 nm and long UV 366.
Total flavonoid content
Total flavonoid content (TFC) was determined using aluminium
chloride method as reported by Cook NC, Samman S. [16]. About 2
ml of methanolic extract of KPDR, RD, CPDR, WPDR (mg/ml) was
dispensed into a test tube, followed by 1.5 ml of methanol, 0.1 ml of
aluminium chloride (10%), 0.1 ml of 1 M potassium acetate and 2.8
ml of distilled water. The reaction mixture was mixed, allowed to
stand at room temperature for 30 min before absorbance was read
at 514 nm. TFC was expressed as chrysin (5, 7-dihydroxy flavone)
equivalent (QE) in µg/ml material.
Principal component analysis (PCA)
Principal component analysis (PCA) is a technique used to emphasise
variation and bring out strong patterns in a dataset. It's often used to
make data easy to explore and visualize. Principal component analysis
provides a method for understanding the meaning of a data set by
extracting a smaller series of important components that account for the
variability in the data. Principal component analysis is a variable
reduction procedure. It is useful when you have obtained data on a
number of variables (possibly a large number of variables) [17-18].
Data analysis
All the physicochemical data were tabulated in two-way matrix
form. One way is a respective sample and another way thermal and
solubility category parameter (LOD, AV and AIA, WSE and MSE). The
single table data were executed for PCA with help of Unscrambler
Camo ® student version.
RESULTS AND DISCUSSION
Physico-chemical analysis
The result of the physicochemical properties of the four Danti
samples are presented in table 1.

Table 1: Physico-chemical parameters of root powder of KPDR, CPDR, RD, and WPDR powder (Result expressed as % w/w, n=3,
meansSD)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test</th>
<th>CPDR</th>
<th>KPDR</th>
<th>WPDR</th>
<th>RD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loss on drying at 110 °C</td>
<td>14.5±1.6%</td>
<td>9±0.12%</td>
<td>1.2±0.23%</td>
<td>9.1±0.26%</td>
</tr>
<tr>
<td>2</td>
<td>Ash value(w/w)</td>
<td>7.5±0.42%</td>
<td>6±0.39%</td>
<td>8.6±0.29%</td>
<td>9.4±0.26%</td>
</tr>
<tr>
<td>3</td>
<td>Acid Insoluble ash</td>
<td>1.8±0.4%</td>
<td>2.1±0.3%</td>
<td>1.9±0.5%</td>
<td>2±0.4%</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble extract</td>
<td>7.6±0.71%</td>
<td>4.3±0.52%</td>
<td>4.6±0.35%</td>
<td>4.1±0.32%</td>
</tr>
<tr>
<td>5</td>
<td>Methanol soluble extract</td>
<td>9.52±0.47%</td>
<td>2.32±0.63%</td>
<td>3.5±0.81%</td>
<td>3.36±0.70%</td>
</tr>
<tr>
<td>6</td>
<td>p. H</td>
<td>7.0±0.42</td>
<td>6.5±0.44</td>
<td>6.5±0.46</td>
<td>6.5±0.39</td>
</tr>
<tr>
<td>7</td>
<td>No of Spots@254 nm</td>
<td>09</td>
<td>10</td>
<td>09</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>No of Spots@366 nm</td>
<td>06</td>
<td>07</td>
<td>06</td>
<td>07</td>
</tr>
</tbody>
</table>

Loss on drying signifies the considerable amount of moisture in order to
control definite strength and prevent decomposition. Loss on drying in
CPDR is 5.4% and that in WPDR is 35% as compared to RD which
suggests that CPDR group was adhered to madhu which is a great source
of oleoresin content and KPDR group was exposed to water directly, so
due to absorption of direct absorption of water during fomentation,
there is evidence of increase in LOD. In rest groups, the value of loss on
drying is in identical range. Ash values were used to detect the presence
of siliceous contamination and water soluble salts in favor of
determining authenticity and purity of drugs. As regards to Ash value,
there is evidence of a decrease of ash value in CPDR i.e. 1.9%, that of
KPDR is 1.28% and in WPDR is 0.8% as compared to R. D. This may be
due to the fact that CPDR after being obtained through shodhana, there
occurs addition of various organic materials like Pipalpat (a good source of
volatile materials), honey, fragments of Kusha which may have been
transformed to different level chemical moieties signaling in variation of
reduced ash value and increased LOD in CPDR group. The water and
alcohol soluble extractive value shows no significant changes indicating
the percentage of soluble polar and moderately polar component like
sugarglycosides etc. remains same in all groups.

Phytochemical analysis
The phytochemical screening results suggest that the methanol
soluble extractives indicate the presence of carbohydrate, flavonoids,
polyphenol, steroidal glycoside, phenolic and tannin content which have
been presented in table 2.

