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STANDARDIZATION OF BILWADI AGADA THROUGH HPTLC

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ABSTRACT

The quality control standards for the Ayurvedic polyherbal medicinal preparation is the need for the day in view of commercialization. Despite availability of modern equipments and techniques only a few Ayurvedic drugs have been standardized. Bilwadi agada (BA), an herbal preparation; mentioned in the text of Ayurveda in the context of treatment of poisoning, is one of those. High-performance thin layer chromatography (HPTLC) is an instrumental techniques and an analytical tool for chromatographic information. In this present study an effort has been made to standardize BA through HPTLC. TLC photo documentation of BA showed 13, 12 and 11 spots under short UV, long UV and under white light after derivatization respectively. Densitometric scan at 254 nm revealed 3 high peaks corresponding to 3 different compounds in the ethanol extract, compounds, at 366 nm and 620 nm there were three high peaks. These physico-chemical constants, TLC photo documentation, the unique Rf values and densitogram obtained at different wavelengths can be used as fingerprint to identify BA.

KEYWORDS: Bilwadi agada, Standardization, HPTLC, Pharmaceutical study.

INTRODUCTION

Ayurveda Pharmaceutics comprises of formulations that have multiple ingredients. These ingredients will have various components and their action is based on the different entities present in the drug. One such formulation is *Bilwadi agada* mentioned for the management of poisons that has many ingredients. Use of medicinal plants in bulk as there is a demand for herbal drugs at present has led to commercialization. To maintain the quality, standardization is needed as it has been said that the indigenous system of medicine has been commercialized in the present scenario leading to the use of quality control standards of formulations.[1] The present study was carried out to evaluate the phytochemical standard for *Bilwadi agada* using the chromatography technique i.e., HPTLC. *Bilvadi agada* (BA) is a herbal formulation, mentioned in the context of treatment of various kinds of poisoning, comprising bilva (*Aegle marmelos*), surasa (*Ocimum sanctum*), karanja (*Pongamia pinnata*), tagara (*Valeriana wallichi*), devadaru (*Cedrus doedara*), hareetaki (*Terminalia chebula*), vibheetaki (*Terminalia bellerica*), amalaki (*Emblica officinalis*), shunti (*Zingiber officinale*), pippali (*Piper longum*), maricha (*Piper nigrum*), haridra (*Curcuma longa*), daruharidra (*Berberis aristata*) and the media used for trituration is goat's urine.[2] It is said that High-performance thin layer chromatography (HPTLC) is a sophisticated instrumental techniques and is a powerful analytical tool for chromatographic information for various samples.[3] So a formulation like BA, comprising multiple ingredients, was subjected for phytochemical standardization for quality control and authentication of preparation to ensure therapeutic efficacy.

MATERIALS AND METHODS

Plant material

The ingredients of BA (Table 1) were collected from the local market. The collected drugs were identified and authenticated at the teaching pharmacy of Department of Dravyaguna, SDM College of Ayurveda and Hospital, Hassan, Karnataka state, India.

Phytochemical standardization

Phytochemical studies through HPTLC were carried out as per the WHO guidelines.[4] The analytical study was carried out at SDM Centre for Research in Ayurveda and Allied Sciences (AYUSH Centre for Excellence and Recognized SIROs by DSIR), Laxminarayana Nagar, P.O. Kuthpady - 574 118, Udupi, Karnataka state, India as per standard procedure.

Pharmaceutical preparation of Bilwadi agada

Table no. 1: Ingredients of *Bilvadi Agada.*

Sl. No	Ingredient	Botanical name	Used part	Quantity
1	Bilva	*Aegle marmelos*	Root	12g
2	Surasa	*Ocimum sanctum*	Flower	12g
3	Karanja	*Pongamia pinnata*	Fruit	12g
4	Tagara	*Valeriana wallichi*	Root	12g
5	Devadaru	*Cedrus doedara*	Heart wood	12g
6	Hareethaki	*Terminalia chebula*	Fruit	12g
7	Vibheethaki	*Terminalia bellerica*	Fruit	12g
8	Amalaki	*Emblica officinalis*	Fruit	12g
9	Shunti	*Zingiber officinale*	Rhizome	12g
10	Maricha	*Piper nigrum*	Fruit	12g
11	Pippali	*Piper longum*	Fruit	12g
12	Haridra	*Curcuma longa*	Rhizome	12 g
13	Daruharidra	*Berberis aristata*	Root	12g
14	Aja mootra (Goat's urine)			600ml

Method of preparation

All ingredients were taken in dry form in equal quantity (12g each). They were pounded well separately and sieved separately through a cotton cloth. Then it was mixed well into a homogenous mixture and bhavana (trituration) was done with aja mutra (Goat's urine) for three days till subhavitha lakshanas (test of perfection) were appreciated. Then pills were made of uniform size, dried and stored in air tight container.

