ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

DOSAGE FORM DEVELOPMENT OF *NAGABALA- ARJUNADI YOGA* AND CHROMATOGRAPHIC ANALYSIS USING HPTLC

Sushya Surendran[1]*, Mandip Goyal[2], Vinay J Sukla[3], Harisha C.R[4]

*[1]PhD Scholar, [2]Associate Professor, Department of Kayachikitsa, [3]Head, Pharmaceutical Chemistry Laboratory, [4]Head, Pharmacognosy Laboratory, ITRA, Jamnagar, India.

Article info

Article History:

Received: 27-07-2022

Revised: 05-08-2022

Accepted: 17-08-2022

KEYWORDS:

HPTLC, *Nagabala-Arjunadi Yoga,* Pharmacognostical, Pharmaceutical.

ABSTRACT

Nagabala -Arjunadi Yoga, is the combination of *Nagabala* and *Arjuna Churna* mentioned in Chakradatta, *Hridroga Chikitsa*, is prepared by giving *Bhavana* of *Rasonadi Kwatha. Hridroga* (cardiovascular disorders) are the most common health concern of the present era. It is the leading cause of death worldwide. Ancient *Samhitas* contain many formulations in the context of *Hridroga*, whose applicability is unexplored. *Churna* and *Kwatha* are the main dosage forms used in clinical practice. But compared to *Churna* and *Kwatha*, tablets are more patient compatible in terms of palatability and possess increased shelf life. Hence, *Nagabala-Arjunadi Yoga,* a tablet dosage form is developed using *Nagabala- Arjuna Churna* and *Rasonadi Kwatha*. No scientific evaluation data for this drug is available to date. The present study was done to evaluate the pharmacognostical and pharmaceutical profile of *Nagabala-Arjunadi Yoga*. The microscopic examination of the *Nagabala- Arjunadi Yoga* showed the presence of rosette crystals, rhomboidal crystals, simple fibres, oil globules and stones cells. The physicochemical analysis showed that pH value, hardness, loss on drying, ash value, water extractive value and methanol extractive value was 5.8, 3.5kg/cm^2, 7.949%, 3.03%, 17.43%, 16.14% respectively. The HPTLC densitograms at UV 254 nm and UV 366nm using Toluene and Ethyl acetate in the ratio 9:1 showed maximum peak height in 3rd peak corresponding to the Rf value 0.18 and 0.17 respectively. The finding observed in the present study can be used as reference for future quality control.

INTRODUCTION

Ayurveda is becoming seemingly important in the present era due to its role in treating chronic diseases. Back in history of time, the world was battling communicable diseases, but the present scenario has changed due to the demographic transition and industrialization. Now the non-communicable diseases like cardiac disease, cancer, chronic pulmonary diseases and diabetes have become the culprit. Herbal medicine is used increasingly in the world to treat such chronic conditions. Most of the Ayurvedic formulations being polyherbal contain high variability of chemical compounds.

Access this article online

Quick Response Code

https://doi.org/10.47070/ijapr.v10i8.2453

Because of this, quality control becomes a challenge. Poly herbalism has its roots in the earliest texts of Ayurveda like *Caraka Samhita*, *Susrutha Samhitha*, *Ashtanga Hrudaya*, which systematizes the pharmaceutical procedures. The traditional Ayurvedic text *Sarangdhar Samhita*, which dates from 1300 AD, highlights the concept of polyherbalism in this ancient medical system.[1] Recent research shows that combining plants of varying potency enhances their effect when compared to individual plant use and the sum of their individual effect. This phenomenon is known as synergy. Some pharmacological actions, from the active constituents of some herbs, have proven to be significant only when potentiated by those of other plants, but are not evident when used alone.[2]

Considering different dosage forms are important for a physician while practising, various dosage forms are also mentioned in Ayurveda classics. *Churna* and *Kwatha* are the main dosage forms used in clinical practice. They are often overlooked due to the

preparation methods and palatability. Moreover, most of the time once the conventional diagnosis is made, the patients opt for Ayurveda treatment alongside the conventional treatment. In chronic conditions this scenario is often seen. Hence, it becomes inevitable for the physician to decrease the number of medicines. Compared to *Churna* and *Kwatha*, tablets are more patient compatible in terms of palatability and possess increased shelf life. Hence, in the present study, *Nagabala Arjuna churna* was given *Bhavana* with *Rasonadi Kwatha* and tablets were made. *Nagabala Arjuna churna* is quoted in *Hridroga chikitsa*, stating that it cures the Hridroga by acting *as Balya, Rasayana* and *Vatahara.*[3] *Rasonadi kwatha* is a known formulation used in *Hridroga*, it is also best *Vatahara Yoga.*[4] The combination is made to enhance the potency of the main drugs i.e., *Nagabala* and *Arjuna*. As no reference standards are available for this compound formulation, an attempt has been made to analyse the physicochemical and analytical profile of *Nagabala Arjunadi Yoga.*

