Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2011, 3(1): 276-286
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Standardization and Evaluation of Laxative Activity of a Poly Herbal Formulation

Priyabrata Pattanayak[1]*, Manaswini Behera[2], Prithwiraj Mohapatra[2], Sangram Keshri Panda[2]

[1]*Indira Gandhi Institute of Pharmaceutical Sciences, IRC Village, Bhubaneswar-751015*
[2]*Jeypore College of Pharmacy, Jeypore (K), Orissa, India-764002*

ABSTRACT

Standardization of herbal formulations is essential in order to asses the quality of drugs for therapeutic value. The World Health Organization (WHO) in 1999, has given a detail protocol for the standardization of herbal drugs comprising of a single content, but very little literature is available for the standardization of poly-herbal drugs. We have developed a simple scheme for the standardization and authentification of Panchasakar Churna comprising of four botanical ingredients. Three samples from different manufactures were procured and subjected to various physicochemical analyses and HPTLC fingerprinting along with in-house formulation. The set parameters were found to be sufficient to standardize the Panchasakar Churna and can be used as reference standards for the quality control/ quality assurance study. The laxative activity of the aqueous extract of Panchasakar Churna was also evaluated to justify the traditional claim.

Keywords: Standardization; Quality tests; Traditional medicine; Physicochemical parameters; Laxative activity.

INTRODUCTION

In the last few decades, there has been an exponential growth in the field of ayurvedic medicine (Indian Traditional System of Medicine) [1]. There are great need of standardization and quality control of ayurvedic formulations. Standardization and quality control depends upon the nature of crude drug and compound drugs, on it's source i.e. factors associated with raw materials which are beyond of human control like seasonal, geographical, age of the plant, time of collection, type of drying etc. due to these natural conditions the percentage of chemical constituents [2] of the drug does no remain uniform as our expectation. The need of quality control for ayurvedic drug is due to the fact that the preparation of drug according to the ancient method has been reduced due to the commercialization of ayurvedic pharmacy during past era

[3]. The absence of post-market surveillance and the paucity of test laboratory facilities also make the quality control of aurvedic medicines exceedingly difficult at this time.

Therefore, an attempt has been made to standardize Panchasakar Churna, an Ayurvedic compound formulation as prescribed in Ayurvedic Formulary, used as herbal laxative. The individual plant powders of the formulation were subjected to various pharmacognostical parameters. Three formulation, one in-house preparation and two samples from different manufactures were procured and subjected to various physicochemical analysis, TLC and HPTLC fingerprinting and botanical characterization using authenticate ingredients as controls. The present study also reports the laxative activity of Panchasakar Churna to justify the traditional claims of Panchasakar churna in the treatment of constipation.

MATERIALS AND METHODS

All the chemicals used in the experiment were of analytical grade. Menthol, Piperine, Thymol, Carvone and Ferulic acid were purchased from Sigma Aldrich, USA. All the solvents used in the experiment were procured from Merck Specialities Pvt. Ltd, Mumbai, India.

Instruments

Spotting device: Linomat IV automatic sample spotter; CAMAG (Muttenz, Swizerland)
Syringe: 100µL Hamilton (Bonadug, Swizerland)
TLC chamber: Glass twin trough chamber (20× 10× 4).
Densitometer: TLC scanner 3 with CATS software; CAMAG
HPTLC Plate: 20×10cm, 0.2 precoted with silica gel 60F_{254}; Merck
pH meter: Elico Ltd., Hyderabad, India.
Flame Photometer: Digital Biomed Flame Photometer, Hyderabad.
Muffle furnace: Dolphin Industries Ltd., Mumbai.

Plant mateials

Panchasakar Churna consists of Terminalia chebula (Combretaceae, dried fruit), Cassia angustifolia (Leguminosae, dried leaves), Zingiber officinale (Zinglberaceae ,dried rhizome), Anethum sowa (Apiaceae, dried seed), Rock salt (Saindhava lavana). All these ingredients were procured from the local market of Jeypore, Koraput, Odissa, India and all the plant material were authenticated by Mr. S.R. Dash H.O.D Dept of Botany Vikram Dev College Jeypore, Koraput Odisa. Voucher specimens (JCP/09/LAB-5/31) of the same have been deposited in the museum of Dept. of Pharmacognosy, Jeypore College of Pharmaceutical Sciences for future reference.

