



MICROBIOLOGICAL, PHARMACOGNOSTICAL AND PHARMACEUTICAL ANALYSIS OF BILVADI ASCHYOTANA YOGA – A COMPOUND AYURVEDIC FORMULATION

¹Dr. Jyoti G. Khokhani, ²Dr. D.B. Vaghela, ³Dr. Deepak K. Pawar, ⁴Harisha C. R.

*¹ 2nd year PG Scholar, ²I/c HOD and Associate Professor, ³Assistant Professor, ⁴Head Pharmacognosy Lab.

^{1,2,3} Department of Shalaky Tantra I.T.R.A., Jamnagar, Gujarat. Email – ¹jpatel61197@gmail.com

ABSTRACT

Aim and Objective: *Bilvadi Aschyotana Yoga*ⁱ is a choice of drug given in *Chakradatta* in the treatment of *Vataja Abhishyanda*ⁱⁱ. Symptoms of the Simple Allergic Conjunctivitis can be compared with classical symptoms of *Vataja Abhishyanda*. In present study attempt has been made to evacuate microbiological and develop pharmacognostical and pharmaceutical standards as per Ayurvedic Pharmacopoeia of India (API) for *Bilvadi Aschyotana Yoga*. **Methodology:** Preliminary Microbiological studies, Pharmacognostical and Pharmaceutical parameters, High-Performance Thin Layer Chromatography has been performed as per Standard method. **Result:** Microbiological study for *Bilvadi Aschyotana Yoga* revealed absence of any microbial contamination under smear and culture study in aerobic atmosphere after 90 days of sample been prepared. The Pharmacognostical study of ingredients of *Bilvadi Aschyotana Yoga* shows the presence of the fibers of *Bilva*, *Agnimantha*, *Gambhari*, *Patala*, Lignified cork cells of *Shyonaka*, *Gambhari*, *Patala*, spiral vessels of *Brihati*, Tannin and Rhomboidal crystal of *Patala* etc. Pharmaceutical analysis of *Bilvadi Aschyotana yoga* showed that loss on drying 2.10%, water soluble extract 2.12% w/w, alcohol soluble extract 1.32% w/w, pH 7.5 etc. and HPTLC at 254 nm resulted into 6 spots and at 366 nm resulted into 8 spots respectively. **Conclusion:** Standard value from this study can be used for further researches and evaluation of *Bilvadi Aschyotana Yoga* helps in Quality control tool for its manufacturing or processing. Analysis through Pharmacognostical and Pharmaceutical shows suitability of drug for *Aschyotana* purpose.

Key words: *Bilvadi Aschyotana Yoga*, Microbiological, Pharmacognostical and Pharmaceutical, *Vataja Abhishyanda*.

INTRODUCTION

In this era people are more aware of diseases and expecting medical science to satisfying their needs on curative plus preventing aspect. Thus, Ayurvedic medicines are on great demand. As traditional science have vast variety and individualist concept there is a more need to research on *Ayurvedic* drugs to standardize so that all over the world medicine can be used with maximum safety.

As eye is an important sense organ in whole body and also more sensitive to external environment or pathogens. *Abhishyanda* is one of the common *Netra Roga* described in *Sushruta Samhita*. The symptoms like *Nistoda* (Pricking Pain), *Sangharsha* (Foreign body sensation), *Ragata* (Redness), *Vishushka Bhava* (Dryness - feeling), *Achhasruta* (Clean/ Watery discharge), *Alpa Shopha* (Mild Chemosis), *Kandu* (Itching), *Daha* (Burning Sensation) are given in *Vataja Abhishyanda*.ⁱⁱⁱ Thus, from the clinical manifestation point of view, symptoms of the Simple Allergic Conjunctivitis can be compared with classical symptoms of *Vataja Abhishyanda*. First treatment protocol in *Netra roga* is *Ashchyotana*. Among all the *Kriyakalpas* *Ashchyotana* is one of the most important and primary procedures.

Here *Bilvadi Aschyotana Yoga* a compound Ayurvedic formulation has been studied with pharmacognostical and pharmaceutical parameters and also microbiological analysis done. *Bilvadi Aschyotana Yoga* is given in *Netra Rogadhikara in Chakradatta*. It is a herbal preparation containing *Kwatha* of *Bilva*, *Agnimantha*, *Shyonaka*, *Patala*, *Gambhari*, *Eranda*, *Brihati*, *Tarkari*, *Madhu Shigru*. *Bilvadi Aschyotana Yoga* indicated in *Chikitsa* of *Vataja Abhishyand* for *Aschyotana* purpose.

