ASSSESSMENT OF EFFECT OF SHODHANA ON PHYTOCHEMICAL AND CHROMATOGRAPHICAL PROFILE OF DIFFERENT LEVELS OF CLASSICAL PROCESSED DANTI (BALIOSPERMUM MONTANUM WILDL.) ROOT

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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 Wild.) 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. Though have not been categorized under poisonous drugs, still association of Official Analytical Chemist [11]. Preliminary phytochemical investigations such as Molisch’s test, Salkowski test,

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 (detoxification technique/processing) in Ayurveda is not only a process after passing through Shodhana procedures. The concept of Shodhana (detoxification technique/processing) in Ayurveda is not only a process to enhance the potency and efficacy of the drugs [2]. More to say a process where different types of purification/detoxification, but also a process to enhance the potency and efficacy of the drugs [2]. More to say a process where different types of purification/detoxification, but also a process to enhance the potency and efficacy of the drugs [2].

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Quantitative UV-VIS analysis

HPTLC study

Chromatography is a powerful analytical method suitable for the separation and quantitative determination of a considerable number of compounds even from complicated matrix [13-15]. HPTLC study was carried out with methanolic extract. Each of 5 ml methanol extract of RD, CPDR, KPDR, and WPDR was spotted on pre-coated Silica Gel GF254 plates by means of Camag Linomat V sample applicator. The mobile phase consisted of Toluene: Chloroform: Acetone 4:2.5:3.5 V/V. After development, the densitometry scan was performed with a Camag T. L. C. scanner III in reflectance absorbance mode at 254 nm and 366 nm under the control of Wincats software. Further spectral comparison was also performed.

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 ethanol, 0.1 ml of methanolic extract of KPDR, RD, CPDR, WPDR (mg/ml) was added and 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].

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, means±SD)

| S. No. | Test                      | CPDR        | KPDR        | WPDR        | RD         |
|--------|---------------------------|-------------|-------------|-------------|------------|
| 1      | Loss on drying at 110 °C  | 14.5±1.6%   | 9±0.12%     | 1.26±0.23%  | 9±0.26%    |
| 2      | Ash value(w/w)            | 7.5±0.42%   | 8.±0±.39%   | 6.4±0.2%    | 9.4±0.26%  |
| 3      | Acid Insoluble ash        | 1.8±0.4%    | 2.±1±.3%    | 1.9±0.5%    | 2±0.4%     |
| 4      | Water soluble extract     | 7.6±0.71%   | 4.3±0.52%   | 4.6±0.35%   | 4±0.32%    |
| 5      | Methanol soluble extract  | 9.52±0.47%  | 2.32±0.63%  | 3.5±0.81%   | 3.36±0.7%  |
| 6      | pH                       | 7.0±0.42    | 6.5±0.44    | 6.5±0.46    | 6.5±0.39   |
| 7      | No of Spots@254 nm        | 09          | 10          | 09          | 10         |
| 8      | No of Spots@366 nm        | 06          | 07          | 06          | 07         |

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 3.5% as compared to RD which suggests that CPDR group was adhered to maddhu 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 shedhana, there occurs addition of various organic materials like Pippali (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 alcoholsoluble 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 extracts indicate the presence of carbohydrate, flavonoids, polyphenol, steroids, 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

| Phytochemical     | Test           | R. D | WPD | KPDR | CPDR |
|-------------------|----------------|------|-----|------|------|
| Steroid           | Salkowski reaction | +    | +   | +    | +    |
| Phenolic and Tannin| Lead acetate solution | +    | +   | +    | +    |
| Flavonoids        | Shinoda Test    | +    | +   | +    | +    |
| Protein           | Biuret Test     | -    | -   | -    | -    |
| Alkaloid          | Dragendorff’s test | -    | -   | -    | -    |
| Glycoside         | Keller-Killiani test | +   | +   | +    | +    |
| Sapponin          | Foam Test       | -    | -   | -    | -    |
| Amino Acid        | Ninhydrine test | -    | -   | -    | -    |
| Carbohydrate      | Molisch’s Test  | +    | +   | +    | +    |

+= present, -= absent, R. D-Raw Danti, WPDR-Water processed Danti root, KPDR-Kusha processed Danti root, CPDR-Classic processed Danti root.
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.

