Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [3] February 2023 : 70-76

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Preparation and Standardization of an Ayurvedic Polyherbal Formulation

Deepthi Viswaroopan[1], Sudhirkumar Pani[1],Prasanna Mathad[1] , Lekshmi C. S.[2]
[1]Parul Institute of Ayurved and Research, Parul University, Vadodara, Gujarat- 391760
[2]Institute for Teaching and Research in Ayurved, Ministry of AYUSH, Jamnagar – 361008
Correspondence Email: deepthi.viswaroopan@paruluniversity.ac.in

ABSTRACT
Balachaturbhadra formulation is popular in paediatric practice for its broad-spectrum usage in gastro intestinal disorders. The four herbal drugs constituting Balachaturbhadra are namely Ativisha (Aconitum heterophyllum), Musta (Cyperus rotundus), Pippali (Piper longum), Karkatashringi (Pistacia integerrima). This formulation is also mentioned in arka form in the textbook of Arkas named Arkaprakasha.This paper deals Preparation of Balachaturbhadra Arka, HPTLC fingerprinting of Arka and Comparative GCMS analysis of Balachaturbhadra Arka and Kashayawith observations and discussion for the purpose of standardisation of this drug. TLC photo documentation of Arka using toluene: ethyl acetate solvent at 9:1 ratio shows 7 spots at UV 254 nm, 3 spots at UV 366 nm and 8 spots at UV 540 nm. In HPTLC densitometric scan of same sample at UV 254 nm, 7 peaks were obtained. There are many numbers of spots which are not identified before during the standardisation of Churna. Hence, it isseen that in Arka there is the presence of a number of volatile phytochemicals. Also, absence of certain spots which are seen in case of Churna is indicative of the absence of those phytochemicals when the dosage form of the formulation has been changed.
Keywords: *Balachaturbhadra Arka, Undernutrition, HPTLC fingerprinting, GCMS*

Received 05.11.2022 Revised 10.01.2023 Accepted 28.01.2023

INTRODUCTION

Balachaturbhadra formulation is popular in paediatric practice for its broad-spectrum usage in gastro intestinal disorders. The formulation is seen in classical Ayurvedic textbooks like *Sharangdhara Samhita* [1], *Bhavaprakasha* [2], *Chakradatta*[3], *Bhaishajyaratnavali* [4], *and Yogaratnakara* [5], in the churna (powder) form. The author found that this powder is difficult for children to take due to its astringent and bitter taste. Consequently, the same formulation in the form of *Arka* (steam distillate) was found in another reference from a medieval textbook named *Arkapradeepika* authored by Vaidya Hariscchandra Mukherji [6] which was indicated in *Karshya* (undernutrition) and as a *Rasayana* (rejuvenator). This formulation is also mentioned in *arka* form in the textbook of *Arkas* named *Arkaprakasha*. This paper is divided into three parts: Preparation of *Balachaturbhadra* Arka, HPTLC fingerprinting of Arka and Comparative GCMS analysis of *Balachaturbhadra* Arka and Kashaya. Each section has been dealt in detail with observations and discussion for the purpose of standardisation of this drug.

MATERIALS AND METHODS

The four herbal drugs (Table 1) constituting *Arka* are namely *Ativisha* (*Aconitum heterophyllum), Musta(Cyperus rotundus), Pippali (Piper longum), Karkatashringi (Pistacia integerrima*) were procured from teaching pharmacy of Parul Institute of Ayurved and Research, Parul University, Vadodara, Gujarat, India. The procured drugs were identified and authenticated at Department of Dravyaguna, Parul Institute of Ayurved, Parul University, Vadodara, Gujarat, India. The preparation of *Arka* was carried out at the teaching pharmacy of Parul Institute of Ayurved and Research.

Preparation of *Arka*

Table 1: Ingredients of *Arka*

Sr. No.	Drug	Latin name	Part used
1.	*Ativisha*	*Aconitum heterophyllum*	Root
2.	*Musta*	*Cyperus rotundus*	Rhizome
3.	*Pippali*	*Piper longum*	Fruits
4.	*Karkatashringi*	*Pistacia integerrima*	Galls

Step 1:
All the drugs were taken in equal quantity (75gms) and coarsely powdered with mortar and pestle. It was then soaked in 16times of water (4800ml) of water and left overnight.