MATERIALS AND METHODS

Collection and authentication of raw drugs

The raw drugs *Arjuna*, *Rasona*, *Karavi* and *Krishna* were obtained from the authorized local vendor, Jamnagar. *Nagabala* and *Shalaparni* were obtained from genuine drug suppliers from Junagadh. The ingredients and the part used are given in Table 1 and Table 2. The API standards based on the morphological features, organoleptic characters and powder microscopy were used for pharmcognostical authentication of *Arjuna*,[5] *Rasona*,[6] *Karavi*,[7] *Krishna*[8] and *Salaparni*.[9] (Figure 1 (a), (c), (d), (e), (f)) *Nagabala* was authenticated with reference to previous works on the same. [10] (Figure 1 (b))

Table 1: Ingredients of *Nagabala Arjuna Churna*

S. No	Drug	Botanical Name	Part Used	Proportion
1	*Nagabala*	*Grewia tenax* (Forssk.) Fiori	Root	10 kg
2	*Arjuna*	*Terminalia arjuna* (Roxb.) W.& A.	Stem Bark	10 kg

Table 2: Ingredients of *Rasonadi Kwatha* (*Bhavana Dravya*)

S. No	Drug	Botanical Name	Part Used	Proportion
1	*Rasona*	*Allium sativum* Linn.	Bulb	5 kg
2	*Karavi*	*Nigella sativum* Linn.	Seed	5 kg
3	*Krishna*	*Piper longum* Linn	Fruit	5 kg
4	*Sthira*	*Desmodium gangeticum* DC.	Root	5 kg

Method of Preparation of *Nagabala- Arjunadi Yoga*

Dried raw materials of *Nagabala* and *Arjuna* in equal proportion were pulverised into fine powder separately. Equal proportions of *Rasona*, *Karavi*, *Krishna* and *Sthira* were pulverised into coarse powder and *Kwatha* was prepared as per the standard reference mentioned in *Sharangdhara Samhita.*[11] *Nagabala* and *Arjuna* were mixed together and potentiated by giving seven *Bhavanas* of *Rasonadi Kwatha* in the edge runner mill. (Figure 2a, 2b) Tablets of 500mg each, were prepared by granulation[12] and compression[13] method using the acacia gum 10% as the binding agent and stored in proper hygienic condition. (Figure 3) The final product was prepared in pharmacy of Gujarat Ayurved University.

Pharmacognostical Evaluation

Pharmacognostical analysis of the finished product, *Nagabala Arjunadi Yoga* was analysed using organoleptic characteristics and microscopic examination. A small quantity of the finished product was dissolved in distilled water and filtered through the filter paper, and the filtrate was studied under the Corl Trinocular microscope attached with camera, with stain and without stain. Microphotographs were also taken under the microscope.[14] The cellular components identified under the microscope were compared with the characters of individual drugs of the finished product.

Pharmaceutical Evaluation

Physicochemical Analysis

The physicochemical analysis of *Nagabala-Arjunadi Yoga* was carried out at Modern Pharmaceutical Chemistry Laboratory, IPGT & RA, Gujarat Ayurved University, Jamnagar. The quality control parameters mentioned for compressed tablets in Ayurvedic Pharmacopoeia of India[15] and CCRAS[16] guidelines i.e., hardness, total ash, pH value, water and alcohol soluble extractives were analysed. The presence of more moisture content in a sample can create a preservation problem. Hence loss on drying was also selected as one of the parameters.[17]

High- Performance Thin Layer Chromatography Study (HPTLC)

Methanolic extract of *Nagabala Arjunadi Yoga* was spotted on pre-coated silica gel GF 60_{254} aluminium plate as 5mm bands, 5mm apart and 1cm from the edge of the plates, by means of a Camang Linomat V sample applicator fitted with a 100µL

Hamilton syringe for comparative analysis. Toluene: Ethyl acetate (9:1) was used as the mobile phase. After development, a densitometric scan was done with Camang TLC scanner III in reflectance absorbance mode at 254 nm and 366nm UV detection.