Preparation of Panchasakar Churna

In-house formulation of Panchasakar Churna was prepared as per Ayurvedic Formulary of India. All ingredients are taken and roasted in a stainless steel pan at a low temperature till it becomes free from moisture. The ingredients are powdered individually in a pulverizer and pass through 80# sieve. Each ingredients *Terminaila chebula* (2 parts), Cassia angustifolia (4 parts), Zingiber officinale (1 parts), Anethum sowa (1 parts) and Rock Salt (1 parts) were weight separately, mixed together to obtain a homogeneous blend.

Marketed samples

The marketed samples of various brands of Panchasakar Churna i.e. Zandu (Z), Baidyanath(B) and Dabur (D) and the in-house preparation(I) were standardized based on their oganoleptic charecters, physical characteristics and physicochemical properties.

Organoleptic Evaluation

Organoleptic evaluation refers to evaluation of formulation by color, odor, taste, texture etc. The organoleptic characters of the samples were carried out based on the method described by Siddique et. Al [4].

Table 1: Organoleptic properties of different Panchasakar Churna formulation

Different Formulation	Appearance	Color	Taste	Odor
In-House(I)	Powder	Light brown	Astringent/ Pungent/ Salty	Characteristic
Shrinivas(S)	Powder	Brown	Pungent/ Salty	Characteristic
Dindayal(D)	powder	Creamish brown	Pungent/ Salty	Characteristic

Microscopic Study

Individual microscopic analysis of each ingredient of the formulation along with in-house formulation (I) and the marketed formulations were carried out to by classical pharmacognostical methods [5]. The authenticity of the individual ingredients was confirmed by comparison of their power characteristics with those given in the literature.

Physicochemical Investigation

Determination of total ash

Total ash [6] determination constitutes detecting the physiological ash (ash derived from plant tissue) and nonphysiological ash (ash from extrageneous matter, especially sand and soil adhering to the surface of the drug). For its detection, 2g of powdered material of each formulation and the individual ingredients of the powers were placed separately in a suitable tared crucible of silica previously ignited and weighed. The powdered drugs were spread into an even layer and weighed accurately. The materials were incinerated by gradually increasing the heat, not exceeding 450°C until free from carbon, cooled in a desiccator, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible & that of crucible with total ash.

Acid insoluble ash

The ash obtained as above was boiled for 5min with 25ml of dilute hydrochloric acid; the insoluble matter was collected on an ashless filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air-dried drug was calculated.

Water soluble ash

The ash was boiled for 5 minutes with 25 ml of water; collected insoluble matter in an ash less filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding

450^0C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash with reference to the air-dried drug was calculated.

Table 2: Quality tests for different Panchasakar Churna formulation

Samples	Extractive values (%)		Ash values (%)		
	Alcohol soluble	Water soluble	Total ash	Acid insoluble	Water soluble
Terminalia chebula	*38.7± 0.96	18.9±0.09	3.9±0.34	3.8±0.33	8±0.56
Zingiber officinale	3.63±0.01	15.80± 0.96	4.9±0.05	0.9±0.05	6.6±0.71
Cassia angustifolia	4.9±0.02	27.45± 0.28	11±1.42	1.2±0.2773	12±0.43
Anethum sowa	5.39±0.11	16.40± 0.98	10±1.33	0.8±0.2	9.2±0.06
Dabur	27.45± 0.28	62.45± 0.82	20±0.05	24.1±0.41	20±0.38
Baidyanath	28.32±0.29	65.03± 0.9	18.9±0.09	22.9±0.08	20.2±0.72
Zandu	23.35± 1.71	59.09± 0.92	21.2±0.07	20.4±0.51	25±0.82
In house formulation	30.35± 1.31	60.56± 0.69	17.1±0.05	20.9±0.05	19.8±0.52

Determination of solvent Extractive values

***Alcohol soluble extractive value* [6]**

5g of coarsely powdered air-dried drug was macerated with 100ml of alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was then filtered rapidly; taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish at 105°C to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug and is represented as % value.

