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Original Article

ASSESSMENT OF EFFECT OF *SHODHANA* ON PHYTOCHEMICAL AND CHROMATOGRAPHICAL PROFILE OF DIFFERENT LEVELS OF CLASSICAL PROCESSED DANTI (*BALIOSPERMUM MONTANUM WILLD.*) ROOT

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ABSTRACT

Objective: Ayurveda recommends the use of *Danti* root after *Shodhana* (Processing/Purification) where the powder *Pippali* (*Piperlongum* Linn.*)* fruit, honey and *Kusha* (*Desmostachya bippinata* 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*

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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 qualities are depicted through which there is '*gunaantardhana*' (transformation in properties) in the primary substance rendering it safe, as well as many desired qualities, are imbibed in it.[3]. Effect of Shodhana on different phytopharmacological properties of different drugs like Guggulu (*Commiphora mukul* Engl [4], *Kupilu*seeds [5], *Vatsanabha* [6] have been reported. *Shodhana* procedures have been advocated for both herbal as well as mineral drugs based on their toxic nature. Certain medicinal plants like *Danti* (*Baliospermum montanum* Willd), *Vacha* (*Acorus calamus* Linn.), *Vriddhadaru* (*Argyreiaspeciosa* Sweet.), *Hingu* (*Ferula foetida* Bioss.), *Kampillaka* (*Mallotusphilippensis* (Lamk.) Muell-Arg.) And *Guggulu* (*Commiphora mukul* (Hook. ex. Stocks) Engl.) etc. though have not been categorized under poisonous drugs, still 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 (Merck, 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 physicho-chemical constant were determined by using the association of Official Analytical Chemist [11]. Preliminary phytochemical investigations such as Molisch's test, Salkowski test,

Keller-Killiani test, Flavonoid test, Dragendorff's test and test for tannins were performed according to Harbone following standard protocol [12].

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 5µl methanol extract of RD, CPDR, KPDR, and WPDR were 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.

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 chyrisin (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, mean±SD)

S. No.	Test	CPDR	KPDR	WPDR	RD
1	Loss on drying at 110 °C	14.5±1.6%	9±0.12%	12.6±0.23%	9.1±0.26%
2	Ash value(w/w)	7.5±0.42%	8.1±0.38%	8.6±0.29%	9.4±0.26%
3	Acid Insoluble ash	1.8±0.4%	2.1±0.3%	1.9±0.5%	2.2±0.4%
4	Water soluble extract	7.6±0.71%	4.3±0.52%	4.6±0.35%	4.1±0.32%
5	Methanol soluble extract	9.52±0.47%	2.32±0.63%	3.5±0.81%	3.36±0.70%
6	p. H	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 decompositionLoss 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 *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 *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 alcohol soluble extractive value shows no significant changes indicating the percentage of soluble polar and moderatly polar component like sugar,glycosides 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, 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	WPDR	KPDR	CPDR
Steroid	Salkowski reaction	+	+	+	+
Phenolic and Tannin	Lead acetate solution	+	+	+	+
Flavonoids	Shinoda Test	+	+	+	+
Protein	Biuret Test	-	-	-	-
Alkaloid	Dragendroff"s test	-	-	-	-
Glycoside	Keller-Killiani test	+	+	+	+
Sapponin	Foam Test	-	-	-	-
Amino Acid	Ninhydrine test	-	-	-	-
Carbohydrate	Molish's Test	+	+	+	+

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

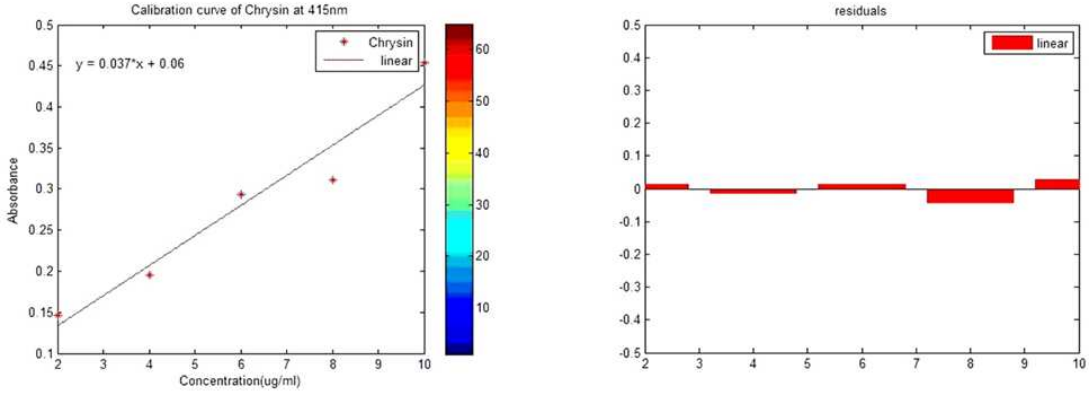

Fig. 1: Linear standard curve of chrysin (dihydroxy flavones) and it's residual

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, r^2 = 0.94.