OBSERVATION AND RESULTS

Pharmacognostical Study

The initial purpose of the study was to evaluate the authenticity of the raw drug used to prepare the *Nagabala- Arjunadi Yoga*. The final product *Nagabala-Arjunadi Yoga* in tablet form was subjected to organoleptic analysis and microscopic examination to authenticate the drug.

Organoleptic Analysis

Organoleptic characteristics like state, colour, odour and taste of *Nagabala- Arjunadi yoga* were recorded as shown in table 2.

Table 3: Organoleptic Characteristics of *Nagabala- Arjunadi Yoga*

S. No.	Parameters	Result
1	State	Tablet
2	Colour	Light Brown
3	Odour	Characteristic
4	Taste	Pungent

Microscopic Examination

The microscopic examination of *Nagabala-Arjunadi Yoga* showed the following features of its individual drug. Plate 1 (Figure 4) shows the brown content [fig.4a], cluster cells [fig.4b], rosette crystals [fig.4c], lignified fibres [fig.4d] and starch grains [fig.4e] that are the microscopic characters of *Arjuna.* Plate 2 (Figure 5) shows the rhomboidal crystals [fig.5a], group of fibres [fig.5b], simple fibres [fig.5c], crystal fibres [fig.5d], brownish content [fig.5e] and pitted vessels [fig.5f] , are the specific microscopic features of *Nagabala.* Plate 3 (Figure 6) showing the simple fibres is the specific microscopic feature of *Rasona* [fig.6a]. Plate 4 (Figure 7) shows the oil globules [fig.4a] and mesocarp cells [fig.4b], are the microscopic character of *Karavi.* Plate 5 (Figure 8) shows the epicarp cells [fig.8a] and stone cells [fig.8b], are specific microscopic features of *Pippali.* Plate 6 (Figure 9) shows the trichome [fig.9a], pitted vessels [fig.9b] and spiral vessels [fig.9c], that are the microscopic character of *Sthira.*

The microscopic evaluation authenticates the individual drugs used in the preparation of the final product i.e *Nagabala Arjunadi Yoga.*

Pharmaceutical Study

Physicochemical Parameters

Physicochemical Parameters of the tablet like uniformity, hardness, ash value and loss on drying were all found to be within the normal range. The water- soluble extractive and methanol soluble extractive values were found to be 17.43% w/w and 16.1410% w/w respectively. (Table 4)

Table 4: Physicochemical Parameters of *Nagabala- Arjunadi Yoga*

Test		Results
Uniformity of Tablet	Average	526.78gm
	Highest	541gm
	Lowest	471gm
Hardness		3.5 kg/cm^2
Loss on Drying		7.949 %
Ash value		3.03%
Water soluble extract		17.43%
Methanol soluble extract		16.1410%
pH value (5% aqueous solution)		5.8

High-Performance Thin Layer Chromatography Study (HPTLC)

The densitogram of methanol extract of *Nagabala Arjunadi Yoga* showed 8 peaks corresponding to the Rf values 0.07, 0.09, 0.18, 0.24, 0.29, 0.72, 0.87 and 0.98 respectively when visualized at 254nm. At 366nm, the densitogram showed 8 peaks corresponding to Rf values 0.07, 0.09, 0.17, 0.24, 0.29, 0.64, 0.72 and 0.87 respectively as shown in table 5. The HPTLC densitogram is showed in Figure 10 (Plate 7).

Table 5: HPTLC of *Nagabala- Arjunadi Yoga*

Sample	Visualization	No. of Peaks	Max Rf	Area %
Nagabala- Arjunadi Yoga	254 nm	8	0.07	6.39
			0.09	2.1
			0.18	35.96
			0.24	12.71
			0.29	16.43
			0.72	12.51
			0.87	13.26
			0.98	0.64
	366 nm	8	0.07	4.09
			0.09	1.25
			0.17	26.88
			0.24	10.66
			0.29	13.57
			0.64	20.04
			0.72	16.53
			0.87	6.98