Total quantity obtained

Total quantity of pills obtained: 120 pills.

Average weight of pill

1.20g in wet form.

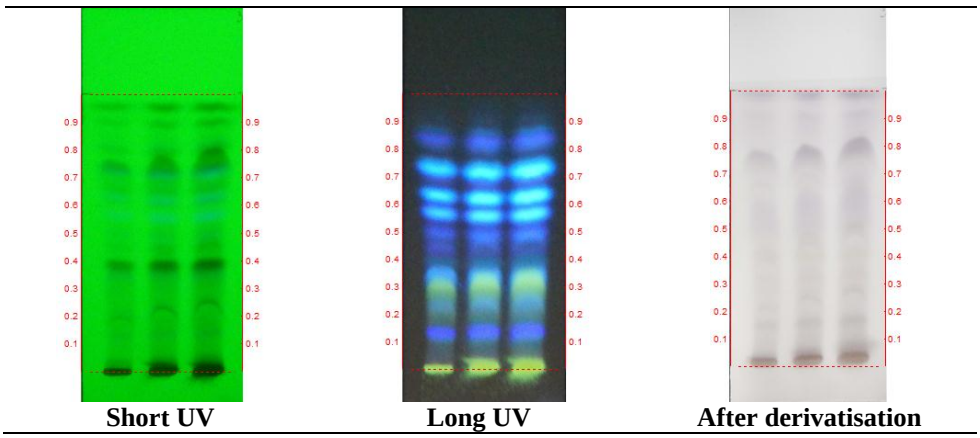
Physical characteristic of pills

- Appearance: Pill form
- Colour: Blackish
- Odour: Goat's urine smell
- Touch: Smooth
- Taste: *Kashaya* (Astringent)
- Solubility: Dissolved in water

HPTLC

1g of Bilwadi agada powder was extracted with 20 ml of alcohol kept for 24hrs for cold maceration then was filtered. 3, 6 and 9µl of the above extract were applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in n-hexane: Ethyl acetate (6.0: 4.0). The developed plates were visualized in short UV, long UV, and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm and 620nm. Rf, colour of the spots and densitometric scan were recorded.

RESULTS



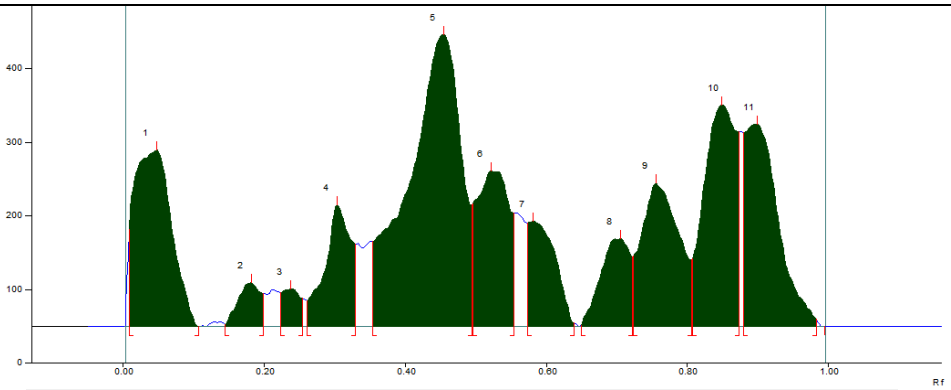
Short UV **Long UV** **After derivatisation**

Track 1- *Bilwadi agada*– 3µl
Track 2- *Bilwadi agada*– 6µl
Track 3- *Bilwadi agada*– 9µl
Solvent system – n-hexane: Ethayl acetate (6.0: 4.0)