An attempt has been made to study *Bilvadi Aschyotana Yoga* by pharmacognostical, pharmaceutical, physio-chemical parameters and develop HPTLC fingerprints of the compound formulation.

MATERIALS AND METHOD

Collection of Raw drug:

Raw drug materials were collected from raw drug store of pharmacy of I.T.R.A., Jamnagar. After obtaining raw drug proper drying and grinding done to make *Yavkuta* form of all the ingredients of *Bilvadi Aschyotana Yoga*. The ingredients and part used are given below. (Table 1)

Table 1: Ingredients of *Bilvadi Aschyotana Yoga*

No.	Name of Drug	Latin/English name	Part used	Proportion
1.	<i>Bilva</i>	<i>Aegle marmelos</i> Corr.	Root bark	1 Part
2.	<i>Agnimantha</i>	<i>Clerodendrum phlomidis</i> Linn.	Root bark	1 Part
3.	<i>Shyonaka</i>	<i>Ailanthus excels</i> Roxb.	Root bark	1 Part
4.	<i>Patala</i>	<i>Stereospermum suaveolens</i> DC.	Root bark	1 Part
5.	<i>Gambhari</i>	<i>Gmelina arborea</i> Linn.	Root bark	1 Part
6.	<i>Eranda</i>	<i>Ricinus communis</i> Linn.	Root bark	1 Part

7.	<i>Brihati</i>	<i>Solanum indicum</i> Linn.	Root bark	1 Part
8.	<i>Tarkari</i>	<i>Premna integrifolia</i> Linn.	Root bark	1 Part
9.	<i>Madhu Shigru</i>	<i>Moringa oleifera</i> Lam.	Stem bark	1 Part

Method of preparation of *Bilvadi Aschyotana Yoga*

After making *Yavakuta* of each drug i.e. *Bilva*, *Agnimantha*, *Shyonaka*, *Patala*, *Gambhari*, *Eranda*, *Brihati*, *Tarkari* and *Madhu Shigru/Shigru* next step is *Kwatha* preparation. For that in one part of *Bilvadi Yavakuta* eight times of water will be added and boil it upto $\frac{1}{4}$ th quantity remains. After preparation of *Kwatha*, it will be filter by using clean cotton cloth. Final preparation will be filled into clean container to avoid any contamination.

MICROBIOLOGICAL EVALUATION:

Microbiological investigation has been carried out of *Bilvadi Aschyotana Yoga* after 90 days from day of preparation at Microbiological laboratory of I.T.R.A., Jamnagar. Smear examination and Aerobic as well as fungal culture study has been carried out for *Bilvadi Aschyotana Yoga* under microscope. Smear examination: Gram's Stain and 10% KOH Preparation *Bilvadi Aschyotana Yoga* has been done. A sterile sample smear collected under aseptic condition. Culture study: Aerobic and Fungal culture has been assessed for the sample of *Bilvadi Aschyotana Yoga*.^{iv}

PHARMACOGNOSTICAL EVALUATION:

A. MICROSCOPIC STUDY: Individual raw drugs identified and verified with API, Finished drug was identified and authenticated by the Pharmacognosy lab, I.T.R.A., Jamnagar. The identification was carried out based on organoleptic features and microscopy of the prepared drug. For pharmacognostical evaluation, drugs studied under the Corl zeiss Trinocular microscope with staining and without staining. The microphotographs were also taken under the microscope.^v

B. ORGANOLEPTIC STUDY: *Bilvadi Aschyotana Yoga* was evaluated for organoleptic characters ie. colour, odour, touch and taste.

PHARMACEUTICAL EVALUATION:

A. Physico-chemical analysis: Physico-chemical Parameters of *Bilvadi Aschyotana Yoga* like loss on drying, water soluble extract and many more were determined as per the API guideline. *Bilvadi Aschyotana Yoga* was further subjected to High Performance Thin Layer Chromatography (HPTLC) study.^{vi}

B. HPTLC: Thin layer chromatography (HPTLC) study was carried out with dry methanol (MeOH) extract on pre-coated silica gel GF 6254 aluminium backed plate as 6mm bands, 8mm apart and 15cm from the edge of the plates, by means of a Camag Linomate V sample applicator fitted with a 100 μ L Hamilton syringe. The mobile phase used was Toluene: Ethyl acetate (9:1v/v). The plates were developed in Camag twin trough chamber (20 x

10 cm²) and spots were detected in short U.V. (254 nm), Long U.V. (366nm). Video Densitometry rTLC shiny app was used for documentation.^{vii}

RESULTS AND DISCUSSION:

Study of *Bilvadi Aschyotana Yoga* has been done to standardize the drug. For that Microbiological, Pharmacognostical and Pharmaceutical parameters were assessed.