Figure 1

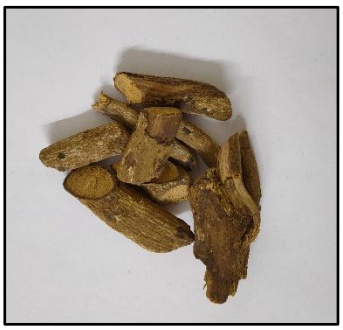
(a) *Arjuna*

(b) *Nagabala*

(c) *Rasona*

(d) *Karavi*

(e) *Karavi*



(f) *Sthira*

Figure: 2

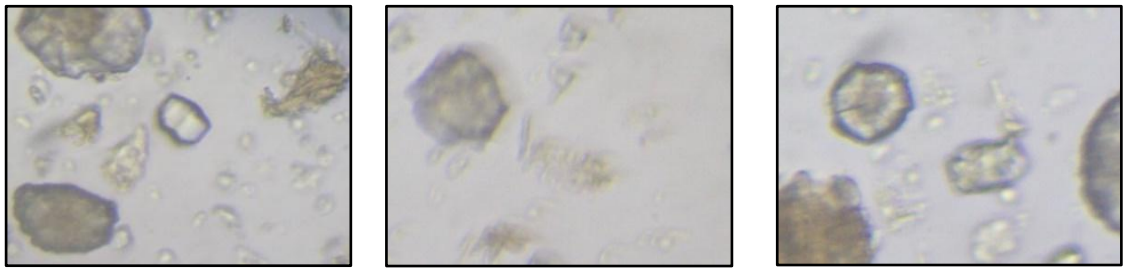
(a) *Nagabala- Arjuna churna*

Figure: 3

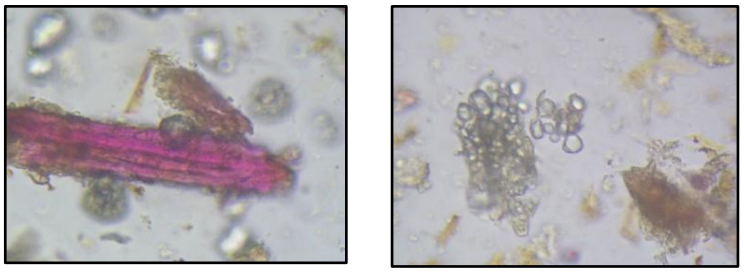
(b) *Rasonadi Kwatha*

(c) *Nagabala- Arjunadi Yoga* Tablet

Figure: 4 (Plate 1)

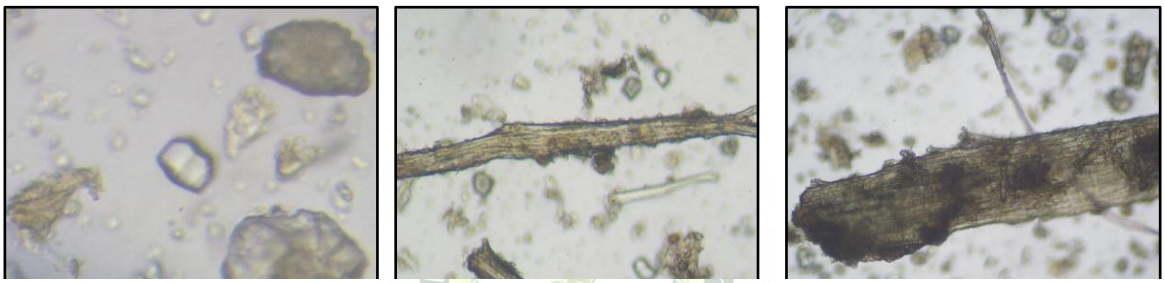


(a) Brown content (b) Cluster Crystals (c) Rosette Crystals

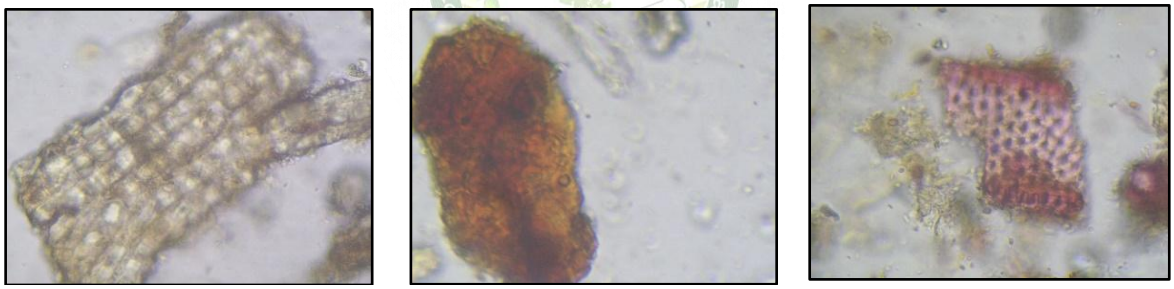


(d) Lignified fibres (e) Starch Grains

Figure: 5 (Plate 2)