Fig. 1: Linear standard curve of chrysin (dihydroxy flavones) and it's residual

Table 3: The content of total flavonoid in four respective samples

| Samples | KPDR       | RD         | WPDR       | CPDR       |
|---------|------------|------------|------------|------------|
|         | Total flavonoid chrysin equivalent (µg/ml) | 5.81±0.02  | 30.02±0.04 | 5.21±0.05  | 10.18±0.06 |

Data were presented as mean±SD (n=3) and also 95% confidential limit. Standard curve for total flavonoids: y = 0.037x+0.06, r² = 0.94.

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-A-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, at 366 nm respected samples six, seven, six and seven peaks are found in favor of target class of Moeyty polyphenol and flavonoid.

| Solvent system | Track No. | Under UV light | Number of spots | Max Rf. Value | Max Height | Area in % |
|----------------|-----------|----------------|----------------|--------------|------------|-----------|
| Toluene: Chloroform: Acetone 4:2.5:3.5 V/V | Track 1 (KPDR) | 09 | 0.02, 0.14, 0.34, 0.38, 0.49, | 693.1, 13.1, 31.4, 24.0, 14.6, | 63.27, 1.00, 3.60, 1.71, 1.71, |
| | | | 0.64, 0.73, 0.89, 0.96 | 72.1, 12.8, 44.9, 186.5 | 7.77, 0.92, 3.06, 16.95 |
| | Track 2 (CPDR) | 10 | 0.03, 0.32, 0.37, 0.43, 0.49, | 334.9, 64.4, 32.9, 17.3, 34.0, | 23.45, 8.22, 3.17, 1.57, 6.44, |
| | | | 0.62, 0.70, 0.80, 0.88, 0.93 | 92.4, 20.5, 140.9, 87.9, 133.7 | 12.44, 1.66, 13.00, 13.50, 16.57 |
| | Track 3 (RD) | 09 | 0.02, 0.32, 0.37, 0.52, 0.62, | 569.6, 31.1, 19.7, 14.2, 43.3, | 55.89, 4.18, 2.81, 1.41, 6.27, |
| | | | 0.70, 0.76, 0.87, 0.95 | 16.5, 35.0, 51.1, 110.4 | 0.85, 6.58, 5.85, 16.15 |
| | Track 4 (WPDR) | 10 | 0.02, 0.10, 0.32, 0.37, 0.48, | 601.5, 12.2, 17.5, 23.2, 26.4, | 32.56, 0.18, 1.09, 1.43, 2.56, |
| | | | 0.62, 0.70, 0.79, 0.84, 0.96 | 151.7, 23.6, 151.1, 208.0, 195.8 | 12.71, 1.08, 10.19, 20.25, 17.95 |

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)

| Solvent system       | Track No | Under UV light | Max Rf. Value | Max Height | Area in % |
|----------------------|----------|----------------|---------------|------------|-----------|
| Toluene: Chloroform: Acetone 4:2.5:3.5 V/V | Track 1 (KPDR) | 06 | 0.03,0.50,0.74, 0.78,0.90,0.96 | 602.9,13.1,14.6 | 19.9,17.2,36.3 | 83.90,1.72,1.45 |
|                      | Track 2 (CPDR) | 07 | 0.03,0.40,0.49 | 334.0,11.1,33.3 | 27.63,0.82,0.99 |
|                      | Track 3 (RD) | 06 | 0.71,0.80,0.84,0.91, 0.02,0.70,0.76, 0.82,0.95,0.97 | 47.6,602.0,17.1 | 23.2,17.2,17.3 | 1.98,2.68,2.17 |
|                      | Track 4 (WPDR) | 07 | 0.02,0.10,0.50 | 543.0,30.6,19.0 | 33.58,1.33,1.88 |

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.

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.
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Graphical parameters contributing to the grouping of sensitive separated peaks were the physicochemical, chromatographical parameters. It appeared that the ASH, LOD, and pH, ASE, 254 and 366, which appeared in the upper right quadrant. From the loading plot, it was observed that the sample WPDR was scattered in the lower right quadrant, except for the higher water, alcohol extractive value and chromatographic pattern as well as lower ASH value content.

CONCLUSION

Comparisons of different physicochemical parametric observations like loss on drying, water-soluble extract, methanolic soluble extractive value, pH, chromatographic fingerprinting between 254 nm and 366 nm, different samples of Danti obtained from various levels 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 chrysin 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 data 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 chrysin 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.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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