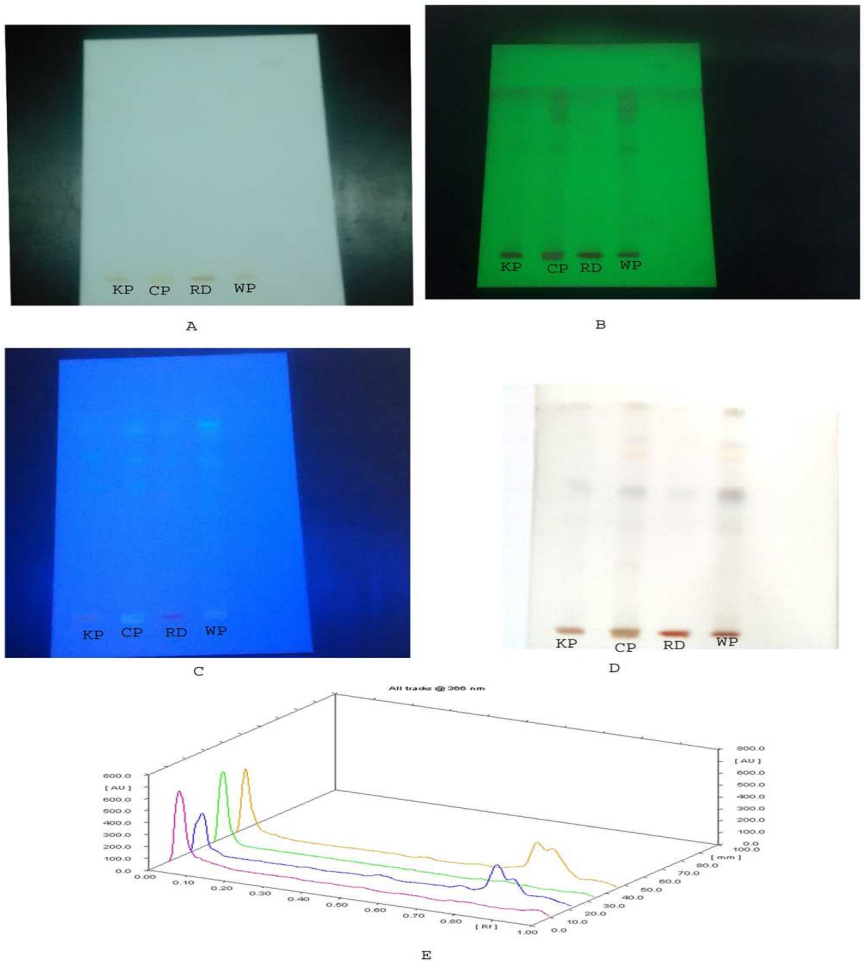

Fig. 2: Separation of methanol extract on HPTLC Si 60 F_{254} with Toluene: Chloroform: Acetone (4:2.5:3.5 V/V), chamber saturation, stained with the vanillin-sulfuric acid reagent. Tracks: 1. KPDR extract, 2. CPDR extract, 3. RD extract, 4. WPDR extract. A-Day light, B–Short UV (254 nm), C–Long UV (366 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, at366 nm respected samples six, seven, six and seven peaks are found in favor of target class of Moeity polyphenol and flavonoid.