Step 2:
The next day, the drugs contents were transferred to a clean round bottom flask. The steam distillation apparatus was then assembled and connected to power source and water source.

Step 3:
The apparatus was switched on at a starting temperature of 50˚ C for the boiling of the contents in the flask. Within the next 30mins the contents started boiling slow bubbling was seen from the sides and from within. The temperature was then increased to 75˚ C and the water tap was switched on. After 30mins, the temperature was again raised to 90˚ C and left for distillation process to start. Soon the contents started bubbling vigorously and there was fear of overflow of the contents. At this point, the water flow was increased and the temperature was reduced to 50˚ C and condensation began. Oil mixed water drops started dripping into the collecting jar. Once the condensation started, the temperature was increased to 75˚ C and maintained till the completion of collection. 150-160 drops/minute was being collected into the jar.

Step 4:
After three and half hours of start of condensation, the apparatus was switched off and the water outlet was kept on for 15 more minutes for the cooling of the pipes. The contents of the flask were reduced to half quantity and approximately 50% yield (2300ml) was obtained. Then the apparatus was dismantled and the collecting jar was labeled and kept aside for cooling. The distillate was then filled into bottles, labeled and dispensed.

Methods

- *Karkatasrungi* and *Musta* are difficult to pound as they are fibrous and hence took more time to become coarse powder.
- The soaked contents looked frothy the next morning due to high amounts of saponins in all the drugs.
- Just before the condensation started, the round bottom flask was being self-cleaned by evaporation of water at the neck of the flask. At this time the water flowing from the tap is cold to touch.
- Once condensation started, golden coloured bubbling was seen inside the flask. A sweet fragrance was coming out of the collecting jar. At this time, the outlet water flow was warm to touch.
- After one hour of condensation, the intensity of foaming inside the flask had increased. The colour of the contents deepened and the sweet smell filled the room. Oil content was clearly visible on top of the collecting jar.
- After 2 hours of start of condensation, the collection rate increased to 200 drops/minute. The foaming reduced and the bubbles were white in colour. A characteristic pungent smell was coming from the collecting jar.
- After 3 hours, the foam became completely white in colour and the contents turned dark blackish brown in colour. There was a mild smoky smell coming from the jar. The collecting jar (2 litre jar) was almost filled up to 2/3rd portion.

KARKATASHRUNGI **PIPPALI** **MUSTA** **ATIVISHA**

Coarsely powdered drug soaked overnight in water

Transferring the contents to a round bottom flask

Boiling the contents in distillation apparatus

Distillation Process

Final distillate left for cooling

Filling bottles

Labeled and ready for dispensing

Figure 1. Preparation of Balachaturbhadra Arka

HPTLC fingerprinting of Arka:

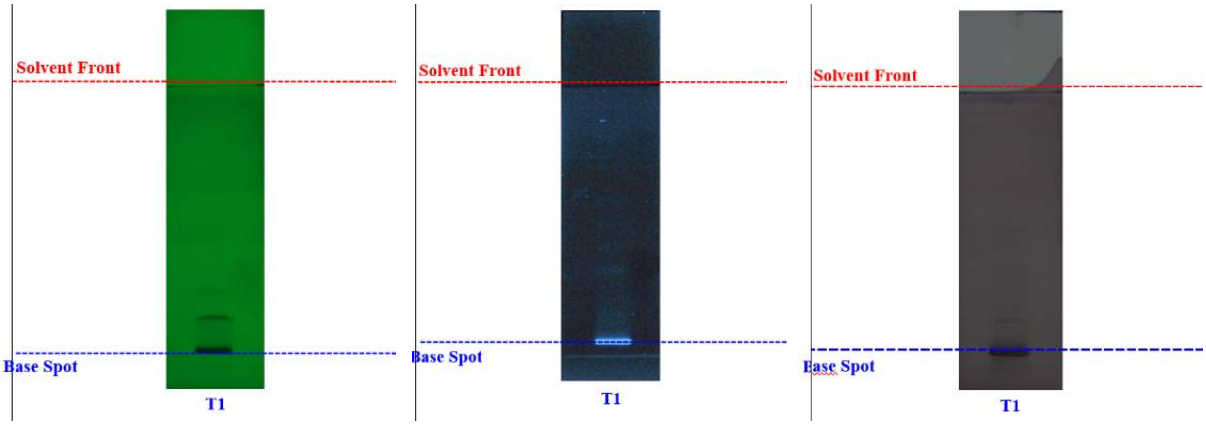
The procedures of standardization and HPTLC Photo documentation, Rf values, densitometric scan, 3-D chromatogram were done at Vasu Research Centre, Division of Vasu Healthcare Pvt. Ltd., 967/4 G.I.D.C., Makarpura, Vadodara – 390010, Gujarat, India. Test solution was prepared by pipetting 20 mL of sample in an evaporating dish. It was then evaporated on a water bath till complete dryness was attained and was allowed to cool down on its own. The sample was then reconstituted with 200 µL of methanol. The test solution thus obtained was used for HPTLC fingerprinting. 40 µl of the test solution was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The applied plate was developed in suitable solvent system and the developed plates were visualized and scanned under UV 254, 366,540 nm and after derivatization in anisaldehyde-sulphuric acid spray reagent. Rf, color of the spots, chromatogram and densitometric scan were recorded. Toluene: ethyl acetate (9:1) was used as the solvent system for development of the test sample. [7]

Comparative GCMS analysis of *Balachaturbhadra* Arka and Kashaya

GC-MS analysis of aqueous extract of the sample has been performed on a GC-MS Perkin Elmer,USA, System XL with NIST Library and Single Quadrapole with prefilter equipped with a DB-5 capillary column (30 m x 0.25 mm id, film thickness 0.25 µm). The oven temperature has programmed from 75-280 °C at the rate of 10°C/min. The ion source has set at 260 °C and electron ionization at 70 eV. Helium used as the carrier gas at a flow rate of 1 mL/min. Scanning range was 20 to 600 amu. Interpretation on mass spectrum of the unknown part has compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials have ascertained.

RESULTS & DISCUSSION:

HPTLC fingerprinting of Arka



At UV 254 nm At UV 366 nm At UV 540 nm

Solvent system – Toluene: Ethyl Acetate (9:1)