Table 2: Preliminary Phytochemical analysis of Coarse Powder of KPDR, CPDR, RD, and WPDR

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Test</th>
<th>R. D</th>
<th>WPDR</th>
<th>KPDR</th>
<th>CPDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid</td>
<td>Salkowski reaction</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic and Tannin</td>
<td>Lead acetate solution</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>Biuret Test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Draganoff's test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycoside</td>
<td>Keller-Killiani test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sapponin</td>
<td>Foam Test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amino Acid</td>
<td>Ninhydrine test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Molisch's Test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Total flavonoid content

Total flavonoid content of the extract was estimated previously explained assay method. The standard calibration curve of chrysin was established. The standard chrysin indicated 0.146, 0.195, 0.294, 0.311, 0.454 absorbance at 2, 4, 6, 8, 10 (µg/ml) respectively (fig. 1). Total flavonoid content of that formulation is described in table 3.

![Calibration curve of Chrysin at 450nm](image)

**Fig. 1: Linear standard curve of chrysin (dihydroxy flavones) and its residual**

<table>
<thead>
<tr>
<th>Samples</th>
<th>KPDR</th>
<th>RD</th>
<th>WPDR</th>
<th>CPDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total flavonoid chrysin equivalent (µg/ml)</td>
<td>5.81±0.02</td>
<td>30.02±0.04</td>
<td>5.21±0.05</td>
<td>10.18±0.06</td>
</tr>
</tbody>
</table>

Data were presented as mean±SD (n=3) and also 95% confidential limit. Standard curve for total flavonoids: $y = 0.037x + 0.06$, $r^2 = 0.94$.

![HPTLC Separation](image)

**Fig. 2: Separation of methanol extract on HPTLC Si 60 F254 with Toluene: Chloroform: Acetone (4:2.5:3.5 V/V), chamber saturation, stained with the vanillin-sulfuric acid reagent. Tracks: 1. KPDR extract, 2. CPDR extract, 3. RD extract, 4. WPDR extract. A–Day light, B–Short UV (254 nm), C–Long UV (366 nm), D–After Visualizing agent. E–3D graph of the respective samples**
Flavonoids are the class of secondary metabolites remains present in a plant in the form of a polyphenolic molecule or in the form of glycoside linkage which is a polar soluble chemical entity as well as aqueous soluble. On keen observation, total flavonoid chrysin equivalent (µg/ml) concentration among all four groups of Danti reveals that KPDR and RD has been evaluated approximately in similar range while a significant marked difference in the value of total flavonoid chrysin equivalent (µg/ml) concentration was found in between RD (i.e.30.02±0.04) and CPDR (10.18±0.06) group. It can be assumed that RD group after being exposed to shodhana, some of the chemical moiety of RD have been absorbed in water resulting in decreased total flavonoid chrysin equivalent (µg/ml) concentration value in CPDR.

High-performance thin layer chromatographic profiling:

In this study, combination of toluene, chloroform and acetone (8:5:7 v/v/v) as mobile phase of HPTLC analysis of four respective samples resulted in well-separated, compact and symmetrical Bands. HPTLC fingerprint profiles of the above explaining samples methanolic extracts are shown in (fig. 6-4-D). HPTLC fingerprinting profiles respective Rf values have been depicted in table 4 and 5. In table 4, the fingerprint patterns of alcoholic extract of the KPDR, CPDR, RD, WPDR at 254 nm are shown nine, ten, nine and ten peaks. On the other hand in table-5, at366 nm respected samples six, seven, six and seven peaks are found in favor of target class of Moey polyphenol and flavonoid.

Table 4: Showing HPTLC profile for Coarse Powder of KPDR, CPDR, RD, and WPDR at 254 nm (Short UV)

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Track No</th>
<th>Under UV light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene: Chloroform: Acetone 4:2.5:3.5 V/V</td>
<td>254 nm (Short UV)</td>
<td></td>
</tr>
<tr>
<td>Track 1 (KPDR)</td>
<td>9</td>
<td>Number of spots: 9</td>
</tr>
<tr>
<td>Track 2 (CPDR)</td>
<td>10</td>
<td>Number of spots: 10</td>
</tr>
<tr>
<td>Track 3 (RD)</td>
<td>9</td>
<td>Number of spots: 9</td>
</tr>
<tr>
<td>Track 4 (WPDR)</td>
<td>10</td>
<td>Number of spots: 10</td>
</tr>
</tbody>
</table>

Fig. 3: HPTLC densitometry chromatogram (at 254 nm) of methanolic extracts of A-KPDR, B-CPDR, C-RD, D-WPDR and HPTLC densitometry chromatogram (at 366 nm) of methanolic extracts of E-KPDR, F-CPDR, G-RD, H-WPDR and spectral comparison of the respective Rf as U,J,K and L with Toluene: Chloroform: Acetone 4:2.5:3.5 V/V as the mobile phase

The densitogram of respective samples are visualized at 254 nm (fig. 3, A-D) and at 366 nm (fig. 3, E-H) along with four samples spectral comparison of various Rf (fig 3, U-L).
Table 5: Showing HPTLC profile for coarse powder of KPDR, CPDR, RD, and WPDR at 366 nm (Short UV)