Water soluble extractive value

5g of coarsely powdered air-dried drug was macerated with 100ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish at 105°C to constant weight and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug and is represented as % value.

Loss on drying

Loss on dying is the loss of mass expressed as percent w/w. About 10g of dug samples of each formulation was accurately weighed in a dried and tared flat weighing bottle and dried at 105^0C for 5hrs. Percentage was calculated with reference to initial weight.

Determination of pH

The pH of different formulations in 1% w/V and 10% w/V of water soluble portions was determined using standard glass electrode at 24^0C according to the prescribed standard method in Indian Pharmacopoeia.

Table 3: pH and loss on drying of Panchasakar Churna formulations

Sample	Loss on drying at 105° (%)	pH	
		10% w/v solution	1% w/v solution
I	1.642±0.0229	3.3	3.3
D	2.88±0.00145	3.8	3.6
Z	2.02±0.0169	3.8	3.7
B	3.33±0.01331	3.9	3.9

Fluorescence analysis

One mg of powdered drugs of each formulation were exposed to ultraviolet light at wavelength of 254nm and 366nm and in daylight [5] while wet after being treated with different reagents.

***Determination of physical Characteristics* [7,8]**

Bulk density and Tap density

The term bulk density refers to a measure used to describe a packing of particles or granules. The equation for determining bulk density (D_b) is:

$$D_b = M/V_b$$

Where M is the mass of the particles and V_b is the total volume of the packing. The volume of the packing can be determined in an apparatus consisting of a graduated cylinder mounted on a mechanical tapping device (Jolting Volumeter) that has a specially cut rotating can. 100gm of weighed formulation powder was taken and carefully added to the cylinder with the aid of a funnel. Typically the initial volume was noted and the sample was then tapped until no further reduction in volume was noted. The initial volume gave the Bulk density value and after tapping the volume reduced, giving the value of tapped density.

Angle of repose

Angle of Repose has been used as an indirect method of quantifying powder flowability because of its relationship with interparticle cohesion. As a general guide, powders with angle of repose greater than 50 degree have unsatisfactory flow properties, whereas minimal angle close to 25 degrees correspond to very good flow properties. The fixed funnel and the free standing cone method employs a funnel that is secured with its tip at a given height, which was taken 2.5 cm (H), above the graph paper that is place on flat horizontal surface. Powder or granulation was carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel.

$$\text{Tan } \alpha = H/R \text{ or } \alpha = \text{arc tan } H/R$$

Where α is the angle of repose, R being the radius of the conical pile.

Hausner ratio

It is related to interparticle friction and as such can be used to predict the powder flow properties. Powders with low interparticle friction such as coarse spheres have a ratio of approximately 1.2, whereas more cohesive, less flowable powders such as flakes have a Hausner ratio greater than 1.6. The equation for measuring the Hausner ratio is: Df / Do, where Df = Tapped density and Do = Bulk density.

Table 4: Powder fluorescence test of different Panchasakar Churna formulations

Material	I			S			D		
	Day light	UV 254 nm	UV 366 nm	Day light	UV 254 nm	UV 366 nm	Day light	UV 254 nm	UV 366 nm
Powder as such	LY	BR	FY	PY	BR	PY	P.Y	BR	PY
In NaOH(1N) in H_2O	Y	GY	Y	Y	GY	Y	O	LY	Y
P + In HCl (1N)	YB	GY	G	YB	Y	GB	PY	GY	G
P + In NaOH (1N) in MeOH	YG	BR	PG	YG	GY	PG	YG	GY	PG
P + 50% KOH	YB	GY	FB	Y	GY	FB	YB	Y	FB
P + 50% H_2SO_4	YB	Y	LG	LB	GY	BG	PY	Y	G
P + 50% HNO_3	ReB	Y	FB	ReB	GY	FB	ReY	GY	FB
P + Conc. HNO_3	ReB	Y	FB	Y	GY	BR	Re	GY	FB
P+ Conc. H_2SO_4	YB.	GY	G	YB	GY	FB	YB	GY	LG
P+Iodine in H_2O	BL	BR	FB	BL	BR.	FB	BL	BL.	BL