Figure 1: HPTLC photo documentation of ethanol extract of *Bilwadi agada.*

Table 2: Rf values of ample of *Bilwadi agada.*

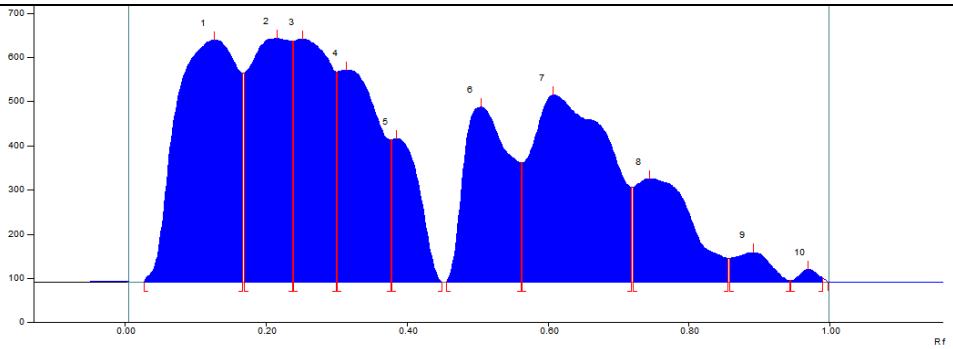
Short UV	Long UV	Post derivatisation
0.14 (D. green)	0.14 (F. blue)	-
-	-	0.16 (D. purple)
0.24 (D. green)	0.24 (F. blue)	0.24 (D. purple)
-	-	0.28 (D. purple)
-	0.32 (F. yellow)	0.32 (D. purple)
-	0.36 (F. blue)	-
0.39 (D. green)	0.39 (F. blue)	0.39 (D. purple)
-	-	0.43 (D. purple)
0.45 (L. green)	0.45 (F. blue)	-
-	-	0.47 (D. purple)
0.50 (L. green)	0.50 (F. blue)	-
-	0.55 (F. blue)	-
0.57 (L. green)	-	-
0.63 (L. green)	0.63 (F. blue)	-
0.65 (L. green)	-	-
-	0.70 (F. blue)	0.70 (D. purple)
0.73 (D. green)	-	-
0.75 (D. green)	-	-
-	-	0.78 (D. purple)
0.80 (D. green)	-	-
-	0.83 (F. blue)	-
-	0.86 (F. blue)	0.86 (D. purple)
0.90 (D. green)	-	0.90 (D. purple)
0.96 (D. green)	-	-



Track 3, ID: Bilwadi gutica

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	130.8 AU	0.05 Rf	238.9 AU	11.11 %	0.11 Rf	0.1 AU	9153.2 AU	11.72 %
2	0.15 Rf	1.9 AU	0.18 Rf	59.1 AU	2.75 %	0.20 Rf	44.9 AU	1374.1 AU	1.76 %
3	0.22 Rf	45.8 AU	0.24 Rf	50.9 AU	2.37 %	0.25 Rf	38.2 AU	937.9 AU	1.20 %
4	0.26 Rf	35.0 AU	0.30 Rf	164.3 AU	7.64 %	0.33 Rf	11.9 AU	4396.9 AU	5.63 %
5	0.35 Rf	114.7 AU	0.45 Rf	395.9 AU	18.41 %	0.50 Rf	64.7 AU	20984.6 AU	26.88 %
6	0.50 Rf	166.3 AU	0.52 Rf	210.9 AU	9.80 %	0.55 Rf	53.7 AU	6951.5 AU	8.90 %
7	0.57 Rf	140.2 AU	0.58 Rf	142.7 AU	6.63 %	0.64 Rf	4.8 AU	3992.1 AU	5.11 %
8	0.65 Rf	1.4 AU	0.71 Rf	118.8 AU	5.52 %	0.72 Rf	94.5 AU	3552.6 AU	4.55 %
9	0.72 Rf	95.6 AU	0.76 Rf	194.5 AU	9.04 %	0.81 Rf	90.0 AU	7442.2 AU	9.53 %
10	0.81 Rf	91.4 AU	0.85 Rf	300.5 AU	13.97 %	0.87 Rf	64.2 AU	9894.7 AU	12.67 %
11	0.88 Rf	262.4 AU	0.90 Rf	274.5 AU	12.76 %	0.98 Rf	9.7 AU	9394.5 AU	12.03 %

