International Journal
Medicine and Pharmaceutical Sciences
Vol.1, Issue 1 (2011) 11-27

PRELIMINARY STUDIES AND EVALUATION OF *DHATUPAUSHTIK CHURNA:* A TRADITIONAL AYURVEDIC FORMULATION

Varun Chaddha*, S. Nayak, Sarita Singh Solanki
Bansal College of Pharmacy, Bhopal.
*160, Chaman Ganj Sipri Bazaar, Jhansi 284003
E-mail ID: - nurav86@gmail.com
Mob n.o. 09450081791

ABSTRACT

Standardization of herbal formulation is essential in order to assess the quality of drugs, based on the concentration of their active principles. The present paper reports on standardization of *Dhatupaushtik Churna (DPC,)* an Ayurvedic formulation which is used as a nutritive tonic in general weakness. Two marketed products of *DPC* have been standardized on the basis of organoleptic characters and physico-chemical properties. The set parameters were found to be sufficient to evaluate the churna and can be used as reference standards for the quality control/quality assurance. In present communication Thin Layer Chromatography was developed for the standardization of *Dhatupaushtik Churna*. Spectroscopic studies were carried out to develop the spectrum of the formulations and validated by Overlain and Linearity study. We have developed a simple scheme for the standardization and authentication of *Dhatupaushtik Churna (DPC).*

KEYWORDS: Standardization; *Dhatupaushtik Churna (DPC)*; U.V.Spectroscopy; Ayurvedic Formulation.

INTRODUCTION

Standardization of drug means confirmation of its identity quality and determination of its purity. Standardization is an essential factor for polyherbal formulation in order to assess the quality of drugs based on the concentration of their active principle. It is very important to establish a system of standardization for every plant medicine in the market, since the scope of variation in different batches of medicine is enormous. Plant material when used in bulk quantity may vary in its chemical content and therefore, in its therapeutic effect according to different batches of collection. The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy. DPCB consist of Shatavari, Gokhru, Bijband, Vanslochan, Kababchini, Chopchini, Kaundbeej, Safed musli, Trikatu, Salam mishri, Baidarikhand, Ashwagandha, and Nishoth. DPCV consists of Shatavari. Gokhru, Vanslochan, Kababchini, Chopchini, Konch, Safed musli. *Dhatupaushtik Churna (DPC)* is a nutritive tonic in general weakness. Therefore an attempt has been made to standardize *Dhatupaushtik Churna (DPC)* on the basis of TLC, and U.V. Spectroscopic fingerprint profile.

MATERIALS AND METHODS

The chemicals used in the experiment were of analytical grade. The U.V. spectra were recorded on the Shimadzu-UVPharmaspac-1700 spectrophotometer. The TLC was performed on a silica Gel G plate. The marketed samples of brands of *Dhatupaushtik churna* i.e. Baidyanath (*DPCB*) and Vyas (*DPCV*) were standardized on the basis of organoleptic and physico-chemical properties. The sample *DPCB* and *DPCV* were purchased from the market of District Jhansi (U.P).

1. Organoleptic properties

Organoleptic evaluation refers to evaluation of formulation by color, odour, and taste, etc. All two batches of *DPC* were observed for color, odor, taste & appearance.

2. Physical Evaluation :

Physical investigations of formulations were carried out, including the determination of extractive values and ash values

A. Determination of Extractive values

This method determines the total solid content in a given amount of medicinal plant material when extracted with various solvents. For determining the extractive values, 5gm of each of *DPCB a*nd *DPCV* were extracted in methanol, chloroform, ether, n-hexane and distilled water separately as per the method given in I.P. and W.H.O guidelines. The results are depicted in table1.