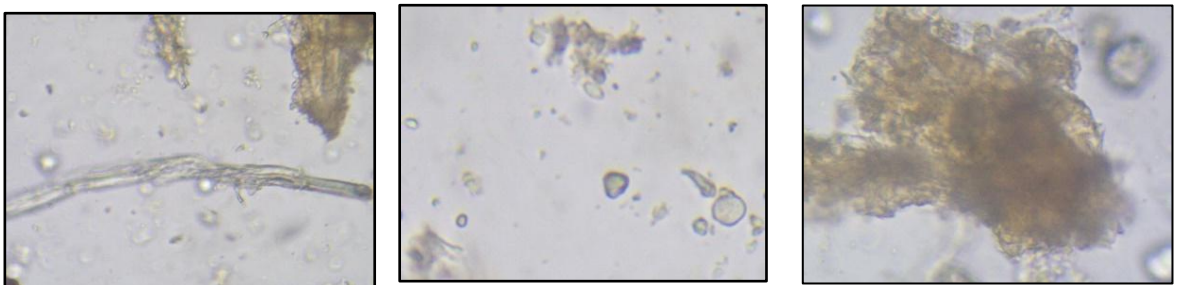


(a) Rhomboid Crystal (b) Simple fibre (c) Group fibre



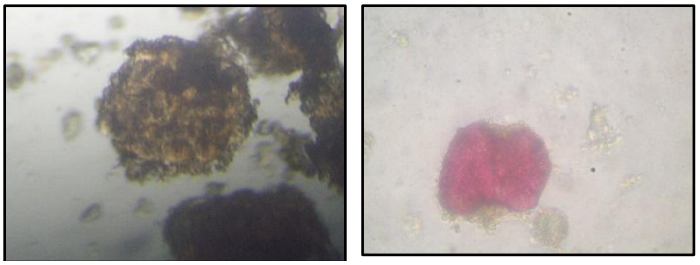
(d) Crystal fibre (e) Brownish content (f) Pitted vessels

Figure: 6 (Plate 3) **Figure: 7 (Plate 4)**



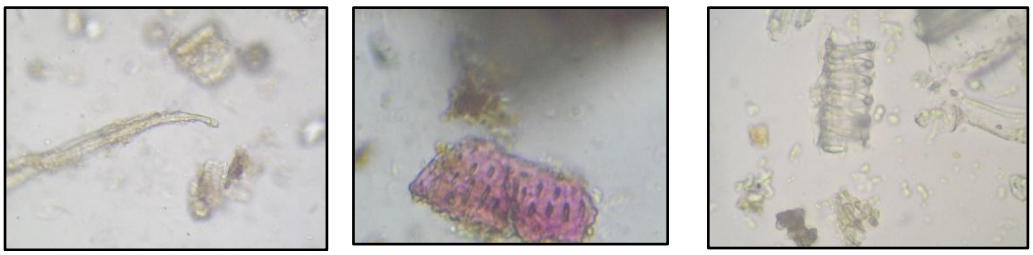
(a) Simple fibres (b) Oil globules (c) Mesocarp cells

Figure: 8 (Plate 5)



(a) Apicarp cells (b) Group of stone

Figure: 9 (Plate 6)