Figure 2: TLC Photo documentation of *Arka*

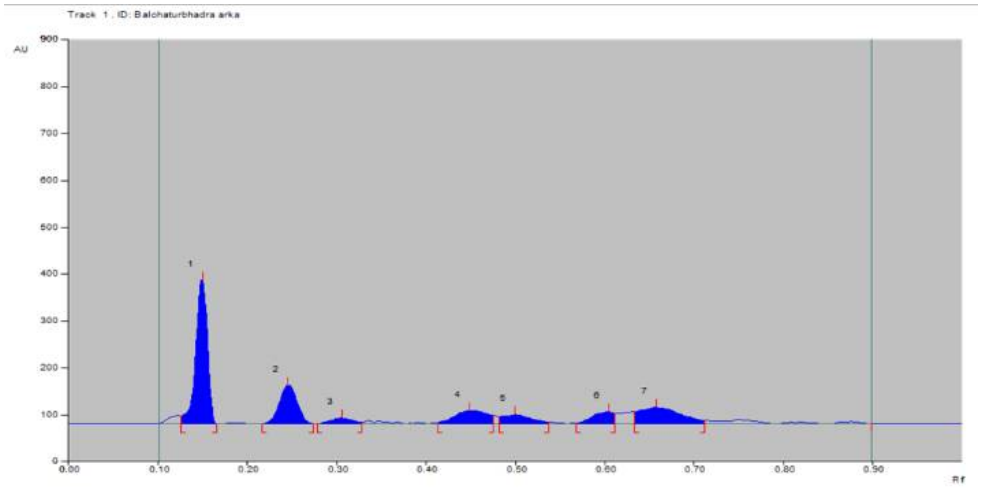


Figure 3: HPTLC Densitometric scan at 254 nm of *Arka*

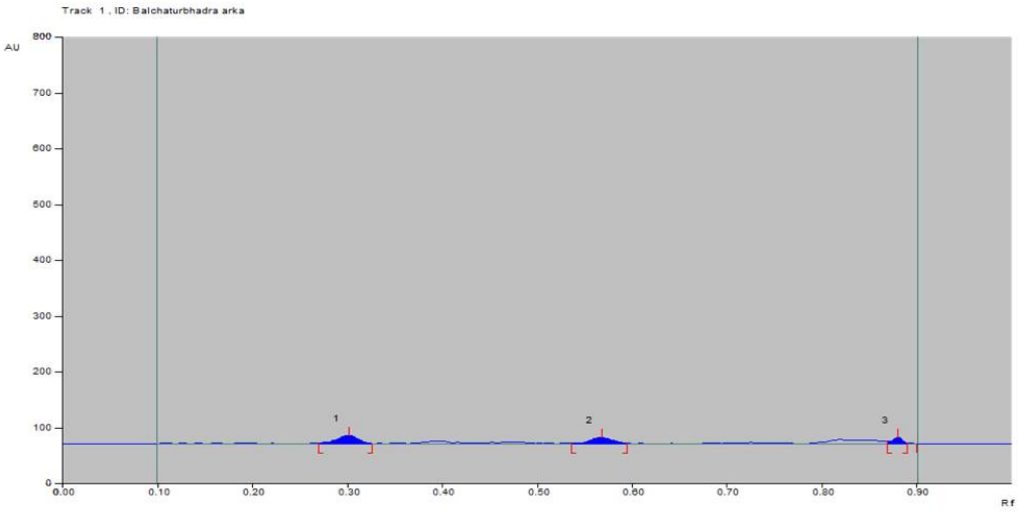


Figure 4: HPTLC Densitometric scan at 366 nm of *Arka*

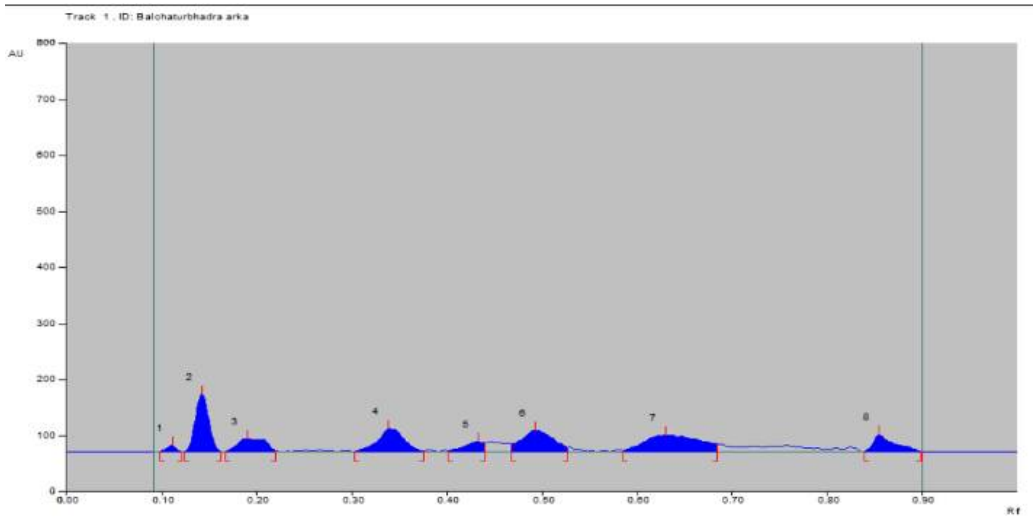


Figure 5: HPTLC Densitometric scan at 540 nm of *Arka*

Table 2: Rf values observed at UV 254 nm, 366 nm and 540 nm for *Arka*

Visualization	UV 254 nm	UV 366 nm	UV 540 nm
Rf values	0.15 0.25 0.31 0.45 0.50 0.60 0.66	0.31 0.60 0.88	0.11 0.15 0.19 0.31 0.45 0.50 0.66 0.88

