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STANDARDISATION OF QUALITY PARAMETER AND QUANTIFICATION OF 6-SHOGOAL IN *CHATURBHADRA KVATHA CHURNA* - A POLYHERBAL AYURVEDIC FORMULATION

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ABSTRACT

Ayurvedic 'finished' medicines belong to diverse classes or categories e.g. *churna, kvatha churna, gulika* (pills), *kashayam, lehyam, gulam, tailam, ghritam, lepa, kuzhambu, asavam, arishtam* (besides *bhasma, sindoora* containing minerals and metals) etc. Standardized ayurvedic formulations of uniform quality are essential for beneficial therapeutic use. As the global market for herbal medicinal product is increasing tremendously, need for quality control parameters which are accepted globally, is being felt. Therefore an attempt has been made in developing standardization parameters covering physico-chemical parameters and TLC/HPTLC/GC profile for the ayurvedic compound formulation *Chaturbhadra kvatha churna.* Three batches of churnas were prepared; physico chemical parameters and extractions were carried out. The HPTLC analysis was performed on pre-coated silica gel plate 60_F-254 plate using n-butanol: acetic acid: water (5: 2: 2) as mobile phase in a CAMAG chamber. Camag TLC Scanner 3 was used for the densitometric scanning at 550nm. Specific marker compounds were used for the quantification. GC was carried out in Agilent GC equipped with HP-5 (5 percent phenyl methyl siloxane) capillary column (30 m x 320 µm x 0.25 µm) with gradient temperature programme. Tests for aflatoxins G1, G2, B1 and B2 were done according to standard procedure. The development of pharmacopoeial standards of *Chaturbhadra kvatha churna* was based on the outcome of physicochemical, TLC, HPTLC, GC finger print profiles and aflatoxin tests. As a quantitative marker for the *churna*, 6-Shogaol and 8-gingerol were tried in the *chloroform* extract (non-sequential) of the *churna*,

using mobile phase *hexane*: *acetone* (7:3). Amount of 6-Shogaol present in the churna samples was quantified and it was found to be 0.007, 0.008, 0.0075% with respect to *churna*. Spectrum of 6-Shogaol and corresponding spot in *churna* samples were compared. HPTLC profiles of three different batches of CBK *churna* were compared. The results were found to be highly accurate, quick and reliable for routine monitoring in compound preparations. With the growing demand of herbal drugs market, it is suggested that this standardization tool will help in maintaining the quality and batch to batch consistency of many ayurvedic raw drug powder based preparations.

KEY WORDS: Polyherbal ayurvedic formulation, chaturbhadra kvatha churna, HPTLC, GC, 6-shogoal.

INTRODUCTION

Recent resurgence of worldwide interest in traditional systems of medicine has focused attention on Ayurveda, the ancient Indian indigenous system of medicine, dating back to the vedic age. It is one of the officially recognized and popularly accepted systems of medicines in India. Ayurvedic 'finished' medicines belong to diverse classes or categories e.g. *churna, kvatha churna, gulika* (pills), *kashayam, lehyam, gulam, tailam, ghritam, lepa, kuzhambu, asavam, arishtam* (besides *bhasma, sindoora* containing minerals and metals) etc. Standardized ayurvedic formulations of uniform quality are essential for beneficial therapeutic use. There is a lack of data regarding the parameters and testing methods to be employed for assessing the quality of traditional systems of medicines. As the global market for herbal medicinal product is increasing tremendously, need for quality control parameters which are accepted globally, is being felt. Therefore an attempt has been made in developing standardization parameters covering physico-chemical parameters and TLC/HPTLC/GC profile for the ayurvedic compound formulation *Chaturbhadra kvatha churna*. *Kvatha churna* is a coarse powder of a drug or drugs which is prepared by mixing clean, coarsely powdered and sieved raw ingredient drugs. *Chaturbhadra kvatha churna* is a polyherbal ayurvedic *kvatha churna* [1] with four ingredients *Tinospora cordifolia* (Willd.) Miers, *Aconitum heterophyllum* Wall., *Zingiber officinale* Rosc. and *Cyperus rotundus* L. It is prescribed for *agnimandya* (digestive impairment), *amagrahani* (sprue associated with indigestion) and *ajirna* (dyspepsia).

MATERIALS AND METHODS

For TLC, HPTLC aluminium plates precoated with silica gel GF_{254} 0.2mm (E.Merck), Camag Linomat IV applicator, automatic developing chamber with humidity controller ADC-2, TLC Densitometric scanner 3 and TLC visualizer for photodocumentation were used. GC was carried out in Agilent GC equipped with HP-5 (5 percent phenyl methyl siloxane) capillary column (30 m x 320 µm x 0.25 µm) with gradient temperature programme.

Thoroughly cleaned and dried (40^0 C for 1 hr) ingredients were separately powdered and sieved (mesh 44 size) and specified quantities (Table-1) were mixed to get a homogeneous blend. This mixture was again passed through 22 mesh and dried at 40^0C for half an hour to get the finished product. Three batches of the *churna* were prepared.