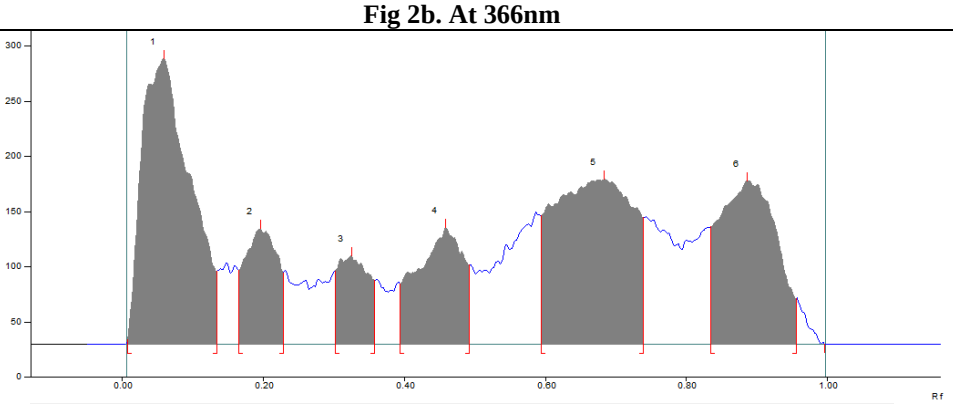
Fig 2a. At 254nm



Track 3, ID: Bilwadi gutica

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.9 AU	0.13 Rf	548.3 AU	15.20 %	0.17 Rf	73.9 AU	34096.8 AU	19.41 %
2	0.17 Rf	474.1 AU	0.22 Rf	551.6 AU	15.30 %	0.24 Rf	44.7 AU	23330.6 AU	13.28 %
3	0.24 Rf	545.0 AU	0.25 Rf	550.5 AU	15.27 %	0.30 Rf	76.1 AU	20064.4 AU	11.42 %
4	0.30 Rf	476.2 AU	0.31 Rf	480.0 AU	13.31 %	0.38 Rf	22.5 AU	20402.8 AU	11.62 %
5	0.38 Rf	322.7 AU	0.39 Rf	325.1 AU	9.02 %	0.45 Rf	0.5 AU	8999.8 AU	5.12 %
6	0.46 Rf	1.4 AU	0.51 Rf	395.8 AU	10.98 %	0.56 Rf	70.0 AU	18614.8 AU	10.60 %
7	0.56 Rf	270.1 AU	0.61 Rf	424.0 AU	11.76 %	0.72 Rf	14.9 AU	33368.9 AU	19.00 %
8	0.72 Rf	215.0 AU	0.75 Rf	234.5 AU	6.50 %	0.86 Rf	54.2 AU	13760.3 AU	7.83 %
9	0.86 Rf	54.3 AU	0.89 Rf	67.2 AU	1.86 %	0.94 Rf	3.2 AU	2496.9 AU	1.42 %
10	0.95 Rf	3.4 AU	0.97 Rf	29.2 AU	0.81 %	0.99 Rf	10.7 AU	519.8 AU	0.30 %

Fig 2b. At 366nm



Track 3, ID: Bilwadi gutica

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	4.1 AU	0.06 Rf	258.6 AU	30.57 %	0.14 Rf	66.0 AU	12788.2 AU	28.60 %
2	0.17 Rf	67.2 AU	0.20 Rf	104.6 AU	12.36 %	0.23 Rf	64.2 AU	3508.8 AU	7.85 %
3	0.30 Rf	66.4 AU	0.33 Rf	79.9 AU	9.44 %	0.36 Rf	57.5 AU	2492.2 AU	5.57 %
4	0.40 Rf	54.8 AU	0.46 Rf	105.4 AU	12.46 %	0.49 Rf	71.8 AU	4953.6 AU	11.08 %
5	0.60 Rf	116.8 AU	0.68 Rf	149.6 AU	17.69 %	0.74 Rf	14.9 AU	12314.6 AU	27.54 %
6	0.84 Rf	106.4 AU	0.89 Rf	148.0 AU	17.49 %	0.96 Rf	40.9 AU	8655.3 AU	19.36 %

Fig 2c. At 620nm

Figure 2: Densitometric scan of *Bilwadi agada.*

DISCUSSION

TLC photo documentation of BA showed 13, 12 and 11 spots under short UV, long UV and under white light after derivatization respectively. Spot with Rf 0.24 and 0.39 were commonly detected in all the three detection methods. All the three methods gave optimum separation of different bands and hence all of them may be used as TLC fingerprint pattern to identify the composition of BA (Table 2). Densitometric scan at 254 nm revealed 3 high peaks corresponding to 3 different compounds in the ethanol extract, compounds with Rf 0.90 (12.03%), 0.85 (12.67%) and 0.45 (26.88%) were the peaks (Figure 2A). At 366 nm there were three high peaks, with Rf 0.22 (13.28%), 0.61 (19%) and 0.13 (19.41%) being the major peaks detected (Figure 2B). At 620 nm there were three high peaks, with Rf 0.89 (19.36%), 0.68 (27.54%) and 0.06 (28.60%) being the major peaks detected (Figure 2C).

The results obtained through this analytical study can be taken as preliminary standards for further studies.

CONCLUSION

The aim of standardization is to maintain the quality and standard of the drug, by which the therapeutic potential can be maintained. The constituents of Bilwadi agada have varied chemical constituents that add up to the therapeutic effect of the drug. The goat's urine (liquid media) used for trituration will also enhance the formulation and add other constituents to the formulation. It is not a single entity but a combination of all the constituents that give the desired result. BA prepared from these ingredients will have combined effects of all the individual herbs. The physicochemical standardization of BA carried out using HPTLC finger print profile for the quality control of the processed pill can be taken as a preliminary standard for this polyherbal preparation.

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