Analytical Study of Bilvadi Ashchyotana and Eye Drops for Evaluation of Phytochemistry by Spectrophotometry and Chromatography

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Abstracts: Background: Pharmacognosy is the scientific study of the structural, physical, chemical and sensory characters of crude drugs of animals, vegetable and mineral origin and includes also their history, cultivation and collection. The quality of finished product entirely depends on the quality of the raw materials. Therefore first step of them is the quality control aspects of raw material. In the present study an attempt had been done authentication of the raw material (Bilvadi Yavakuta) used for the clinical study. Objectives: To evaluate the organoleptic characters of the test drugs; To assess the physicochemical parameters of the test drugs. Method: Present study was carried out in Department of Shalakyatanta, I. P. G. T. & R. A., Jamnagar during the period of May '09 to Jan '10. The phytochemical study was carried out using various physio-chemical parameters for both trial drugs i.e. 1. Bilvadi Ashchyotana and 2. Bilvadi Eye Drops. Results: HPTLC of Bilvadi Ashchyotana and Bilvadi eye drops shows 7 spots and 8 spots under 254nm wave length and 5 spots and 4 spots under 366nm wave length respectively. Conclusion: pH of the Bilvadi Ashchyotana and Bilvadi Eye Drops are comes under the crieteria of the pH (7.1) according to modern ophthalmology pharmaceutical. For identification of a compound, after isolation we need to adopt bulk handling facility. [Udani J NJIRM 2017; 8(3):120-126]

Key Words: Bilvadi Yoga, Ashchyotana, Chromatography, Spectrophotometry

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Introduction: Today, Ayurvedic science is spreading its wings all over the world where the drug lore of this system has been the center of global interest. Ayurveda has quoted that; as the Prakriti varies from person to person similarly every drug has got its own physical and chemical characteristics which help to separate it from other closely related drug. The Phyto-chemical studies of these drugs done by making use of various parameters help in the drug authenticatation. So to sustain its valuable contribution in allaying disease in this modern era it is expected an imminent need for a well coordinated research plan touching phytochemical study of drug. It is essential to gratify the international standards and quality control of the drug used by convincing the drug regulatory authorities. However, to maximize their ultimate efficacy, it is to ensure that preparations derived from medicinal plants are of acceptable quality, safety and efficacy.

The phytochemical study was carried out for both trial drugs i.e. 1. Bilvadi Ashchyotana & 2. Bilvadi Eye Drops.

Objectives: 1. To evaluate the organoleptic characters of the test drugs; 2. To assess the physicochemical parameters of the test drugs; 3. To analyse the test drug U.V. spectrophotometrically; 4. To evolve suitable thin layer chromatography (TLC) pattern of the drug.

Methods: The Bilvadi Yavakuta was used to prepare both of formulations by Kwatha method and by distillation procedure. The Yavakuta was prepared at the pharmacy of IPGT & RA, G.A.U, Jamnagar, up to distillation procedure of the Eye Drops was done at Pharmaceutical department of the institute. Sterile packing of the Eye Drops was done in Indian Ophthalmics Surendranagar.

Preparation of Bilvadi Ashchyotana: (by Kwatha method) 1 part of Bilvadi Yavakuta was mixed with 10 parts of water and boiled it up to half quantity remains. Instillation of the Kwatha after its become luke warm.

Preparation of Bilvadi Eye Drops: 1 part of Bilvadi Yavakuta was mixed with 10 parts of distilled water; kept soaked for 24 hours and subjected for distillation and 6 parts of the distillate was collected in sterile conical flask. After that content was transferred to autoclaved glass bottle and sterile packing in bottles of 10 ml was done.

Organoleptic parameters: The organoleptic character of Ayurvedic drugs are very important and give the

general idea regarding the genuineness of the sample.

Besides quality control measures Rupa (colour), Rasa (taste), Gandha (odour) and Sparsha (texture) pertaining to Panchajnanendriya Pariksha are noted. These primary subtle parameters are important, the affirmation of which generates confidence in patient as well as in the physician.