(a) Trichome (b) Pitted vessels (c) Spiral vessels

Figure 10 (Plate 7): Densitogram of *Nagabala Arjunadi Yoga*

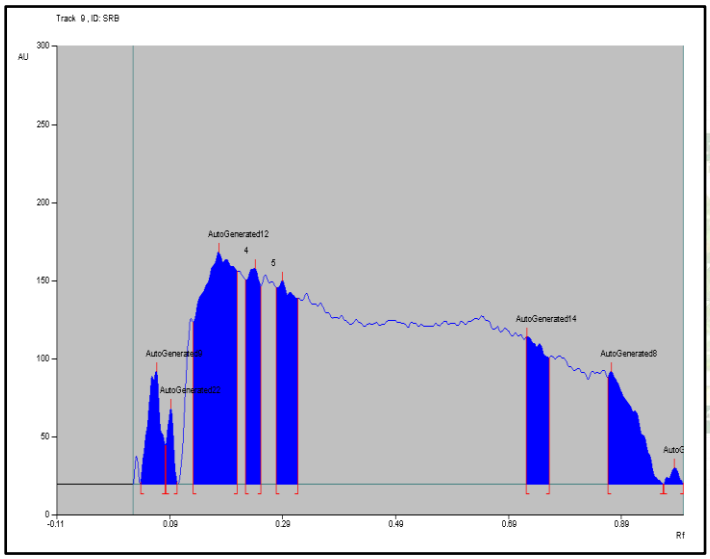


Fig 7A at 254nm

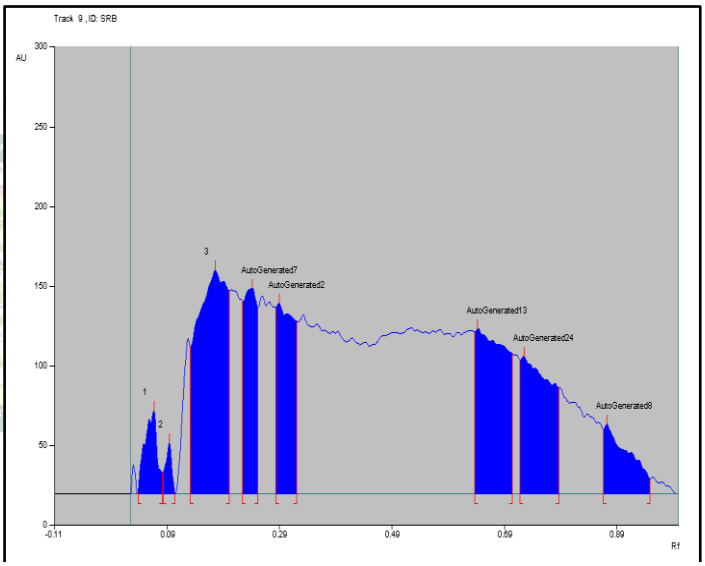


Fig 7B at 366n

DISCUSSION

Pharmacognostic evaluation helps to screen the commercial varieties, substitutes, adulterants and any other quality control of the drugs. It is a simple and reliable tool, helps to obtain information about biochemical and physical properties of crude drug.[18] The pharmacological study of the final product *Nagabala Arjunadi Yoga* revealed all the striking features of the individual drug used for the manufacturing process. This confirms the authenticity of the finished product. Moreover, there was no major change in the characteristics of the microscopic features observed in the final product. The physicochemical analysis was done to establish the quality of the finished product. All the parameters used for the physicochemical analysis of *Nagabala Arjunadi Yoga* was found within limits. Hardness was found to be 3.5kg/cm^2 which indicate that the finished product was durable to withstand the packing and shipping. Ash value of 3.03% indicates the presence of foreign inorganic matter which was within the permissible limit. Water extractive values were little higher than alcohol extractive values which indicate water is better solvent for extraction of the formulation. HPTLC, is used for the identification of constituents, identification and determination of impurities, and quantitative determination of active substances. The high performance thin layer chromatographic analysis (HPTLC) of the finished product showed 8 peaks at UV 254 nm corresponding to the Rf values 0.07, 0.09, 0.18, 0.24, 0.29, 0.72, 0.87 and 0.98. At UV 366nm visualization, 8 peaks were spotted corresponding to the Rf values 0.07, 0.09, 0.17, 0.24, 0.29, 0.64, 0.72 and 0.87. The maximum area percentage i.e., 35.96 corresponds to the Rf value 0.18 at UV 254nm visualization. At UV 366 nm, maximum area percentage i.e., 26.88 corresponds to the Rf value 0.17.

The max area percentage corresponding to the Rf values 0.18 and 0.17 signifies the highest quantitative presence of chemical compound of the final product.

CONCLUSION

The microscopic examination of the *Nagabala-Arjunadi Yoga* showed the presence of rosette crystals, rhomboidal crystals, simple fibres, oil globules and stones cells which are the components of individual drugs of the final product. The physicochemical analysis showed that pH value, hardness, loss on drying, ash value, water extractive value and methanol extractive value was 5.8, 3.5kg/cm^2 , 7.949%, 3.03%, 17.43%, 16.14% respectively. HPTLC analysis showed maximum area percentage corresponding to the Rf value 0.18 and 0.17. As no study is available to date for the quality control for the given finished product, present study can be used as a standard reference for further quality control research. Further analytical studies can be proposed for precise identification of the chemical compounds which helps in drug development and understanding the therapeutic potential.