Table 1: **Formulation Composition**

Sanskrit name	Botanical name	Part used	Quantity
Guduci	Tinospora cordifolia (Willd.) Miers	Stem	1 part
Ativisa	*Aconitum heterophyllum* Wall.	Root	1 part
Sunti	*Zingiber officinale* Rosc.	Rhizome	1 part
Musta	*Cyperus rotundus* L.	Rhizome	1 part

Physicochemical analysis; loss on drying at 110^0 C, total ash, acid insoluble ash, sulphated ash, alcohol and water soluble extractive percentage, pH of filtrate of 10 percent w/v aqueous solution and total phenolics were done according to the standard methods [2,3] for all the samples. Volatile oil isolation was done by hydro distillation of *churna* (100g) for 4hrs and its percentage (v/w) is noted. It was dissolved in petroleum ether and made up to 10ml in volumetric flask. The volatile oil of *Z. officinale* and *C. rotundus* were prepared according to the procedure. *Churna* (10 g) extracted with *hexane* (50 ml x 3) by refluxing on a water bath for 30 min. It was filtered and extract collected and pooled. The marc dried from traces of *hexane* and extracted with *chloroform* (50 ml x 3) followed by *methanol* (50 ml x 3). After each solvent extraction the weight percentage of the extractives was recorded. All the three extractives were dissolved in respective chromatography grade solvents and made up to 10ml in volumetric flask. As reference standards 4 ingredient standards 5g each were extracted with *methanol* (25 ml x 3), solvent evaporated and the residue dissolved in chromatography grade *methanol* and made up to 10ml in volumetric flask. For quantification 10g of *churna* soxhleted with *chloroform* for 3hrs and concentrated to 10ml in a volumetric flask. These solutions were used for TLC, HPTLC and GC analysis.

The mobile phase used was *hexane* : *ethyl acetate* : *methanol* (10 : 2 : 0.2) for *hexane* extract, *toluene* : *chloroform* : *ethanol* (5 : 5 : 2) for *chloroform* extract, *ethyl acetate* : *methanol* : *acetic acid* : *water* (6 : 2 : 1 : 1) for *methanol* extract and *hexane* : *ethyl acetate* (10: 0.5) for volatile oil. The plates were dried and visualized under UV 254 and 366 nm and under visible light after derivatization with *anisaldehyde - sulphuric acid* reagent [4]. GC analysis was done in temperature programme 60^0 to 250^0 at 5^0 rise per min, injector and detector temperatures were 230^0C, nitrogen was used as carrier gas at flow 1ml per minute. For quantification of *6-Shogaol* in *churna* samples a stock solution was prepared by dissolving 5 mg of compound in *chloroform* and volume was made up to 10ml in a volumetric flask. From this solution 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 µl spots were applied corresponding to 0.25, 0.50, 0.75, 1.0, 1.25, 1.5 µg per spot and a calibration curve prepared by densitometric scan at 254 nm. HPTLC profiles were carried out to find out the consistency of product of three different batches. Tests for aflatoxins G1, G2, B1 and B2 were done according to standard procedure [2].

RESULT AND DISCUSSION

The samples were found to be sandy brown coloured, moderately fine powdered, with reminiscent smell of ginger and pungent taste. The powder completely passes through sieve number 22 and not less than 50 percent through sieve number 44. The ash and sulphated ash contents shows the amount of inorganic matter present in the sample and the acid insoluble ash almost within 1% which expresses low siliceous matter present in the sample. The value of loss on drying at 110^0 shows the moisture content along with the volatile matter present in the sample. The alcohol, water soluble extractive percentages and total phenolics content were comparatively same in all the samples (Table 2). *Hexane, chloroform* and *methanol* extractive values indicate less, medium and high polar group of chemical entities (Table 3).

TLC of *hexane* extract of *churna* (5□l) along with 10µl of *methanol* extract of individual drugs in UV 366 nm (Fig.1) shows corresponding bluish fluorescent spot at R_f 0.16, 0.28, 0.33, 0.46 with *Z. officinale*, reddish fluorescence spot at R_f 0.38 with *T. cordifolia*. In UV 254 nm (Fig.2) shows matching spots at R_f 0.29, 0.34, 0.81 with *Z. officinale* and at R_f 0.62 with *T. cordifolia* and spot at R_f 0.49 with *C. rotundus*. Plate after spraying with *anisaldehyde -sulphuric acid* reagent and heating for 10 min at 105^0 in visible light (Fig.3) shows corresponding spots at R_f 0.88 (blue), 0.63 (pink), 0.31 (blue) with *C. rotundus*, spots at R_f 0.09 (pink), 0.11 (pink), 0.33 (pink), 0.80 (pink) with *Z. officinale*, spot at R_f 0.27 (greenish black) with *A. heterophyllum* and spots at R_f 0.38 (violet), 0.43 (violet) with *T. cordifolia* (Table 4).

TLC of *chloroform* extract of *churna* (5□l) along with 10µl of *methanol* extract of individual drugs in UV 366 nm (Fig.4) shows corresponding pink fluorescence spots at R_f 0.20 and 0.32 with *T. cordifolia*, bluish fluorescence spot at R_f 0.60 with *Z. officinale*. In UV 254 nm (Fig.5) shows corresponding spot at R_f 0.70 with *C. rotundus*. Plate after spraying with *anisaldehyde -sulphuric acid* reagent and heating at 110^0 for 10 min in visible light (Fig.6) shows corresponding spots at R_f 0.50 (pink), 0.55 (pink), 0.63 (pink) with *T. cordifolia* and shows spot at R_f 0.37 (violet) with *Z officinale* (Table-5).

TLC of *methanol* extract of *churna* (5□l) along with 5µl of *methanol* extract of individual drugs in UV 366 nm (Fig.7) shows corresponding yellowish fluorescence spots at R_f 0.34 and 0.42 with *T. cordifolia*. Plate observed under UV light at 254 nm (Fig.8) shows matching spots at R_f 0.70 and 0.88 with *C. rotundus* and spot at R_f 0.21 with *A. heterophyllum*. Plate after spraying with *anisaldehyde -sulphuric acid* reagent and heating for 10 min at 105^0 (Fig.9) shows corresponding spots at R_f 0.32 (black), 0.23 (black), 0.15 (black) 0.11 (black), 0.10 (black) with *A. heterophyllum* and spots at R_f 0.67 (greenish black) 0.32 (greenish black) with *Cyperus rotundus*. 5 µl of *methanol* extract of *churna* along with 5µl of *methanol* extract of individual drugs applied on TLC plate and developed to a distance of 85 mm using *chloroform*: *methanol*: *acetic acid* (3 : 1 : 0.3) and after derivatization with dragendorff reagent shows corresponding spot at R_f 0.20 with *T. cordifolia* and at R_f 0.33 with *A. heterophyllum* (Table 6).