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

Solvent system	Track No	Under UV light			
Toluene: Chloroform: Acetone 4:2.5:3.5 V/V		254 nm (Short UV)			
		Number of spots	Max Rf. Value	Max Height	Area in %
	Track 1 (KPDR)	09	0.02, 0.14, 0.34, 0.38, 0.49, 0.64, 0.73, 0.89, 0.96	693.1, 13.1, 31.4, 24.0, 14.6, 72.1, 12.8, 44.9, 186.5	63.27, 1.00, 3.60, 1.71, 1.71, 7.77, 0.92, 3.06, 16.95
	Track 2 (CPDR)	10	0.03, 0.32, 0.37, 0.43, 0.49, 0.62, 0.70, 0.80, 0.88, 0.93	334.9, 64.4, 32.9, 17.3, 34.0, 92.4, 20.5, 140.9, 87.9, 133.7	23.45, 8.22, 3.17, 1.57, 6.44, 12.44, 1.66, 13.00, 13.50, 16.57
	Track 3 (RD)	09	0.02, 0.32, 0.37, 0.52, 0.62, 0.70, 0.76, 0.87, 0.95	569.6, 31.1, 19.7, 14.2, 43.3, 16.5, 33.0, 51.1, 110.4	55.89, 4.18, 2.81, 1.41, 6.27, 0.85, 6.58, 5.85, 16.15
	Track 4 (WPDR)	10	0.02, 0.10, 0.32, 0.37, 0.48, 0.62, 0.70, 0.79, 0.84, 0.96	601.5, 12.2, 17.5, 23.2, 26.4, 151.7, 23.6, 151.1, 208.0, 195.8	32.56, 0.18, 1.09, 1.43, 2.56, 12.71, 1.08, 10.19, 20.25, 17.95