1. MICROBIOLOGICAL EVALUATION: Microbiological study under microscopic examination till 90 days. it shows absence of any microorganisms on gram's stain and no organisms isolated after 48 hours of incubation at 37 degree Celsius under Aerobic Atmosphere. Similarly, for fungal culture, reveals absence of fungal filaments in 10% K.O.H preparation and on culture. No fungal pathogens found as shown in (Plate no. 1).

2. PHARMACOGNOSTICAL EVALUATION

2.1. MICROSCOPIC STUDY: Diagnostic microscopic characters of ingredients of *Bilvadi Aschyotana Yoga* showed the crystal fibers of *Bilva*, Fibers, Microsphenoidal crystals and calcium oxalate crystals of *Agnimantha*, Stone cells, lignified cork cells, rosette and prismatic crystals of calcium oxalate of *Shyonaka*, Fibers, lignified cork cells, tannin and rhomboid crystals of *Patala*, Fibers, lignified cork cells, stone cells and lysigenous cavities of *Gambhari*, Calcium oxalate crystals, starch grain, resin and tannin of *Eranda*, Fibers, spiral vessels, Stellate trichome and starch grains of *Brihati*, Fibers and calcium oxalate crystals of *Tarkari*, Sphaerocrystals, prism and fibers of *Madhu Shigru*. (Plate no. 2).

2.2. ORGANOLEPTIC STUDY: *Bilvadi Aschyotana Yoga* was evaluated for organoleptic characters ie. colour, odour, touch and taste as shown in (Table 2).

3. PHARMACEUTICAL EVALUATION:

3.1. PHYSICOCHEMICAL TESTS: Pharmaceutical analysis of *Bilvadi Aschyotana Yoga* showed that loss on drying 2.10% w/w, ash value 1.63% w/w, water soluble extract 2.12% w/w, alcohol soluble extract 1.32% w/w, pH 7.5, specific gravity 1.0031 and refractive index 1.2997 (at room temp.). (Table 3).

3.2. HPTLC STUDY RESULTS: Chromatographic study (HPTLC) was carried out under 254 and 366 nm UV to establish fingerprinting profile of which showed 6 spots at 254 nm and 8 spots at 366nm with Rf values were recorded which may be responsible for expression of *Bilvadi Aschyotana Yoga* pharmacological and clinical actions. (Table 4) (Plate no. 3).

Table 2: Organoleptic characters of *Bilvadi Aschyotana Yoga*

Color	Light brown
Taste	Bitter
Odour	Characteristic
Texture/ consistency	Watery/liquid

Table 3: Physio-chemical analysis of *Bilvadi Aschyotana Yoga*

Loss on drying	2.10%
Ash value	1.63%
Water soluble extract	2.12% w/w
Alcohol soluble extract	1.32% w/w
pH value	7.5
Specific gravity	1.0031
Refractive index	1.2997(at room temperature)

Table 4: High performance thin Layer Chromatography (HPTLC)

Sample	No. of spots	observation	Max. R _f value
<i>Bilvadi Aschyotana Yoga</i>	6	Observed under short U.V. Light (254 nm)	0.01, 0.19, 0.41, 0.52, 0.71, 0.77
<i>Bilvadi Aschyotana Yoga</i>	8	Observed under Long U.V. Light (366 nm)	-0.05, 0.01, 0.08, 0.28, 0.51, 0.57, 0.81, 0.99

PLATE 1: MICROBIOLOGICAL INVESTIGATIONS OF *BILVADI ASCHYOTANA YOGA*

Dr. Jyoti Khokhani - Shikshya

INSTITUTE FOR POST GRADUATE TEACHING & RESEARCH IN AYURVEDA
GUJARAT AYURVED UNIVERSITY JAMNAGAR
CULTURE REPORT FOR MICROBIOLOGICAL INVESTIGATIONS