B. Determination of Ash values

This determination measures the presence of silica especially sand and siliceous matter. Ashing involves an oxidation of the component present in product. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the crude drugs for marketing. The total ash usually consists of carbonates, phosphates, silicates and silica that include the physiological ash which is derived from the plant tissue itself and non physiological ash which is the residue of the adhering material to the plant material e.g. sand and soil. For determining the total ash values, 2gm of *DPCB & DPCV* were taken in a tarred china dish, then it was subjected to incinerate under muffle furnace at, temp 450°C. After cooling in a dessicator, the weight of white ash was taken to determine total ash value in % w/w. While determining the total ash at very high temperature (more than 600°C) may result in the conversion of carbonates to oxides. Acid insoluble ash is the part of total ash which is insoluble in acid. For determination of acid insoluble ash total ash was

boiled with 25ml of 2M hydrochloric acid for 5 minutes. The insoluble matter was collected on whattmann filter paper, washed with hot water, & ignited. Cooled in a dessicator & weight was measured. The percentage of acid insoluble ash was calculated in % w\w. Water soluble ash was determined by taking total ash in a silica crucible & boiled with 25ml of water. After that insoluble matter was collected on whattmann filter paper. The residue was ignited in crucible & cooled. Then the residue was weighed and water soluble ash was calculated on dry wt. basis by differentiates water insoluble ash from total ash. The results are depicted in table1.

C. Determination of Moisture Content

Moisture triggers the enzymatic activity or facilitates growth of microbes which leads to its deterioration. Moisture content of DPCB & DPCV was determined by subjecting the 2gm of *DPCB* and *DPCV* at 105°C. Cool in a dessicator & weight was measured till the constant weight was achieved. Results are depicted in Table 1.

Table 1. Physiochemical evaluation of DPCB & DPCV

Analytical Parameters	**Mean of *DPCB (n=3)***	**Mean of *DPCV (n=3)***
Extractive Values		
Water soluble	60.77 ± 0.067	35.17 ± 0.083
Methanol soluble	17.55 ± 0.133	32.19 ± 0.138
Chloroform soluble	6.31 ± 0.080	4.84 ±0.107
n-hexane soluble	11.18 ± 0.052	10.80 ±0.076
Ether soluble	6.78 ± 0.085	6.64 ± 0.044
Ash values		
Total	4.20±0.057	6.71±0.217

Water soluble	2.22±0.080	2.06±0.111
Acid in soluble	2.08±0.029	4.18±0.076
Moisture Content	5.15 ± 0.106	5.59± 0.119
Micromeretic parameters		
Bulk Density	0.517±0.007	0.528±0.003
Tapped Density	0.836±0.026	0.844±0.020
Compressibility index	37.936±1.878	37.196±1.912
Angle of repose	39.292±0.468	33.028± 0.231

D. Determination of Micromeretic Parameters

Physical characteristics like bulk density, tap density, and angle of repose were determined for different formulations. The term bulk density refers to packing of particles or granules. Angle of repose has been used as an indirect method for quantifying powder flow ability because of its relationship with interparticle cohesion. The physical characteristics of the formulations were determined in accordance the method given in a Pharmacopeia of India. Results are depicted in Table 1.

E. Phytochemical Evaluation

The methanol, chloroform, water, n-hexane, & ether extract of *DPCB & DPCV* were subjected to phytochemical test as per the method given in WHO Guidelines and Ayurvedic Pharmacopoeia. Results are depicted in Table 2.

Table 2. Phyto-constituents present in DPCV extracts

S.No.	***Phytoconstituents***	***DPCV***				
		Wt.	***Et.***	***$CHCL_3$***	***MeOH***	***Hex***
1.	*Alkaloids*	+	-	+	+	-
2.	*Glycosides*	+	+	-	+	+
3.	*Steroids*	+	-	-	+	+
4.	*Proteins*	+	+	-	+	-
5.	*Carbohydrates*	+	-	-	-	-
6.	*Saponins*	+	+	-	+	-
7.	*Tannins*	+	-	-	+	-
8.	*Flavanoids*	-	-	+	+	-

Wt. = water extract, Et. = ether extract, *$CHCL_3$*= chloroform extract, *MeOH*= methanol extract and Hex = hexane extract

Spectrophotometric Studies:

The chemical constituents present in the drug possess the characteristic features which can be characterized by using various spectrophotometric methods. *DPCB and DPCV* were extracted separately in distilled water, chloroform, methanol, ether & n-hexane for spectrum study. The Overlain spectrum & linearity studies were performed with only distilled water & methanol soluble extract of the formulations. This study was performed to find out the reproducible peaks in U.V. range 200-400nm (Overlain study) & content uniformity (linearity study) in finished products. All the extract from each batch of *DPC* were dissolved in water, methanol, chloroform, ether and n-hexane separately. Their U.V- spectrum were recorded at the range of 200-400nm.In each case the baseline were cleared against the solvent in which the particular solution of extract is prepared. The scanned spectrums were recorded & the peak of the maximum absorbance was noted. Results are depicted in Table 3.

Table 3 : Phyto-constituents present in DPCB

S.No.	***Phytoconstituents***	***DPCB***				
		Wt.	***Et.***	***$CHCL_3$***	***MeOH***	***Hex***
1.	*Alkaloids*	+	-	-	+	-
2.	*Glycosides*	+	+	+	-	+
3.	*Steroids*	-	+	+	+	+
4.	*Protein*	+	-	-	+	-
5.	*Carbohydrates*	+	-	-	+	+
6.	*Saponins*	+	+	-	+	-
7.	*Tannins*	+	-	-	+	-
8.	*Flavanoids*	+	-	+	+	-

Wt. = water extract, Et. = ether extract, *$CHCL_3$*= chloroform extract, *MeOH*= methanol extract and Hex = hexane extract

The overlain UV- spectrums were recorded only aqueous & alcohol soluble extract of *DPCB* & *DPCV* were recorded. The linearity studies were carried out with aqueous & alcoholic solutions only. The Alc. & aq. Stock solution of *DPCB and DPCV* were prepared by extracting 1.25 gm of each with 25ml of methanol & distilled water separately for 1 hr. Then solutions were centrifuged at 100rpm at temp 25°±1°C in cooling centrifuge. The prepared Alc. & aq. Stock solution of *DPCB & DPCV* were diluted in the range of 0.5- 3.0ml of stock solution upto 10ml with respective solvents. The linearity curve was prepared for all aqueous samples of *DPCB a*nd *DPCV* at wavelength 283.40nm & for all the alc. sample of *DPCB* and *DPCV* at 275.60nm respectively. The linear correlation between these concentrations (X- axes) & absorbance (Y- axes) were graphically presented & the slope (b), intercept (a) & correlation coefficient (r) were calculated out for linear equation by regression analysis by Microsoft Excel. Results are depicted in Table 4.

Table 4: Absorption maxima of DPC

S.No	*Batch n.o*	*Wt. extract*	*MeOH extract*	*$CHCL_3$ extract*	*Et. extract*	*Hex extract*
1.	*DPCB*	*283.40nm*	*275.00nm*	*265.40nm*	*364.60nm*	*259.00nm*
2.	*DPCV*	*282.40nm*	*275.60nm*	*292.20nm*	*321.60nm*	*321.60nm*

Wt. = water extract, Et. = ether extract, *$CHCL_3$*= chloroform extract, *MeOH*= methanol extract and Hex = hexane extract

Table 5: Linearity study of DPCB and DPCV

S.No	*Extracts*	*□ max*	*Volume of the solution*	*DPCB*	*DPCV*
1.	*Aq. Extact*	*283.40*	*0.5 ml*	*0.103*	*0.092*
			1 ml	*0.194*	*0.181*
			1.5 ml	*0.259*	*0.262*
			2 ml	0.340	0.343
			2.5 ml	0.415	0.475
			3ml	*0.484*	*0.568*
2.	*Alc. Extact*	*275.60*	*0.5 ml*	*0.115*	*0.070*
			1ml	*0.159*	*0.138*
			1.5 ml	*0.228*	*0.222*
			2 ml	0.319	0.291
			2.5 ml	0.370	0.334
			3 ml	*0.453*	*0.413*

Table 6 : Regression data for linearity Studies

S.No	*Extract*	*Regression*	
		DPCB	*DPCV*
1.	Aq. Extract	y=0.151x+0.034 Regression=0.998	y=0.191x-0.014 Regression= 0.993
2.	Alc. Extract	y=0.137x+0.032 Regression=0.992	y=0.135x+0.007 Regression= 0.994

F. Chromatographic studies

Chromatographic studies were performed TLC fingerprinting for *DPCB* and *DPCV* manufacturers. Rf values were calculated for both samples.