BL: Black, BR: brown, PY: Pale yellow, Y: yellow, G: Green, LG: Light green, LY: Light yellow, GY: Greyish yellow, FY: Fluorescencent yellow. F.B.: Fluorescencent blue, , Re: red, GB:Greenish brown, LB:Light brown, , YB:Yellowish brown, BG:Brownish green, ReB:Reddish brown, ReY:Reddish yellow, YG: Yellowish green, PG:Pale green. P: Powder.

Carr's index

Another indirect method of measuring the powder flow from bulk density is Carr's index. The equation for measuring Carr's index is: % compressibility = (Df-Do/Do) ×100 where Df = Tapped density and Do = Bulk density

Estimation of sodium contents

Sodium content [9, 10] was estimated by flame photometry by using a flame photometer. A stock solution of NaCl 100µg/ml was prepared in distilled water and further dilutions were made to get 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml respectively for preparing the standard graph. Sodium contents of the formulations were estimated by flame photometric method based on the measurement of emission intensity. The method was validated for linearity, precision and accuracy. The method obeyed Beer's law in the concentration range 1-10 µg/ml. 10 g of the powdered sample was shaken with 100 ml water in a mechanical shaker for 20 min, filtered and used for determination of the unknown concentrations in the different samples.

HPTLC finger printing profile

HPTLC study [11, 12] of methanolic extracts of the individual ingredients, in-house formulation and marketed formulations were carried out along with the different marker compounds corresponding to the active ingredients to ensure the presence of active ingredients in all the

formulations. For HPTLC, 2gm of each sample (Formulation-Z, B and D) and the in-house formulation(I) were extracted with 25ml of methanol on boiling water bath for 25minutes consecutively three times using fresh potion of 25ml methanol, filtered and concentrated. The chromatograph was performed by spotting standards and extracted samples on pre coated silica gel aluminium plate 60F-254 (10cm×10cm with 250µm thickness) using Camag Linomat IV sample applicator and 100µl Hamilton syringe. The samples, in the form of bands of length 5mm, were spotted 15mm from the bottom, 10mm apart, at a constant application rate of 15nl/s using nitrogen aspirator. Plates were developed using mobile phase consisting of toluene-ethyl acetate. Subsequent to the development, TLC plates were dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance/reflectance mode. The HPTLC finger print profiles of the formulations are presented in Fig 3a-d.

Table 5: Physical characteristic and Sodium contents (%) of different Panchasakar Churna formulations

Parameters	In House formulation	Baidyanath	Dabur	*Zandu*
Tap density	0.45 ± 0.77	0.43 ± 0.24	0.58± 0.17	0.35± 0.01
Bulk density	0.59± 0.02	0.456	0.52±0.001	0.503
Angle of repose	34.44 ± 0.67	32.35± 0.53	32.97 ± 0.42	32.88 ± 0.37
Hausner ratio	1.74 ± 0.04	1.69 ± 0.01	1.82±0.02	1.78 ± 0.01
Sodium contents (%)	3.1	4.8	4	4.1

Animals

Healthy Wistar adult male and female albino rats between 2 and 3 months of age and weighing about 150-200g were used for the study. Housed individually, in polypropylene cages, maintained under standard conditions (12-hr light: 12 -hr dark cycle; 25±3°C; 35-60% humidity), the animals were fed with standard rat pellet diet (Hindustan Lever Ltd., Bombay, India). The study was conducted after obtaining institutional animal ethical committee clearance (Regd no.-HP1/07/60/IAEC/0013 of date 07-05-2007).