TLC of volatile oils of *churna* (2µl) and volatile oils of *Z. officinale* and *C. rotundus* in UV 254 nm (Fig.10) shows corresponding spots at R_f 0.23 and 0.79 with *Z. officinale* and corresponding spots at R_f 0.11, 0.17, 0.31, 0.34 with *C. rotundus*. Plate after spraying with *anisaldehyde -sulphuric acid* reagent and heating for 10 min at 105^0 in visible light (Fig.11) shows corresponding spots at R_f 0.13, 0.20, 0.25, 0.57, 0.78 (violet) with *Z. officinale* and R_f 0.25 (pink), 0.51 (orange), 0.57 (dark blue) with *C. rotundus* (Table-7). TLC of volatile oils after spraying with 2,4 DNP reagent shows corresponding orange coloured spots at R_f 0.44, 0.56, 0.67 with *C. rotundus*. GC profile of the *churna* shows matching peaks with both the profile of *Z. officinale* and *C. rotundus* (Fig.12).

Table 2: Physico-chemical parameters of *Chaturbhadra kvatha churna*.

Parameters	Sample 1	Sample 2	Sample 3
Form	Coarse powder	Coarse powder	Coarse powder
Colour	Sandy brown	Sandy brown	Sandy brown
Odour	Reminiscent of ginger	Reminiscent of ginger	Reminiscent of ginger
Taste	Pungent	Pungent	Pungent
pH (20%)	4-5	4-5	4-5
Loss on drying at 110^0 C	11.1	11.2	11.0
Total ash	4.64	4.67	4.66
Sulphated ash	10.9	10.8	11.1
Acid insoluble ash	1.1	1.2	1.2
Alcohol soluble extractive (%)	6.62	6.78	6.73
Water soluble extractive (%)	14.83	14.97	14.92
Total phenolics	0.22	0.21	0.23
Volatile oil (%)	0.4	0.4	0.4
Aflatoxins	Nil	Nil	Nil

Table 3: Sequential solvent extractive (%)

Parameters	Sample 1	Sample 2	Sample 3
Hexane extractive (%)	2.1	2.3	2.74
Chloroform extractive (%)	5.1	4.9	4.5
Methanol extractive (%)	9.6	8.8	9.2