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Track No</th>
<th>Under UV light</th>
<th>Max Rf Value</th>
<th>Max Height</th>
<th>Area in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene: Chloroform: Acetone 4:2.5:3.5 V/V</td>
<td>Track 1 (KPDR)</td>
<td>06</td>
<td>0.03, 0.60, 0.84</td>
<td>602.9, 13.1, 14.6</td>
<td>83.90, 1.72, 1.45</td>
</tr>
<tr>
<td></td>
<td>Track 2 (CPDR)</td>
<td>07</td>
<td>0.03, 0.40, 0.49</td>
<td>334.0, 1.1, 33.3</td>
<td>27.63, 0.82, 5.9</td>
</tr>
<tr>
<td></td>
<td>Track 3 (RD)</td>
<td>06</td>
<td>0.02, 0.70, 0.76, 0.91</td>
<td>39.82, 5.7, 16.0</td>
<td>5.48, 35.15, 17.5, 8.68</td>
</tr>
<tr>
<td></td>
<td>Track 4 (WPDR)</td>
<td>07</td>
<td>0.02, 0.10, 0.50</td>
<td>543.0, 30.6, 19.0</td>
<td>33.58, 1.33, 1.88</td>
</tr>
</tbody>
</table>

Chromatographic performance of CPDR, KPDR, WPDR and RD on silica gel at 254 nm using mobile phase toluene, chloroform and acetone (8:5:7 v/v/v) shows that the compound fraction separated at Rf 0.03 found to be similar as UV-Vis spectrum shows similarity while component separated at 0.32, 0.79 and 0.87 shows similarity between KPDR and WPDR chromatographic curve while RD and CPDR chromatographic curve remains to each other distinct respectively. It suggests that in CPDR, the process of steaming with vapour in water as well with the help of pippali and honey modulates some of the components to produce a new chemical entity that has produced a distinct chromatographic graph to RD.

Multivariate analysis

PCA was executed to provide a data structure study in a reduced dimension, covering the maximum amount of information present in the data. It is value revealing that PCA is among the most versatile of all chemometric methods as it involves a mathematical procedure that reduces data dimensionality. The data matrix corresponding to the physicochemical parameters along with chromatographic data (table 1) was submitted to PCA in order to show possible trends in their values and emphasize the similarities and differences between various samples on a score plot.

![PCA score plot](image1)

![Loading plot](image2)

![Bi-plot](image3)

Fig. 4: (A) PCA score plot and (B) loading plot (C) Bi-plot of three samples based on its physico-chemical and chromatographic separation behavior showing the distribution pattern of samples and various physicochemical parameters contributing to the groups respectively. The ellipse represents the Hotelling T2 with 95% confidence in score plot.

The score plot in fig. 4-A showed that the Danti samples (KPDR-RD) were grouped together in the upper left quadrant of the score plot, though one samples RD appear below the horizontal line of the score plot. CPDR samples were well separated from the other samples.
scattered in the lower right quadrant, except for the sample WPDR which appeared in the upper right quadrant. From the loading plot in fig. 4-B, it appeared that the ASH, LOD, and pH, ASE, 254 and 366 sensitive separated peaks were the physicochemical, chromatographical parameters contributing to the grouping of Danti samples, and that these attributes corresponded to the PCI which explained about 96% of the total variance. It should be noted that CPDR samples are differentiated from other samples by their higher water, alcohol extractive value and chromatographic pattern as well as lower ASH value content.

CONCLUSION

Comparisons of different physicochemical parameteric observations like loss on drying, water soluble extract, methanolic soluble extractive value, pH, chromatographic fingerprinting at 254 nm and 366 nm between different samples of Danti obtained from various level of Danti shodhana (purificatory conditions) shows the level of discriminations in between water processed Danti root, classical processed Danti root, kusha processed Danti root, raw Danti root groups. With respect to physicochemical variables among all groups of Danti, classical processed Danti root remains at upper hand among them. Discrimination upon one target group like total flavonoid chrysine equivalent (µg/ml) concentration between raw Danti root and classical processed Danti root has justified that shodhana (purification) has produced an impact upon the between raw Danti root and classical processed Danti root. Based on physicochemical data, it is observed that pattern recognition techniques such as PCA have shown potent discrimination at various levels of Danti samples. Analysis of all these datas shows water soluble extractive value is the most prominent value that has been disturbed to a significant level in classical processed Danti root as compared to raw Danti. The overall decrease in Ash value, increase in loss on drying, decrease in total flavonoid chrysine equivalent (µg/ml) concentration and other chromatographic findings of classical processed Danti root clearly discriminates it from raw Danti. Hence on the basis of these findings, it can be concluded that shodhana (Purification) has a definite impact upon Danti and the observed parameter may act as a referencing tool for further scientific advancement.

ACKNOWLEDGEMENT

The authors thank to the Director, IPGT & RA Jamnagar, for providing financial support. The author is exclusively thankful to Jayanta Kumar Maji and all the associate staffs of pharmaceutical lab, the Gujarat Ayurveda University for the support.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

REFERENCES


How to cite this article