Table 6: Laxative activity of aqueous extracts of panchasakar churna formulations

Treatment	Dose mg/kg	Faecal output(g) 8 hour	Faecal output(g) 8-16 hour
Control	-	0.086 ± 0.05	0.06 ± 0.01
Agar-Agar	300	1.24 ± 0.97*	0.46 ± 0.06
In-house formulation(I)	100	1.18±0.64**	0.44±0.34*
	300	1.83 ± 0.05**	0.61 ± 0.22

** p<0.01 and ** p<0.001 compared with vehicle-treated control group. Results are expressed as mean ±SEM.*

Evaluation of Laxative activity

The test was performed according to method of Bose et al [13] on rats of either sex, fasted for 12 h before the experiment, but with water provided ad libitum. The animals were divided into four groups of six in each. The first group of animal is served as control, received vehicle (1%

Tween-80 in normal saline, 25ml/kg), the second group serving as references, received reference standard agar-agar (300 mg/kg, p.o.), while third and fouth groups received aqueous extracts of Panchasakar churna (In-house formulation) at a dose of 100 and 300 mg/kg. Immediately after dosing, the animals were separately placed in cages suitable for collection of faces. After 8 h of drug administration, the faces were collected and weighed. Thereafter, food and water were given to all rats and faecal outputs were again weighed after a period of 16h.

Statistical analysis

The data obtained in the laxative studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's t-test. A p-value less than <0.05 were considered to be significant. All the values were expressed as Mean ± SEM.

RESULT AND DISCUSSION

In house formulation was prepared in accordance with the Ayurvedic Fomulay of India. As part of standardization procedure, the finished product Panchasakar Churna was tested for relevant physical and chemical parameters along with samples from three different manufacturers, Z, B and D for a comparative study.

All the samples were brown in color except formulation D which is green in color. The powders were smooth, having characteristic odor, possessing pungent/salty taste. The organoleptic properties of the marketed formulations and the in-house formulations were reported in table 1.

Microscopic examination was carried out for individual ingredients present in the formulation along with different Panchasakar Churna to see the presence of *Terminalia chebula, Cassia angustifolia, Anethum sowa,* and *Zingiber officinale* in the different formulations of churna. In the in-house formulation (I) the epidermal cells with paracytic stomata and glandular stomata indicated the presence of *Cassia angustifolia*, Criss cross fibers and fibers with peg like out growth indicated the presence of *Terminalia chebula* , parenchyma cells with adherent oleoresin indicated the presence of *Zingiber oficinale* , endosperm cells with micro rosette crystals of calcium oxalate and oil globules and sclerides of the wing indicate presence of *Anethum sowa*.

Quality tests for different Panchasakar Churna and its individual ingredients were performed for moisture content, ash content, water soluble extractive, methanol soluble extractive, acid insoluble ash and water insoluble ash, and were found to be within standard ranges. The extractive values and ash values of individual ingredients of churna, in-house formulation and different marketed formulations are given in table 2. The results are expressed as mean (n=6) ± Standard deviation (SD).Variations were observed in most of the physicochemical parameters studied. The total ash value of formulation D was found to be higher than that for I, B and Z. Acid insoluble ash value for in house formulation (I) was found to be 20.9±0.05 and in case of marketed formulation D, B and Z this was found to be 24.1±0.41, 22.9±0.08 and 20.4±0.51 respectively. On the contrary, water soluble ash percentage of I, D and B were comparable except Z which was comparatively high.