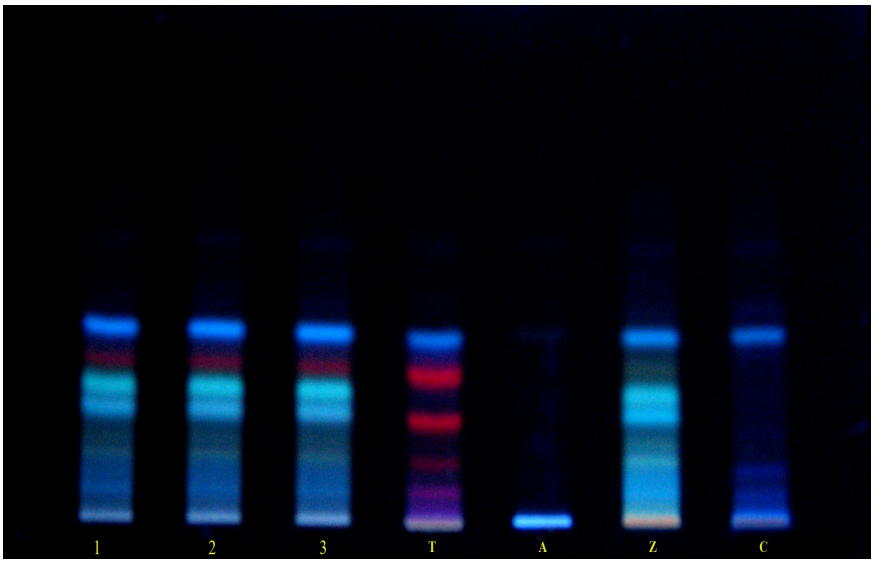


Fig. 1: TLC of *hexane* extract at 366 nm

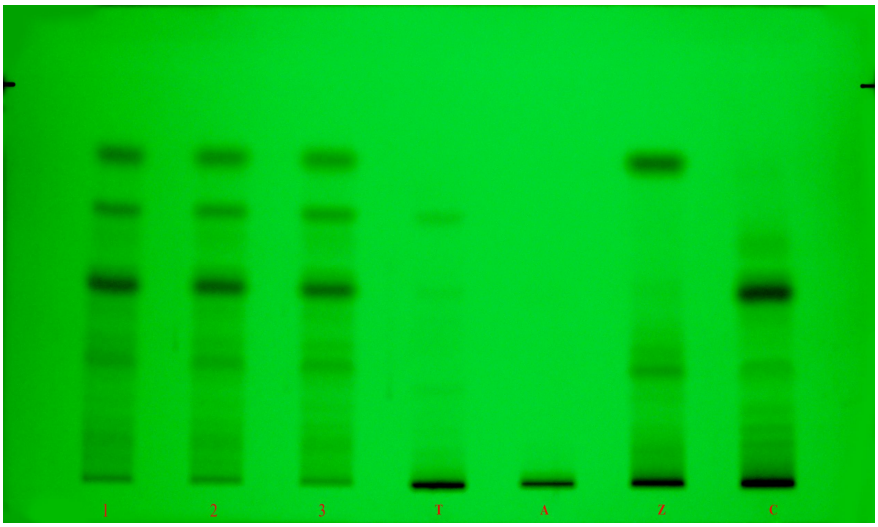


Fig. 2: TLC of *hexane* extract at 254 nm

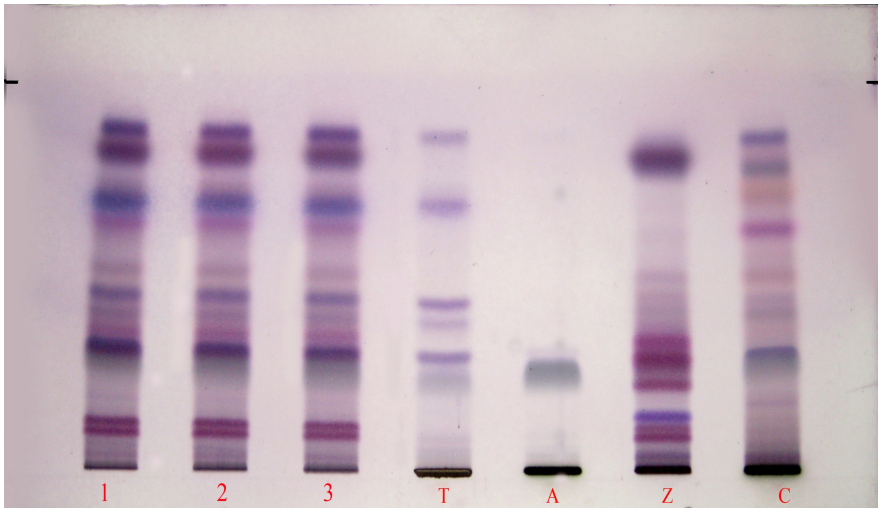


Fig. 3: TLC of *hexane* extract after ANS derivatization

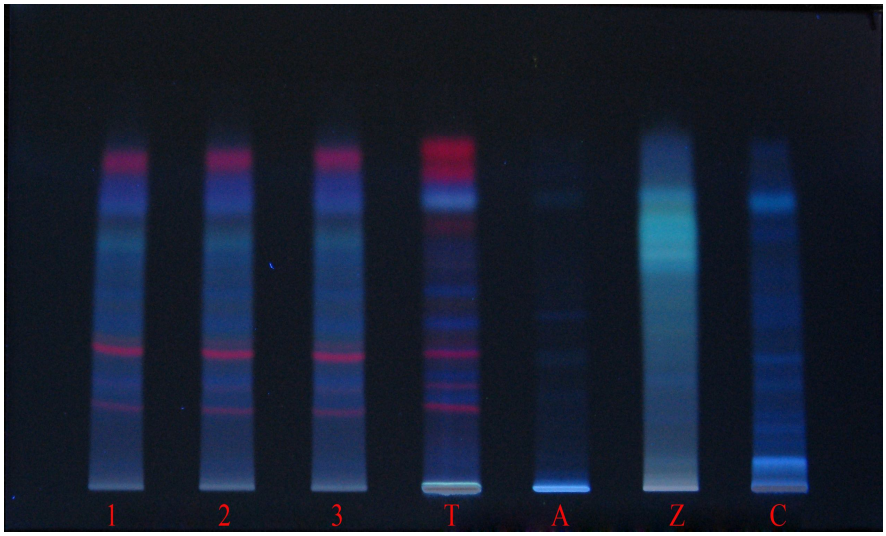


Fig. 4: TLC of *chloroform* extract at 366 nm

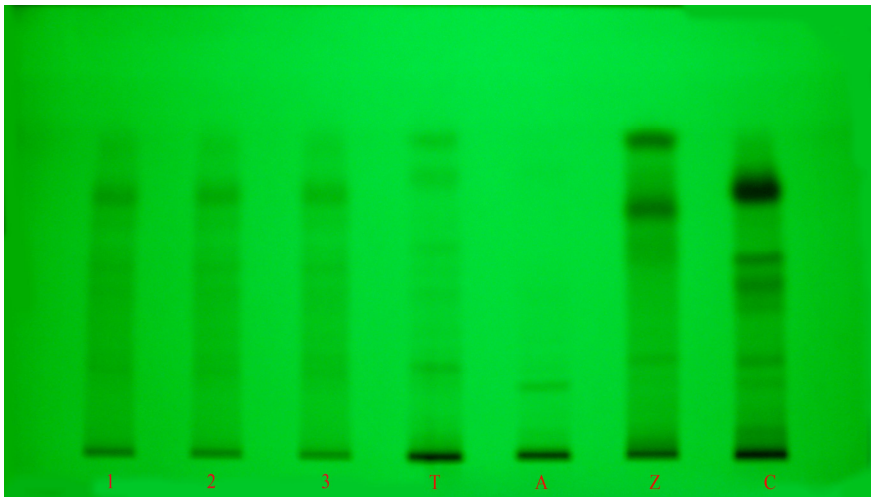


Fig. 5: TLC of *chloroform* extract at 254 nm

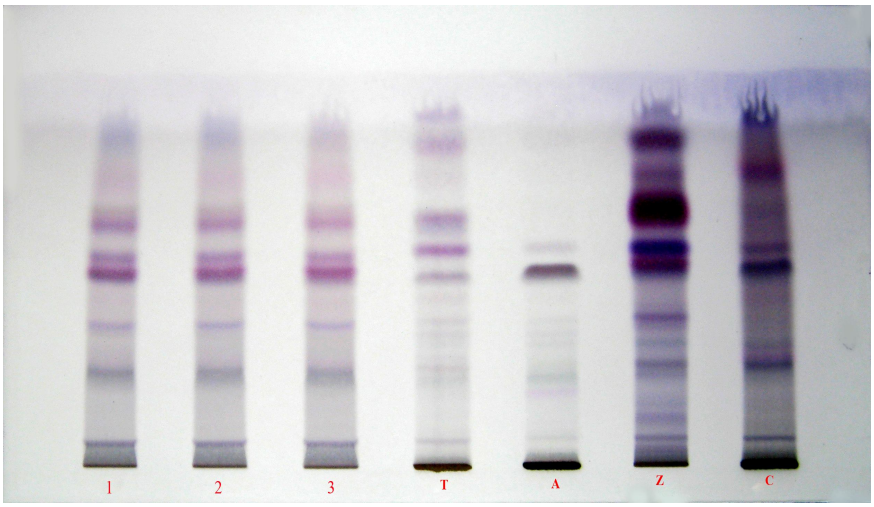


Fig. 6: TLC of *chloroform* extract after ANS derivatization

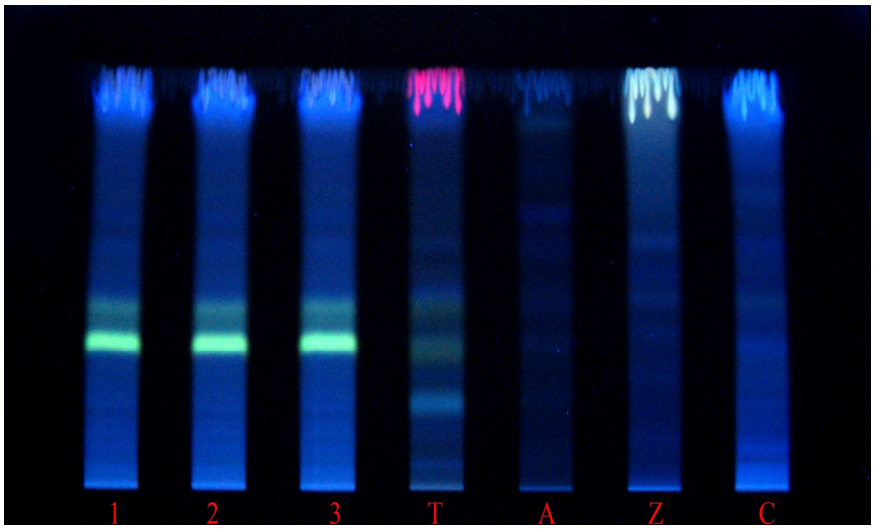