Date of Request	Drug Preparation Date	Drug Sample Detail	Aerobic Culture Report	Fungal Culture Report
29/08/2022	09/05/2022	Bilvadi Aschyotan Kwath Decotion Form Randomly selected from Pouch no. 04 Container - 1 Opened	Microscopic Examination: Gram's Stain: Smear shows absence of microorganisms. On Culture : No organisms isolated. After 48 hrs. of incubation at 37°C Under Aerobic Atmosphere	Microscopic Examination: 10% K.O.H. Preparation: Structure resembling fungal filaments not seen. On Culture : No fungal pathogen isolated. After 07 days of incubation at 37°C Under Aerobic Atmosphere
29/08/2022	09/05/2022	Haridrakhandha Granules Randomly selected from Pouch no.03 Opened	Microscopic Examination: Gram's Stain: Smear shows absence of microorganisms. On Culture : No organisms isolated. After 48 hrs. of incubation at 37°C Under Aerobic Atmosphere	Microscopic Examination: 10% K.O.H. Preparation: Structure resembling fungal filaments not seen. On Culture : No fungal pathogen isolated. After 07 days of incubation at 37°C Under Aerobic Atmosphere

MB - No - 408, 409 DDr - AC - 31/08/2022 & FC - 05/09/2022

Microbiologist

PLATE 2: MICROSCOPIC STUDY OF *BILVADI ASCHYOTANA YOGA*


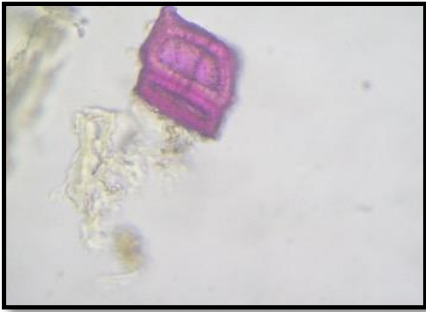



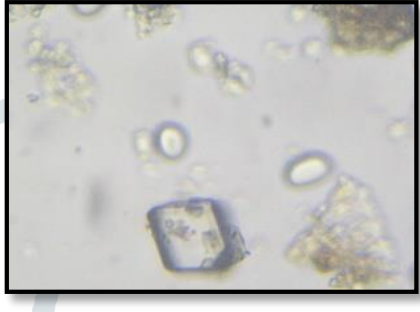
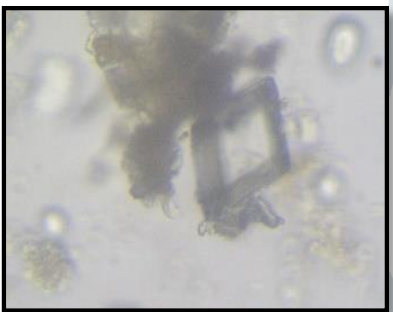




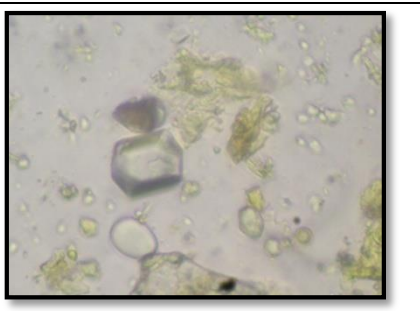
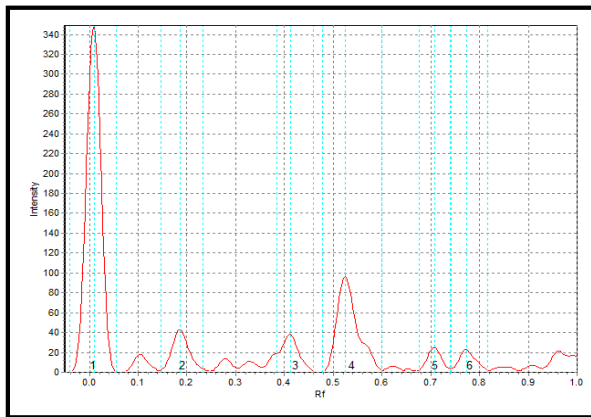
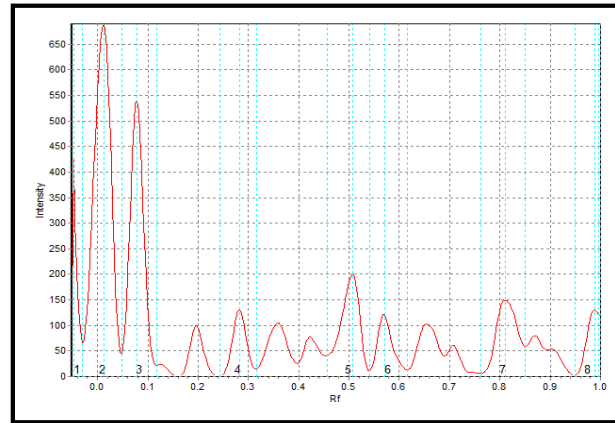
		