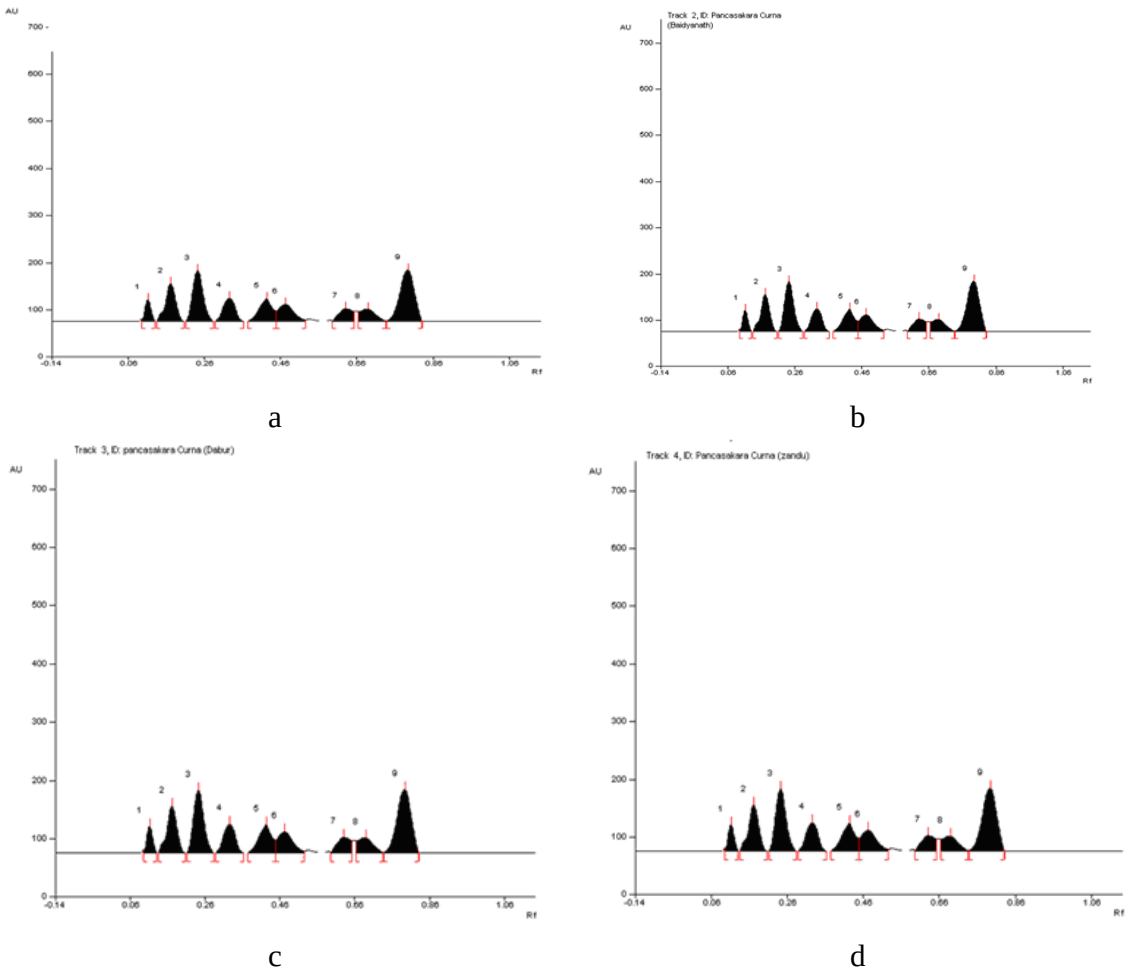

Figure 1: HPTLC fingerprinting of: (a) Fomulation I; (b) Fomulation B: (c) Fomulation D; (d) Fomulation Z

The extractive values of formulations in water were found to be much higher than alcohol extractive values. However value of I (30.35± 1.31) in alcohol is more when compared to D (27.45± 0.28), B (28.32±0.29) and Z (23.35± 1.71). The extractive values in water was found to be different for I,S and D. These variations may be due to variation in the quality of raw materials used, their season of collection and storage time. Loss on drying at (105^0C) and pH of 1% w/v and 10% w/v aqueous solution are also presented in Table 3. pH of 1% and 10%w/v solution revealed that the formulations are acidic. In fluorescence analysis the power samples were exposed to ultraviolet light at wavelength of 254nm and 366nm and to day light after being treated with different reagents as reported in table 4. Fluorescence analysis results shows whether any fluorescent ingredients are present or not, here we have found there was no such material found in any of formulation and individual ingredients either.