Fig. 7: TLC of *hexane* extract at 366 nm

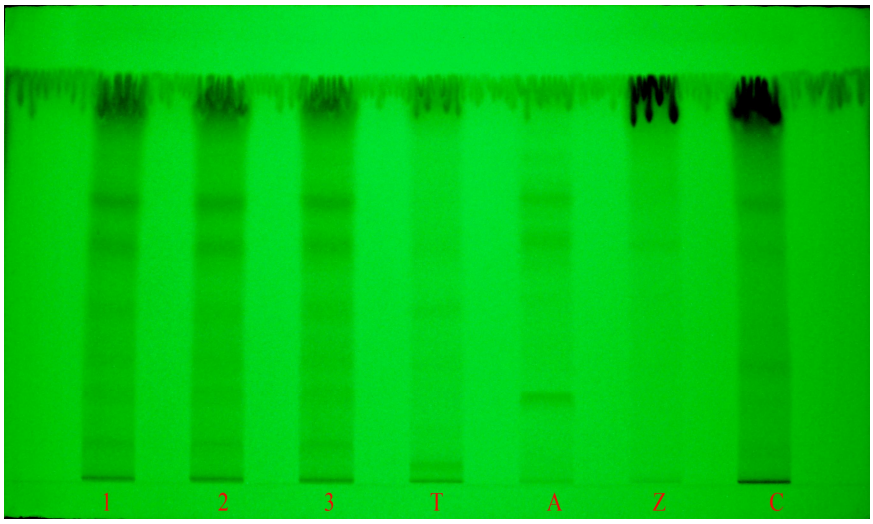


Fig. 8: TLC of *hexane* extract at 254 nm

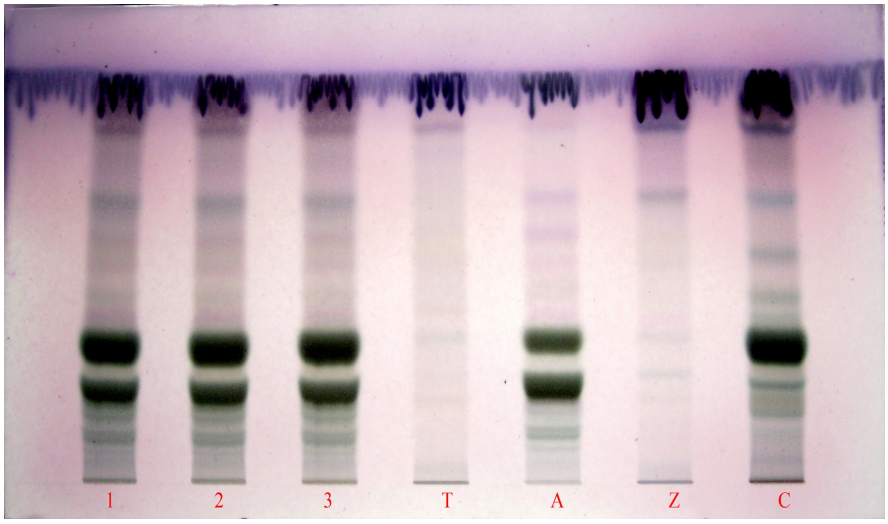


Fig. 9: TLC of *methanol* extract after ANS derivatization

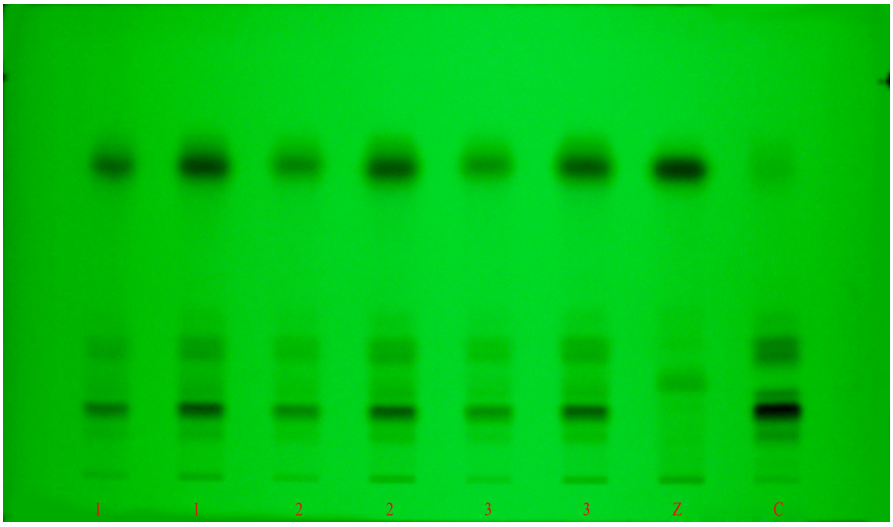


Fig. 10: TLC of volatile oil at 254 nm

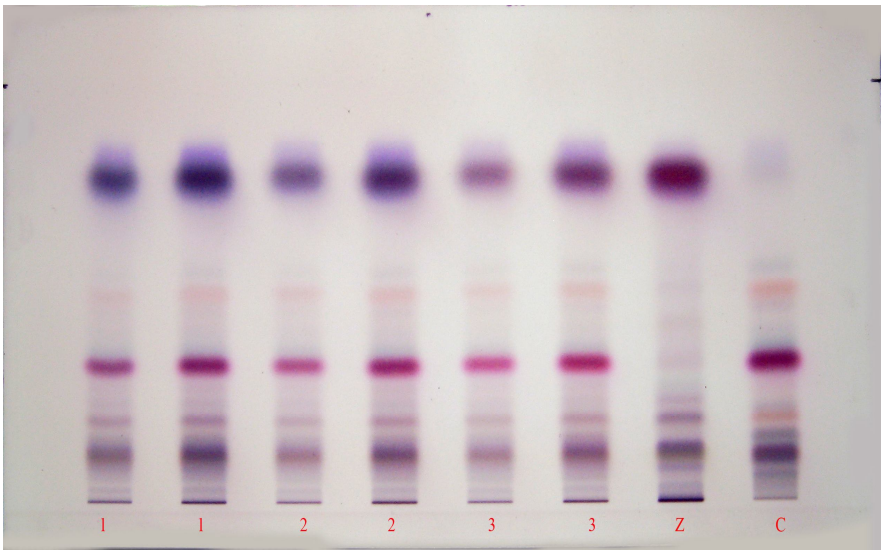


Fig. 11: TLC of volatile oil after ANS derivatization

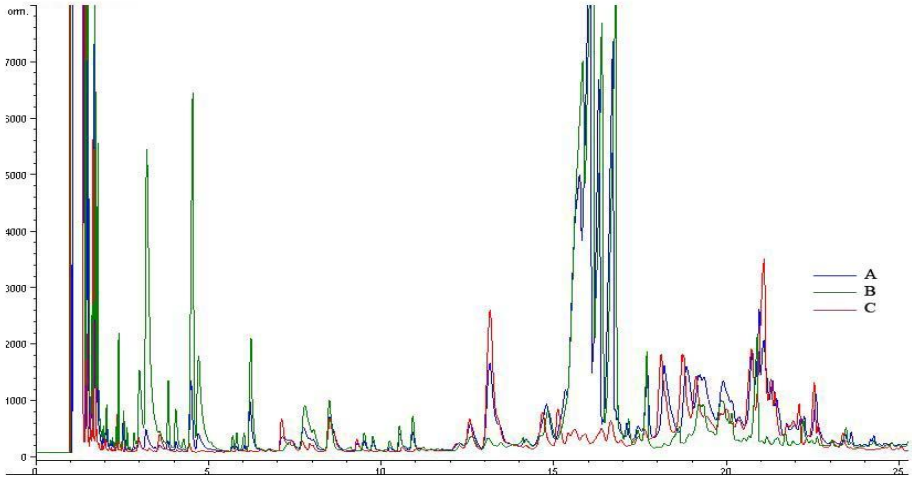


Fig. 12: GC Profile of volatile oil of Churna (A), *Z.offficinale* (B) and *C.rotundus* (C).

Table 4: R_f values of TLC of *Hexane* extract

Visualisation	Sample 1	Sample 2	Sample 3	*T*	*A*	*Z*	*C*
366 nm	0.16	0.16	0.16			x	
	0.28	0.28	0.28			x	
	0.33	0.33	0.33			x	
	0.36	0.36	0.36			x	
	0.38	0.38	0.38	x			
254 nm	0.29	0.29	0.29			x	
	0.34	0.34	0.34			x	
	0.49	0.49	0.49				x
	0.62	0.62	0.62	x			
	0.81	0.81	0.81			x	

After spray with ANS reagent	0.09	0.09	0.09			x	
	0.11	0.11	0.11			x	
	0.27	0.27	0.27		x		
	0.31	0.31	0.31				
	0.33	0.33	0.33			x	
	0.38	0.38	0.38	x			
	0.43	0.43	0.43	x			
	0.48	0.48	0.48				
	0.63	0.63	0.63				x
	0.80	0.80	0.80			x	
	0.88	0.88	0.88				x
T *– T. cordifolia*; ***A*** *- A. heterophyllum*; ***Z*** *- Z. officinale*; ***C*** *- C rotundus*							