REFERENCES

1. Srivastava S, Lal VK, Pant KK. Polyherbal formulations based on Indian medicinal plants as antidiabetic phytotherapeutics. Phyto-pharmacology. 2013; [2]:1-15
2. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: concept of Ayurveda. Pharmacogn Rev. 2014; 8[16]: 73-80
3. Cakrapanidatta. Cakradatta. 1st editio. Chaukhambha orientalia, Varanasi; 2014. p. 325.
4. Aravattazhikathu K V Krishnanvaidyan ASG. Rasonadi kashayam. In: Sahasrayogam. 28th editi. Vidyarambham Publishers; 2009.
5. Anonymous. The Ayurvedic Pharmacopoeia of India. Part 1. Vol.- 1. 1st ed. New Delhi; Ministry of Health and Family Welfare, Department of AYUSH Government of India; 2001. p. 5
6. Anonymous. The Ayurvedic Pharmacopoeia of India. Part 1. Vol.- 3. 1st ed. New Delhi; Ministry of Health and Family Welfare, Department of AYUSH Government of India; 2001. p. 163
7. Anonymous. The Ayurvedic Pharmacopoeia of India. Part 1. Vol.- 1. 1st ed. New Delhi; Ministry of Health and Family Welfare, Department of AYUSH Government of India; 2001. p. 202
8. Anonymous. The Ayurvedic Pharmacopoeia of India. Part 1. Vol.- 4. 1st ed. New Delhi; Ministry of Health and Family Welfare, Department of AYUSH Government of India; 2001. p. 142
9. Anonymous. The Ayurvedic Pharmacopoeia of India. Part 1. Vol.- 3. 1st ed. New Delhi; Ministry of Health and Family Welfare, Department of AYUSH Government of India; 2001. p. 258
10. Sulieman, A. M. E., & Mariod, A. A.Grewia tenax (guddaim): Phytochemical constituents, bioactive compounds, traditional and medicinal uses. In Wild Fruits: Composition, Nutritional Value and Products. 2019; 165–173. Springer International Publishing. https://doi.org/10.1007/978-3-030-31885-7_14
11. Sharangdhar A. Sharangdhar samhita. Madhyam Khanda. Ch. 9 Varanasi; Choukhambha Surbharti Publication; 2004. Verse no.3-4
12. Troy DB. Remington. Lippincott Williams and wilkins.The science and practice of pharmacy. vol.1, Baltimore; 21st edition, Indian edition; 2005. p-896
13. Troy DB. Remington. Lippincott Williams and wilkins. The science and practice of pharmacy. vol.1, Baltimore; 21st edition, Indian edition; 2005. p-901
14. Anonymous. The Ayurvedic Pharmacopoeia of India. New Ministry of Health and Family welfare. Department of AYUSH Government of India. Delhi; Part 2. Vol.- 1, 1st edition, 2008.136-139.
15. Anonymous. Protocol for testing of Ayurveda. Siddha & Unani medicines, Pharmacopeial laboratory for Indian medicines. Ghaziabad, Ministry of AYUSH, Government of India.
16. Anonymous. Parameters for qualitative assessment of Ayurveda, Siddha drugs, CCRAS, New Delhi; 2005.
17. Anonymous. The Ayurvedic Pharmacopoeia of India. Part II (Formulation),.Volume I, First edition, Ministry of AYUSH. Government of India. New Delhi; 2007. 140-147.
18. Pharmacognostical Studies. Prog Drug Res. 2016; 71: 5-10. PMID: 26939259.

Cite this article as:
Sushya Surendran, Mandip goyal, Vinay J Sukla, Harisha C.R. Dosage Form Development of Nagabala- Arjunadi Yoga and Chromatographic Analysis Using HPTLC. International Journal of Ayurveda and Pharma Research. 2022;10(8):27-33.
https://doi.org/10.47070/ijapr.v10i8.2453
Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Dr. Sushya Surendran
Phd Scholar,
Kayachikitsa Department,
ITRA, Jamnagar.
Email: drsushyasu@gmail.com