Thin layer chromatography: A 0.6gm of powdered churna was taken in an iodine flask. Than 12.5ml of distilled water is added and 6 hrs continuous shaked and kept for 24 hours. Water extract was filtered and kept for drying .The TLC plate was prepared by silica gel G. The plate was then get activated in hot air oven at 110□ c for 30 min. Solvent system was prepared from n-butanol: acetic acid: water (4:1:5). Then the spot was made on the plate then the plate was kept in the solvent system until it reaches to 75% of the plate. The plate was kept in iodine chamber and spot was examined. Calculation was done and Rf value was calculated.

RESULTS AND DISCUSSION

The present investigation was carried out with an aim to establish some evaluation parameter which would be helpful for standardizing Ayurvedic churna. By the organoleptic evaluation it was found that both churna *DPCB and DPCV* are similar in taste and color, both are sweet in taste and creamish in color but slightly different in odor like *DPCB* have characteristic odor evaluate sweet in taste, creamish in color, and characteristic odor while *DPCV* have acrid odor. This is may be because of great variation in composition of both the churna. The moisture content of *DPCB* and *DPCV* were found to be *5.15 ± 0.106 and 5.59± 0.119* respectively. Total ash value indicates the amount of minerals and earthy materials present in a raw material which was used to prepare a formulation content of *DPCB* and *DPCV* were *4.20±0.057* and 6.71±0.217 respectively. Results of acid insoluble and water soluble ash for *DPCB* were *2.08±0.029 and 2.22±0.080* and for *DPCV* were *4.18±0.076 and 2.06±0.111* respectively. The extractive values of *DPCB* and *DPCV* in water, methanol, chloroform, hexane and ether indicated the presence of almost polar and semi polar components in the formulation. The results of preliminary

phytochemical analysis of *DPCB* show the presence of alkaloids, glycosides, protein, carbohydrates, saponin, tannins and flavanoids in water extract, glycosides, saponin, and steroids in ether extract, glycosides, steroids, and flavanoids in chloroform extract, alkaloids, steroids, proteins, tannins, saponin, carbohydrates and flavanoids in methanol extract and steroids, glycosides and carbohydrates in n-hexane extract while in *DPCV* it shows the presence of alkaloids, glycosides, steroids, protein, carbohydrates, saponin and tannins in water extract, glycosides, proteins and saponins in ether extract, alkaloids, and flavanoids in chloroform extract, alkaloids, glycosides, steroids, proteins, tannins, saponin and flavanoids in methanol extract and glycosides, and steroids in n-hexane extract. The micromeretic results shows that the two samples have fair flow properties. The TLC was performed for fingerprinting of *DPCB* and DPCV. The results of TLC shows that the Rf value of *DPCB* is 0.83 (light yellow) and 0.86 (yellow) for *DPCV*. For fingerprinting of the *DPCB* and *DPCV* an attempt has been made with the help of spectroscopic studies because of its simplicity. The U.V-Spectrums of solutions form of *DPCB* and *DPCV* indicates prominent and identical peaks for aqueous and alcoholic extracts respectively. The peaks are sharper at lower concentration while diffuse as the concentration is increase. An overlain spectrum reflects the studied extract to have some component in same ratio.

The absorption maximum at designated wavelength can be utilized to evaluate consistency of the product and constituents. It may be utilized for product evaluation for adulteration where addition of excess of inorganic substance and substituted constituents, etc.