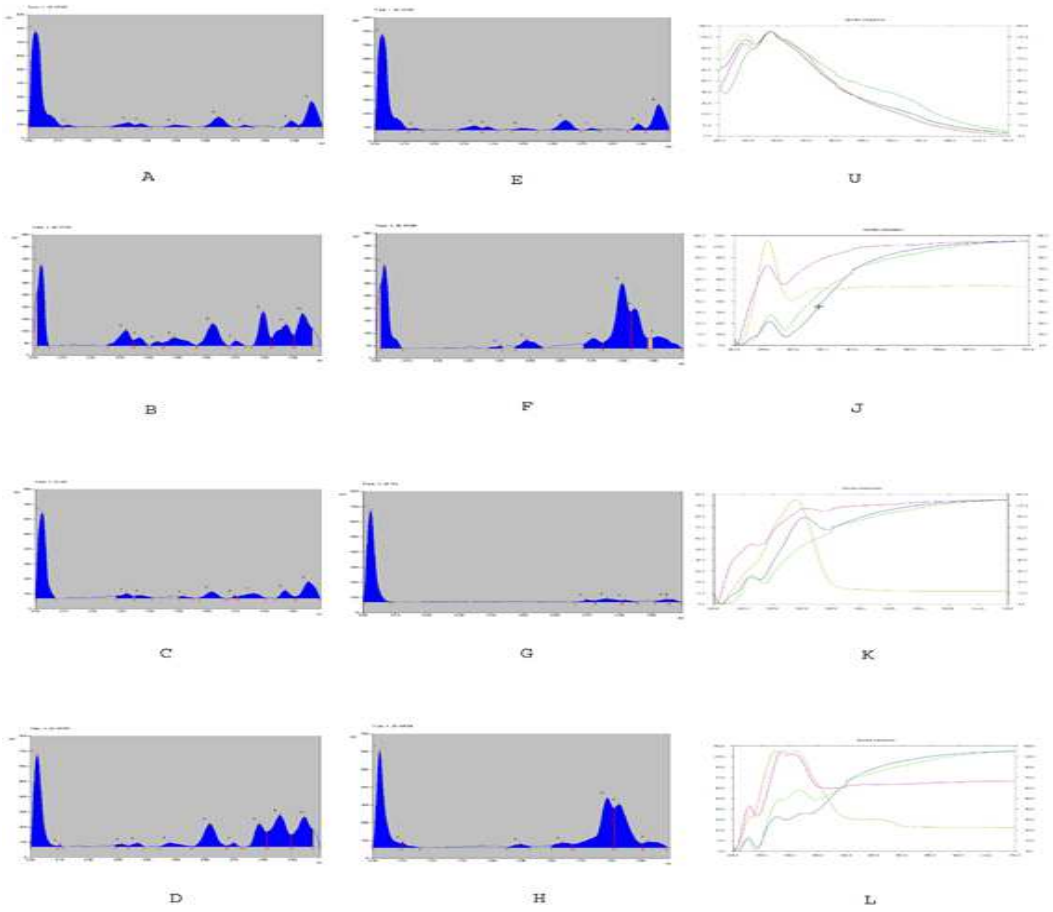

Fig. 3: HPTLC densitometry chromatogram (at 254 nm) of methanolic extracts of A-KPDR, B-CPDR, C-RD, D-WPDR andHPTLC densitometry chromatogram (at 366 nm) of methanolic extracts of E-KPDR,F-CPDR, G-RD, H-WPDR and spectral comparison of the respective Rf as U,J,K and L with Toluene: Chloroform: Acetone 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			
Toluene: Chloroform: Acetone 4:2.5:3.5 V/V		366 nm (Short UV)			
		Number of spots	Max Rf. Value	Max Height	Area in %
	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 3.93,2.02,6.99
	Track 2 (CPDR)	07	0.03,0.40,0.49 0.71,0.80,0.84,0.91	334.0,11.1,33.3, 39.8,259.6,160.0	27.63,0.82,5.09 5.48,35.15,17.15,8.68
	Track 3 (RD)	06	0.02,0.70,0.76, 0.82,0.95,0.97	47.6,602.0,17.1 23.2,17.2,17.3	83.98,2.55,6.65 1.98,2.68,2.17
	Track 4 (WPDR)	07	0.02,0.10,0.50 0.64,0.79,0.83,0.92	543.0,30.6,19.0 27.3,279.2,242.6,32.8	33.58,1.33,1.88 3.22,30.19,25.48,4.27

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.

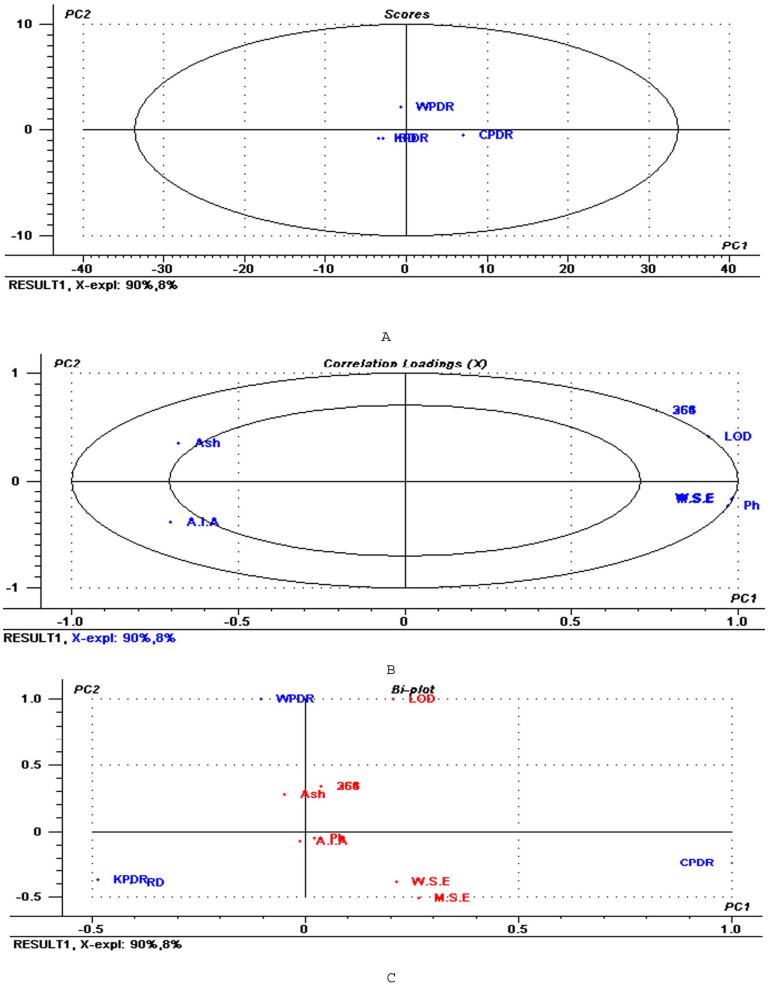

Fig. 4: (A) PCA score plot and (B) loading plot (C) Bi-plot of three samples based on its physicho-chemical and chromatographic separation behavior showing the distribution pattern of samples and various physicochemical parameters contributing to the groups respectively. The ellipse represents the Hotelling 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 p. H, ASE, 254 and 366 sensitive separated peaks were the physicochemical, chromate-graphical parameters contributing to the grouping of *Danti* samples, and that these attributes corresponded to the PC1 which explained about 98 % 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 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 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 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 imact 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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