The formulation contains 4 major ingredients namely Musta (Cyprus rotundus Linn.), Pippali (Piper longum Linn.), Ativisha (Aconitum heterophyllum Wall) and Karkatashringi (Pistacia integerrima Stew.). Figure 2 shows the TLC photo documentation of *Arka* at UV 254 nm and UV 366 nm using toluene: ethyl acetate solvent at 9:1 ratio and Table 2 gives the Rf values of the same.7 spots were seen at UV 254 nm, 3 spots at UV 366 nm and 8 spots at UV 540 nm. In HPTLC densitometric scan of same sample at UV 254 nm, 7 peaks were obtained (Figure 3). Maximum peak height is observed in the 1st peak with Rf value of 0.15. The next two prominent peaks are observed at Rf values 0.25 and 0.66 respectively. At UV 366 nm only 3 peaks were observed among which the peak at Rf value 0.88 was less prominent (Figure 4). Figure 5 shows chromatogram at UV 540 nm, in which 8 peaks are observed. Out of the 8, maximum peak height is observed at Rf value 0.15. The other peaks observed in decreasing order of prominence are at Rf values 0.31,0.66,0.50,0.19 and 0.88. The peaks obtained at Rf value 0.88 at visualisations UV 366 nm and 540 nm corresponds to volatiles present in *Musta* (*Cyperus rotundus*)[8]. Spots which are indicative of presence of piperine in *Pippali*(*Piper longum*), which is to be observed at Rf value of 0.44 is absent in the TLC of the sample under all visualisations[9]. The spots observed at Rf values 0.15, 0.31 and 0.60 under UV 256 nm

and 0.31 and 0.60 under UV 366 nm corresponds to phytoconstituents present in *Ativisha*(*Aconitum heterophyllum*) [10]. The specific Rf values indicative of certain phytochemicals present in *Karakatashringi* (*Piatachia interigemma*) are absent [8]. The peaks observed at Rf value of 0.45 under visualisations at UV 256 nm and UV 540 nm is suggestive of the presence of Vascicinone [11]. HPTLC was run for a time period of 30 mins and hence the first set of compounds observed are those which are acid-base strength compounds [12]. Since there are many a number of spots which are not identified before during the standardisation of *Churna*, it can be assumed that in *Arka* there is the presence of a number of volatile phytochemicals. Also, absence of certain spots which are seen in case of *Churna* is indicative of the absence of those phytochemicals when the dosage form of the formulation has been changed [8, 13].