The prepared sample dilution for aqueous (0.5-3.0ml) and alcoholic (0.5- 3.0ml) solution of *DPCB* and *DPCV* shows linearity in the absorbance at 283.40nm and 275.60nm respectively. The absorption maxima at designated wavelengths can be utilized to evaluate consistency of the product and constituents. Linearity studies make feasible quantitative estimation of

components in *DPC* contributing for generating the maxima at designated wavelength. The regression data for linearity studies of *DPCB* water extract shows 0.998 and for *DPCB* alcohol extract shows 0.992 while of *DPCV* water extract shows 0.993 and for *DPCV* alcohol extract shows 0.994 respectively. Static analysis shows significant difference in data obtained among all batches.

CONCLUSION

The present investigation was carried out with an aim to establish some evaluation parameter which would be helpful for standardizing Ayurvedic churna. Ayurvedic medicine *Dhatupaushtik churna* has been standardized by intervention with modern scientific quality control measures and for the development of finger printing methods. Phytochemical tests and U.V analysis. These finding may be applicable for quality control and finger printing of different companies of same Ayurvedic products. So far since no attempt has been made to standardization and comparable studies of an Ayurvedic formulation of *Dhatupaushtik churna* of different manufacturer's products. This is the small step towards the development of quality control methods and finger printing from different companies. This will also helps to produce uniform standard product and will be helpful in detecting adulteration or substandard product in traditional Ayurvedic formulation.

The TLC analysis also showed as a comparable finger printing of two companies of a *DPCB* and *DPCV*. Hence these parameters and developed methods for their determination may be considered as a tool for quality control, and identification methods of traditional formulations. This will also assist the regulatory authorities, scientific organizations and manufacturers in identification their product with other company same product.

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FIGURES

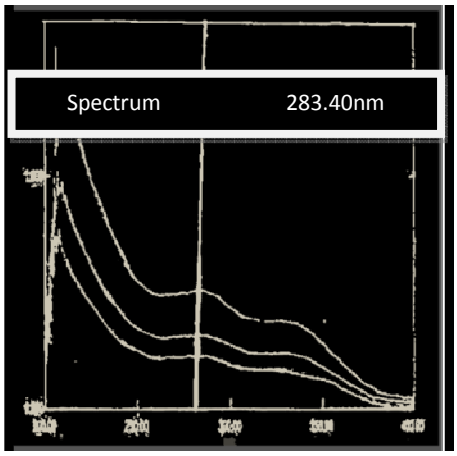

Figure 1. Linearity study spectra for aqueous extract of DPCB.

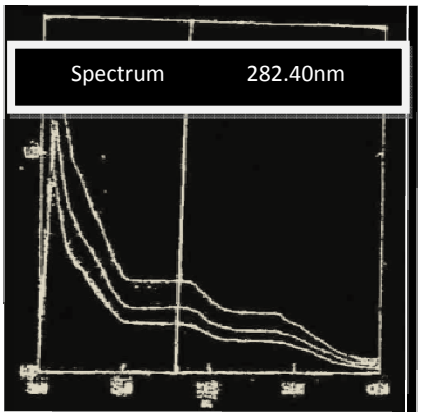

Figure 3 Linearity study spectra for aqueous extract of DPCV.

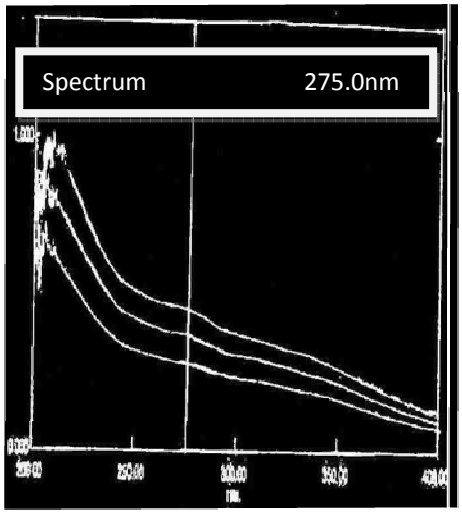

Figure 2 Linearity study spectra for alcoholic extract of DPCB

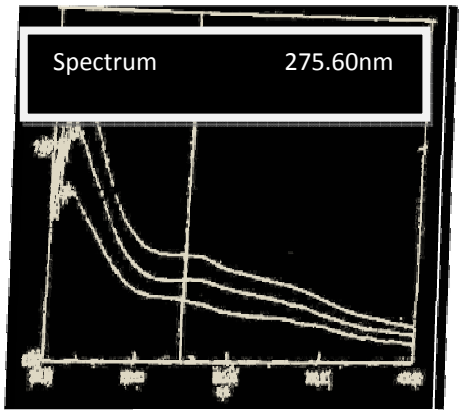

Figure 2. Linearity study spectra for alcoholic extract of DPCV.