The physical characteristics of the in house formulation (I) and two market formulations (average value along with standard deviation) are shown in Table 5. The results of the market formulations and in house formulation were found to be comparable. The flowability of the formulation was found to be poor in both market formulation and in house formulation, which

was further confirmed by high values of Hausner ratio and Carr's index. For the estimation of sodium by flame photometer the emission intensity of different concentrations are presented in table 5. The sodium content was found to be less in in-house formulation (3.1%) and highest in Baidyanath formulation (4.8%).

HPTLC fingerprint profile of the Panchasakar Churna formulations are depicted in figure 1a-d indicates the presence of all the ingredients in proportional quantity in the formulations. This confirms the brand-to-brand consistency of the finished products.

Results of the evaluation of laxative activity in Table 6 revealed that the aqueous extract produced significant activity (1.83 ± 0.05 and 0.61 ± 0.22) at the tested dose level (300 mg/kg, p.o.) compared to the standard (1.24 ± 0.97 and 0.46 ± 0.06). Even at a dose of 100mg/kg the aqueous extract of the formulation shows comparable activity (1.18±0.44 and 0.84±0.34) compared to standard (1.24 ± 0.97 and 0.46 ± 0.06).

Ayurvedic medicine Panchasakar Churna has been standardized by intervention of modern scientific quality control measures described in classical texts. Hence, the physicochemical parameters, quantitative analysis, HPTLC fingerprint profiles and the microscopic characteristics together may be used for quality evaluation and the standardization of compound formulations. The present study also justifies the traditional claims of Panchasakar churna in the treatment of constipation. The exact mechanism of laxative activity exhibited by the extracts can only be established after further investigation.

Acknowledgements
Authors gratefully acknowledge the valuable inputs provided by Dr Prithwiraj Mohapatra during the course of preparation of the manuscript.

REFERENCES

[1]. D. Govind, Bhaishajya Ratnavali, Motilal Banarasidas publishers, New Delhi, **2002**, pp. 461.
[2]. L. V. Asokar, K. K. Kakkar, O. J. Chakra, Glossary of Indian medicinal plants with active pinciples, Publication and Information Diectorate, New Delhi, **1992**.
[3]. Anonymous , The Ayurvedic Formulary of India, Govt. of India, Ministry of Health and Family Welfare, New Delhi, **1976**.
[4]. A. Siddiqui, M. A. Hakim, Format for the pharmacopoeial analytical standards of compound formulation, wokshop on standardization of unani drugs, (appendix), Central council for research in unani medicine, New Delhi, **1995.**
[5]. Anonymous, Pharmacopeial standards for Aurvedic formulations, Central council for research in Ayurveda and Siddha, Govt. of India, Ministry of Health and Family Welfare, New Delhi, 1987.
[6]. P. K. Mukerjee, Quality control of herbal drugs, Business horizons pharmaceutical publisher, New Delhi, **2002**.
[7]. L. Lachman, H. A. Liberman, J. L. Kanig, The theory and practice of industrial Pharmacy, 3rd edn, Varghese publishing house, Bombay, **1987**.
[8]. M. E. Aulton, Phamaceutics, The science of dosage forms design, 2nd edn, Churchill Livingstone, New Delhi, **2002**.

[9]. A. D. Skoog, D. M. West, F. J. Holler, Fundamentals of analytical chemistry, 17th edn, Saunders College publishing, New york, **1991**.
[10]. J. Mendham, R. C. Denney, J. D. Barnes, M. Thomas, Vogel's text book of quantitative chemical analysis, 6th edn., Pearson Education Pvt. Ltd. Singapur, **2002**.
[11]. Anonymous, Quality standards of Indian medicinal plants, vol-1, Indian council of medical research, New Delhi, **2003**.
[12]. Sante, Organization Mondiale De La., Quality control methods for medicinal plant mateials, World Health Organization, **1992**, 559, rev. 1.
[13]. A. Bose, S. Mondal, J. K. Gupta, G. K. Dash, T. Ghosh, S. Si (). *Phcognosy Magazine*, **2006**, 2, 178-182.