Comparative GCMS analysis of *Balachaturbhadra* Arka and Kashaya

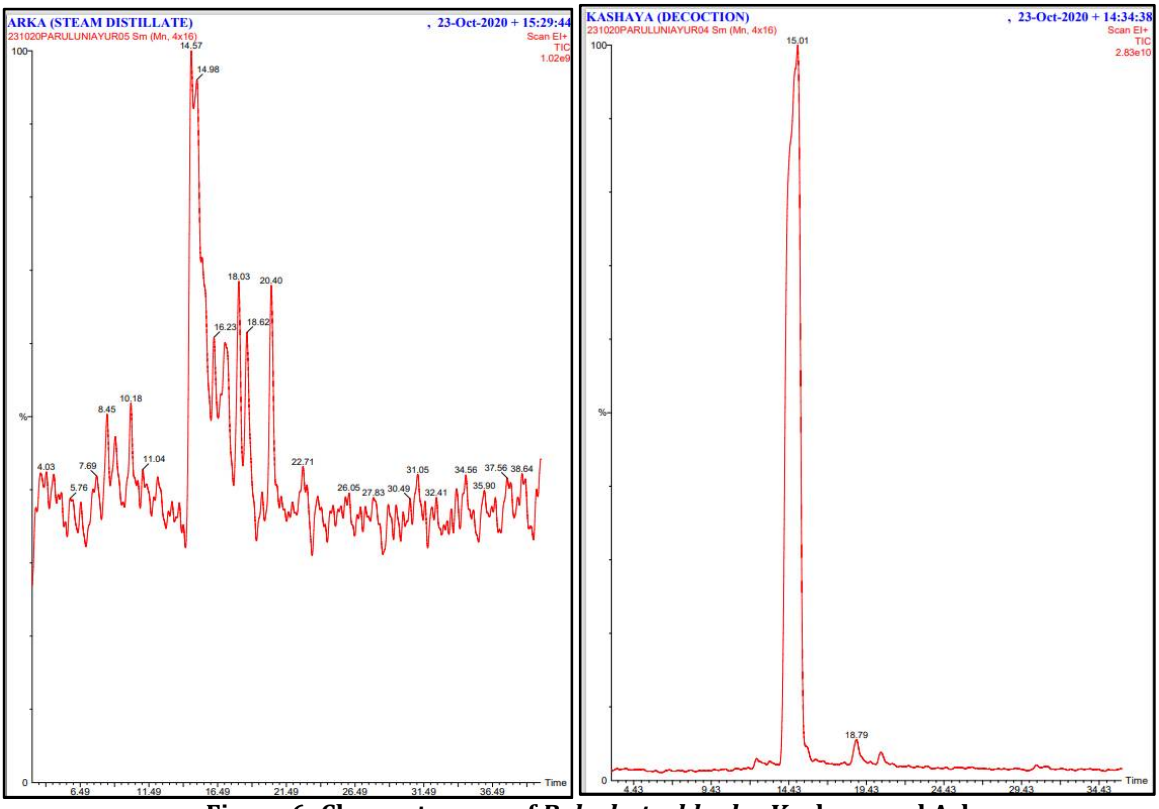


Figure 6: Chromatogram of *Balachaturbhadra* Kashaya and Arka

Table 3: Compounds identified by MS interpretation in Kashaya and Arka

Sl. No	***Balachaturbhadra* Kashaya**	***Balachaturbhadra* Arka**
1	N-(4-Carboxymethyl)-N'-Phenyl-Urea	Salicyl hydrazide
2	Benzoic acid,3-Hydroxy-Methyl ester	Benzoic acid,3-Hydroxy-Methyl ester
3	1-Chloro-1-Ethyl-1-Silacyclopentane	Benzyl O-Nitro benzoate
4	Methyl anthranilate	Benzoic acid,4-hydroxy,2-hydroxypropyl ester
5	Piperonyl alcohol	Propanoic acid, 3-chloro,2-chloroethyl ester
6	Cyclopropanecarboxylic acid,1-Cyano-2-ethenyl-ethyl ester	13-Docosenoic acid, methyl ester
7	N-Methylsalicylamide	Nicotonic acid,2-amino-hydrazide
8	Nicotonic acid,2-amino-hydrazide	Benzoic acid,4-hydroxy,hydrazide
9	Benzaldehyde	
10	Benzoic acid,3-[[2-(Methoxysulfonyl)Acetyl]Amino]-,methyl ester	
11	Benzooxazole-2-thiol	
12	3-(3,4,5-trimethoxyphenyl) propionic acid	

Apart from these identified compounds, many other novel compounds were found in Arka when compared to Kashaya, which indicates the importance of the pharmaceutical processing. The method of preparation of Arka involves distillation and preserves many thermolabile and volatile compounds. This might be the probable reason behind the presence of this number of bioactive chemical compounds in Arka. Benzoic acid,3-Hydroxy-Methyl ester and Nicotonic acid,2-amino-hydrazide were found in common in both the formulations. Benzoic acid,3-Hydroxy-Methyl ester is a natural product. Esters detected in the

formulation can be due to the presence of Musta [1] in the formulation. Aconitum and Piperine has not been identified through GCMS in both Kashaya and Arka.