<p><i>Bilvadi Aschyotana Yoga</i></p>	<p>Lignified stone cell of <i>Bilva</i></p>	<p>Fibers of <i>Agnimantha</i></p>
		
<p>Crystal of <i>Shyonaka</i></p>	<p>Cork cell of <i>Patala</i></p>	<p>Crystal of <i>Patala</i></p>
		
<p>Crystal of <i>Gambhari</i></p>	<p>Spiral vessels of <i>Brihati</i></p>	<p>Trichome of <i>Brihati</i></p>
		
<p>Cork cells of <i>Eranda</i></p>	<p>Stone cells and crystal fibre of <i>Tarkari</i></p>	<p>Rhomboidal crystal of <i>Madhu Shigru</i></p>

PLATE 3: HPTLC STUDY: HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC STUDY (HPTLC)



Observed under short U.V. light (254nm)
Bilvadi Aschyotana Yoga



Observed under long U.V. light (366nm)
Bilvadi Aschyotana Yoga

DISCUSSION:

Study of *Bilvadi Aschyotana Yoga* has been done to standardize the drug formulation. Pharmacognostical study reveals the fibers of *Bilva*, *Agnimantha*, *Gambhari*, *Patala*, Lignified cork cells of *Shyonaka*, *Gambhari*, *Patala*, spiral vessels of *Brihati*, Tannin and Rhomboidal crystal of *Patala* etc. The presence of all contents of raw drugs shows the purity of final product. Pharmaceutical analysis of *Bilvadi Aschyotana Yoga* showed pH, water soluble extract, alcohol soluble extract etc. suitable for *Achyotana*. No any microbial growth found in final preparation till 90 days of preparation. The HPTLC is one of the advanced instrumental techniques for quantitative and qualitative analysis of herbal drugs. Study of this compound reveals the components are more sensitive to long U.V. light than short U.V. light. Further the HPTLC results can also be compared with standard of individual raw drug for obtaining and concluding standards for *Bilvadi Aschyotana Yoga*.

CONCLUSION:

Microscopic evaluation is mandatory to avoid adulteration and contamination in *Bilvadi Achyotana Yoga*. Analysis through Pharmacognostical and Pharmaceutical shows suitability of drug for *Aschyotana* purpose.

The results of these studies can be used for the reference standard, authentication and further researches.

REFERENCE:

- ⁱChakrapani Datta, *Chakradatta*, Editor Shivdasasen, *Netraroga Chikitsa Prakarana*, 59/13, Chaukhambha Sanskrit Sansthan, Varanasi, 1st Edition 1993, p. 658
- ⁱⁱKaviraj Ambikadutta Shastri, *Sushruta Samhita* of Acharya Sushruta, *Uttartantra* 6/6, reprinted edition (2017), Chaukhambha Sanskrit Sansthan Varanasi, p. 34
- ⁱⁱⁱKaviraj Ambikadutta Shastri, *Sushruta Samhita* of Acharya Sushruta, *Uttartantra* 6/6, reprinted edition (2017), Chaukhambha Sanskrit Sansthan Varanasi, p. 34
- ^{iv}Dr. P. Gunasekaran, *Laboratory Manual in Microbiology*, reprint 2005, New Age International (P) Limited, Publishers, New Delhi
- ^vAnonymous, the *Ayurvedic Pharmacopoeia of India*, Part-I, Vol. 1-4, Govt. of India, Ministry of Health & FW, Dept. of ISM and H. New Delhi; Dept. of Ayush, 1999; 155-56

^{vi}Baxi A.J., Shukla V.J. and Bhatt U.B., Methods of Qualitative Testing of Some Ayurvedic Formulation, Gujarat Ayurveda University, Jamnagar, June 2001; 05-12

^{vii}Anonymous, Planner Chromatography, Modern Thin layer Chromatography, Switzerland, 1999; 2-1