Table 5: R_f values of TLC of *chloroform* extract

Visualisation	**Sample 1**	**Sample 2**	**Sample 3**	***T***	***A***	***Z***	***C***
366 nm	0.16	0.16	0.16				
	0.20	0.20	0.20	x			
	0.32	0.32	0.32	x			
	0.60	0.60	0.60			x	
254 nm	0.70	0.70	0.70				x
After spray with ANS reagent	0.37	0.37	0.37			x	
	0.50	0.50	0.50	x			
	0.55	0.55	0.55	x			
	0.63	0.63	0.63	x			
T *– T. cordifolia*; ***A*** *- A. heterophyllum*; ***Z*** *- Z. officinale*; ***C*** *- C rotundus*							

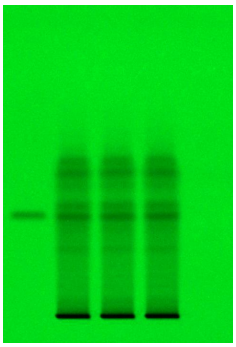
Table 6: R_f values of TLC of *methanol* extract

Visualisation	**Sample 1**	**Sample 2**	**Sample 3**	***T***	***A***	***Z***	***C***
366 nm	0.34	0.34	0.34	x			
	0.42	0.42	0.42	x			
254 nm	0.70	0.70	0.70				x
	0.88	0.88	0.88				x
After spray with ANS reagent	0.10	0.10	0.10		x		
	0.11	0.11	0.11		x		
	0.15	0.15	0.15		x		
	0.23	0.23	0.23		x		
	0.32	0.32	0.32		x		x
	0.67	0.67	0.67				x
T *– T. cordifolia*; ***A*** *- A. heterophyllum*; ***Z*** *- Z. officinale*; ***C*** *- C rotundus*							

Table 7: R_f values of TLC of volatile oil

Visualisation	Sample 1	Sample 2	Sample 3	*Z*	*C*
254 nm	0.11	0.11	0.11	x	
	0.17	0.17	0.17		x
	0.23	0.23	0.23		x
	0.31	0.31	0.31		x
	0.34	0.34	0.34		x
	0.79	0.79	0.79	x	
After spray with ANS reagent	0.13	0.13	0.13	x	
	0.20	0.20	0.20	x	
	0.25	0.25	0.25		x
	0.51	0.51	0.51		x
	0.57	0.57	0.57		x
	0.78	0.78	0.78	x	
Z *- Z. officinale*; ***C*** *- C rotundus*					

As a quantitative marker for the *churna*, 6-Shogaol and 8-gingerol were tried in the *chloroform* extract (non-sequential) of the *churna*, using mobile phase *hexane*: *acetone* (7 : 3) (Fig.13 plate under 254 nm and Fig.14 after ANS derivatization, Table 8). Amount of 6-Shogaol present in the churna samples was quantified (Fig. 15) and it was found to be 0.007, 0.008, 0.0075% with respect to *churna*. Spectrum of 6-Shogaol and corresponding spot in *churna* samples were compared (Fig.16). HPTLC profiles of three different batches of CBK *churna* were compared (Fig. 17 A & B, 18 A & B & 19 A & B).



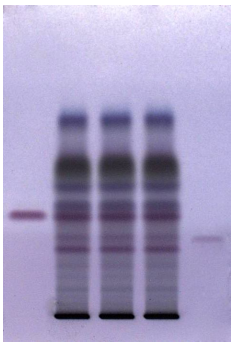
T_1 T_2 T_3 T_4 T_5

Fig. 13

T_1 : 6-Shogaol

T_2 -T_4: Test solution

T_5 : 8 -Gingerol



T_1 T_2 T_3 T_4 T_5

Fig. 14

T_1 : 6-Shogaol

T_2 -T_4: Test solution

T_5 : 8 -Gingerol

Table 8 TLC details of *chloroform* extract (non-sequential)

254 nm		After ANS derivatisation	
R_f value	**Colour**	**R_f value**	**Colour**
0.28	Green	0.11	Blue
0.41	Green	0.16	Blue
0.46	Green	0.21	Blue
0.59	Green	0.28	Purple
0.60	Green	0.33	Purple
0.65	Green	0.41	Purple
		0.46	Dark Blue
		0.54	Dark Blue

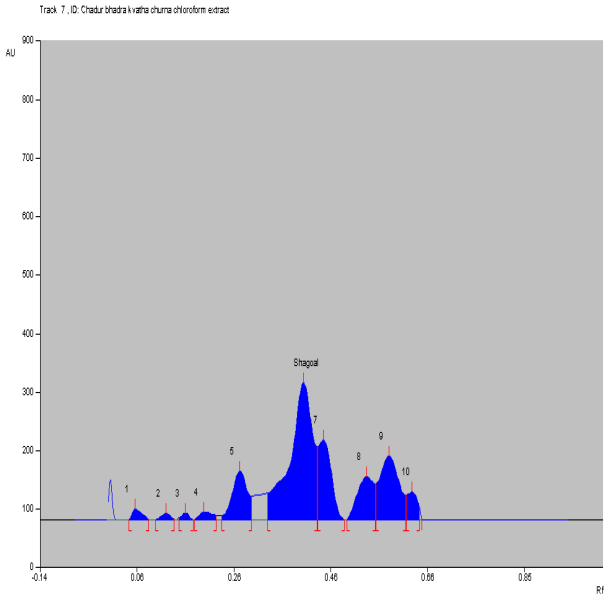


Fig. 15: HPTLC profile of CBK *churna chloroform* extract

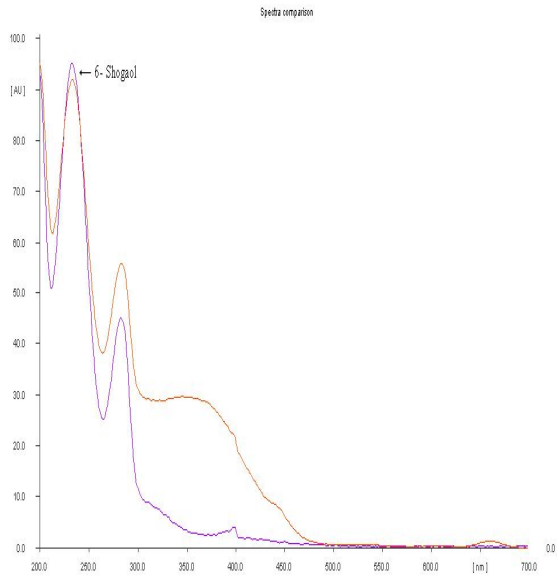


Fig. 16: Spectrum comparison.