Total of 3 peaks and 22 peaks were observed in the chromatogram of *Balachaturbhadra* Kashaya and *Balachaturbhadra* Arka respectively. Number of prominent peaks identified for *Balachaturbhadra* Kashaya and Arka are respectively 3 and 10.

Table 4: Prominent peaks and retention times of *Balachaturbhadra* Kashaya and Arka

Sample	No. of peaks	Retention time
Balachaturbhadra Kashaya	3	15.008,18.795,20.365
Balachaturbhadra Arka	10	8.451, 9.041,10.176,14.568,14.983,16.229, 17.029,18.029,18.625,20.395

CONCLUSION

Standardization is needed to establish quality control parameters for each traditional drug before it is released for use without the fear of toxicity and contamination. The novel concentrated *Arka* thus developed yield better acceptability in terms of palatability, increased shelf life and as compared to prescribed forms in a lesser dose. Here efforts have been made to provide scientific data on formulation and standardization of *Arka*.

REFERENCES

1. Srivasta, S. (2011), Sharangdhara Samhita of Acharya Sharangadhara, Chaukambha Orientalia, Varanasi. 1st edition, 6/16. pp. 175
2. Misra, B. S. (2011), Bhavaprakasha of Sri Bhavamisra, Chaukambha Sanskrit Bhavan, Varanasi. 1st edition, (2), 71/151. pp 803
3. Sharma, P. (2011). Chakradatta, Chaukambha Orientalia, Varanasi. 1st edition, 64/23, pp. 547
4. Misra, B. S. (2016) Bhaishajyaratnavali of Sri Govind Das, Chaukambha Prakashan, Varanasi. 18th edition, 71/39,pp. 1076
5. Pandey, R. (2011) Yogaratnakara, Chaukambha Visvabharati, Varanasi. 1st edition, 2011 71/39, pp.878
6. Mukherji, V. H.(1967). Balarogadhikara, Anandavana Publications, Bombay Press, Bombay, 2nd edition, pp. 63
7. Sethi, P. D. (1996). *HPTLC: high performance thin-layer chromatography; quantitative analysis of pharmaceutical formulations*. CBS publishers & distributors.pp.1-56.
8. Buddhadev, S.S.(2014). Standardisation of an ayurvedic pediatric formulation "balachaturbhadrika churna". *International Journal of Ayurveda and Pharmaceutical Chemistry*, 1(1),1-8
9. Vyas, A., Jain, V., Singh, D., Singh, M., Shukla, S. S., Pandey, R., ... & Saraf, S. (2011). TLC densitometric method for the estimation of piperine in ayurvedic formulation Trikatu churna. *Oriental Journal of Chemistry*, *27*(1), 301.
10. Rajakrishnan, R., Lekshmi, R., & Samuel, D. (2016). Analytical standards for the root tubers of Ativisha-Aconitum heterophyllum Wall. ex Royle. *Int J Sci Res Publ*, *6*(5), 531-534.
11. Suthar, A. C., Katkar, K. V., Patil, P. S., Hamarapurkar, P. D., Mridula, G., Naik, V. R., ... & Chauhan, V. S. (2009). Quantitative estimation of vasicine and vasicinone in Adhatoda vasica by HPTLC. *Journal of Pharmacy Research*, *2*(12), 1893-1899.
12. Gokarn, R., Rajput, D., Wanjari, A., Rathi, B., & Kharabe, P. M. (2016). Drug Dosage Modification and Standardization of Balachaturbhadra Syrup. *Journal of Indian System of Medicine*, *4*(1), 15.
13. Arora, A., Kashyap, C. P., & Arya, V. (2015). Quality control procedures in respect of preparation and evaluation of potential Ayurvedic compound powder formulation Balchaturbhadrika churna. *Journal of Pharma Research*, *4*(12), 406-409.

CITATION OF THIS ARTICLE

Deepthi Viswaroopan, Sudhirkumar Pani, Prasanna Mathad, Lekshmi C. S. Preparation and Standardization of an Ayurvedic Polyherbal Formulation. Bull. Env. Pharmacol. Life Sci., Vol 12[3] Feb 2023: 70-76.