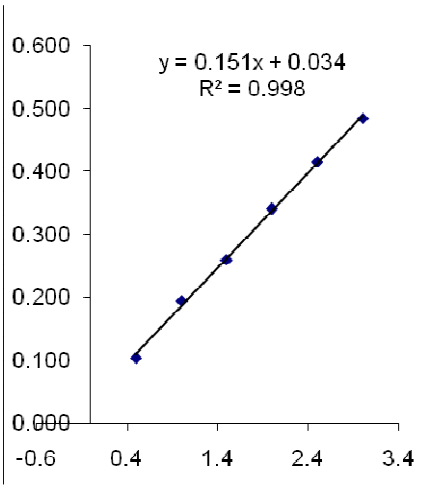

Figure 5 : Linearity of Aq. Extract of DPCB

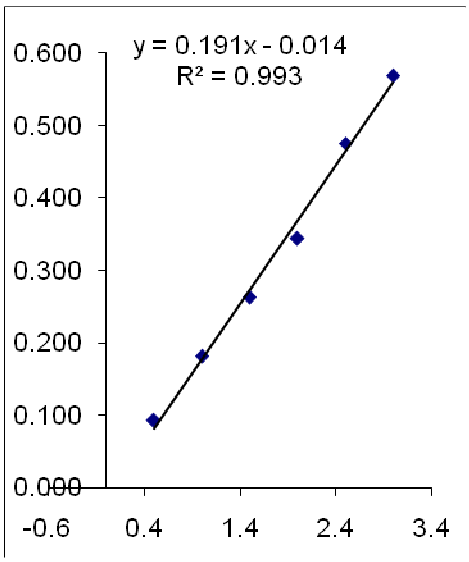

Figure 6 Linearity of Aq. Extract of DPCV

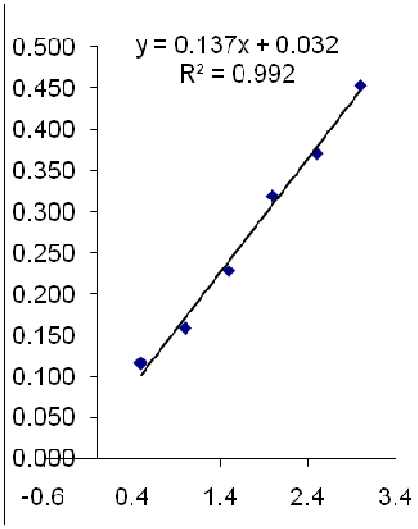

Figure 7 : Linearity of Alc. Extract of DPCB

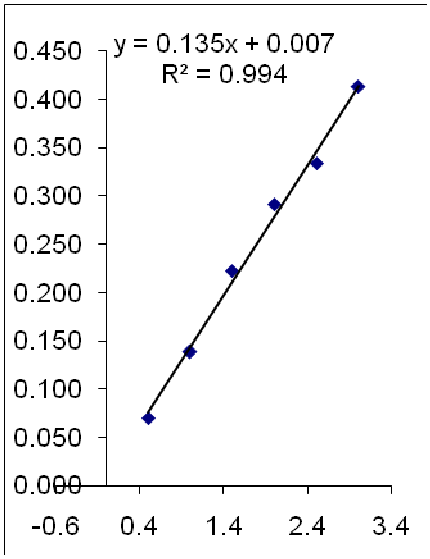

Figure 8 Linearity of Alc. Extract of DPCV