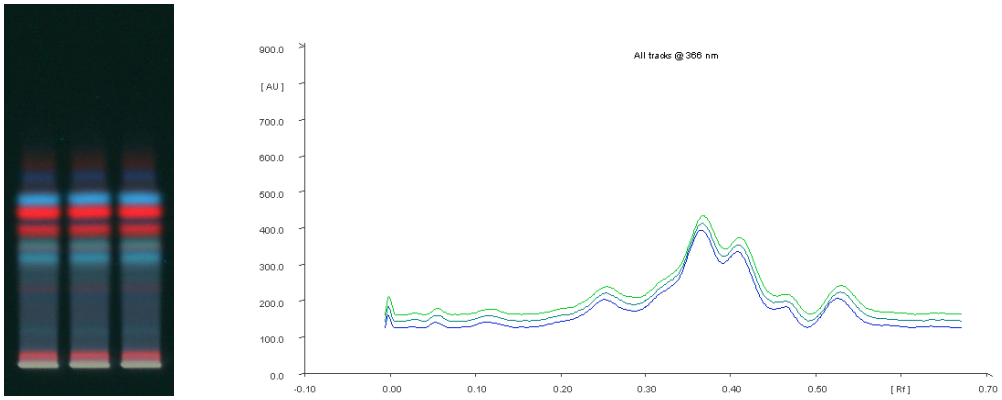


Fig. 17: TLC (A) and HPTLC (B) of three different batches of *churna* at 366 nm

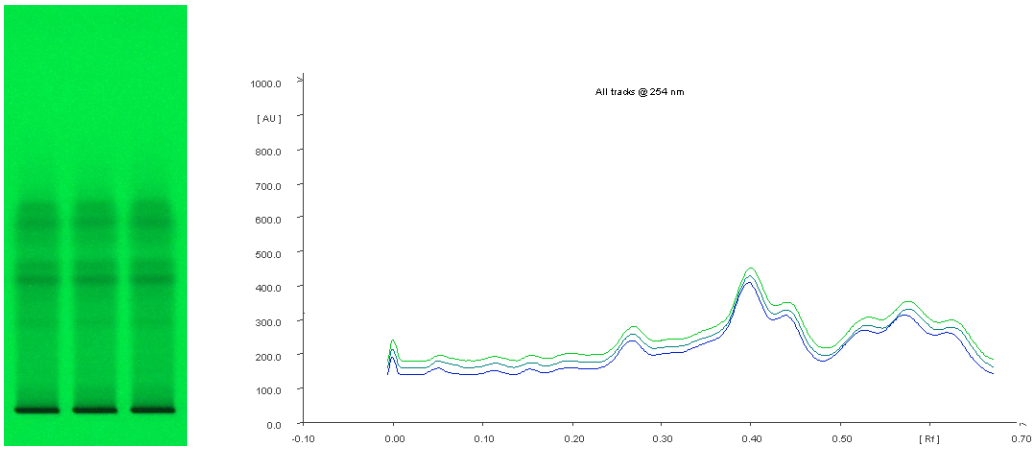


Fig. 18: TLC (A) and HPTLC (B) of three different batches of *churna* at 254 nm

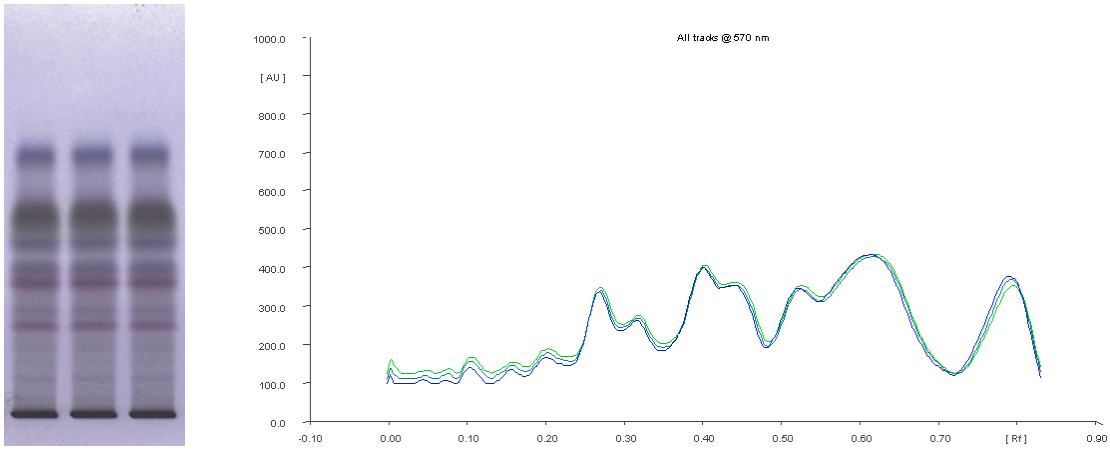


Fig. 19: TLC (A) and HPTLC (B) of three different batches of *churna* after derivatisation with ANS

CONCLUSION

The development of pharmacopoeial standards of *Chaturbhadra kvatha churna* was based on the outcome of physicochemical, TLC, HPTLC, GC finger print profiles. The results were found to be highly accurate, quick and reliable for routine monitoring in compound preparations. With the growing demand of herbal drugs market, it is suggested that this standardization tool will help in maintaining the quality and batch to batch consistency of many ayurvedic raw drug powder based preparations.

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