Physico-chemical parameters of Bilvadi Ashchyotana and Bilvadi Eye Drops:

(1) Specific gravity

Methods: A clean and dry 25-ml capacity pycnometer was taken and its weight was noted. It was filled with the sample, cleaned properly from outside and the weight was taken at 40^0C. Then it was cleaned, rinsed and filled with distilled water, dried from outside and the weight was noted at 40^0C. The weight of sample and the distilled water was calculated. Then the specific gravity was determined by dividing the weight of the sample by the weight of the water.

(2) Refractive index

Method: Refractive index of a substance varies with temperature. Hence, temperature is to be noted while determining R.I. The R.I. of the sample was measured in Abbe's Refractometer at 40^0C. The temperature was maintained at 40^0C by circulating warm water.

(3) D**etermination of pH value**:

Procedure: 10ml of test drug sample was measured and taken in a beaker. pH meter was standardized with the buffer solution of known pH i.e. 7 pH. The electrode was rinsed with distilled water and introduced into the test solution taken in a beaker. pH value of solution was read as per the indicator of the meter.

(4) Total solid contains:

Procedure: 25 ml of the Bilvadi Ashchyotana and Bilvadi eye drops were taken in previously dried and weighed evaporating dish and was heated on hot water bath. After complete evaporation of the liquid, it was kept in hot air oven at 110°C till constant weight of the residue obtained. The total solid content was calculated with reference to volume taken and expressed in terms of % w. /v.

(5) Tannin matter content:

Qualitative test: Take 5 ml liquid Sample added with 5% lead acetate solution. Tannins gives white precipitate which turns red on addition of KOH solution, on excess addition precipitate were dissolved. And With iodine solution sample give red colouration due to the presence of tannins.

Quantitative test: (Method- Indigo Carmine Method - titration Method)

Take 50 ml Sample filtrate it with whatmen No. 1 filter paper. Wash it by distilled water up to 5oo ml volume (Stock Solution).

Take 10 ml of filtrate of Stock Solution in a 1000ml conical flask. Add 25 ml of Indigo Carmine reagent to the Extract and make up to the volume to 800 ml with distilled water. Filtrate this solution against 0.1 N $KMNO_4$ Solution. The end point is indicated by the appearance of yellowish gram colour. How much volume of $KMNO^4$ is required to neutralize the acid content of the tannin was noted.

(6) Alkaloids matter content:

Qualitative test : Take the sample and added few drops of dilute 2N HCl and 0.5 ml Mayer's reagent – Alkaloids give a white precipitate, while using Warner's reagent - brown flocculent precipitate with alkaloids and with Dragondroff's reagent alkaloids give brown precipitation.

Quantitative test: 20 ml of sample was mixed with 5 ml 2 N HCl and shake. Then 10 ml chloroform was added, shacked and kept for 10 minutes. This is transferred to a seperative funnel and lower layer is moved and upper layer is transferred to a 50 ml beaker. To this 3 ml ammonia was added and shake well. After 15 ml chloroform was added and transferred to a separating funnel. The lower aqueous layer is removed and upper layer is collected and transferred to a evaporating dish and evaporated up to dryness. Then the dish is kept in hot oven and after 1 hour it is taken, allowed to cool and weighed.

(7) Volatile matter content: Volatile matters are odorous principles, found in various plant parts. These evaporate when subjected to heating. So the drug was analyzed for its volatile matter content. The volatile matter content of the sample (coarse powder) was determined in a steam distillation

apparatus by taking 100 gm coarsely powdered sample. After distillation the volatile matter content was noted and the percentage of volatile matter was calculated with reference to air-dried powder sample. Further it was taken up for chromatographic analysis.

(8) UV Visible Spectrophotometric Analysis:

Principle: Different chemicals when subjected for photometry in white light (including UV) have specific affinity to absorb or to transmit a particular range of wavelength, which is related to that compound. Spectrophotometric analysis involves the measurement of the ability of the dissolved substance to absorb electromagnetic radiation of definite and narrow wavelength ranges. These absorptions are measured at wavelengths that are generally a characteristic of the chemical composition of a dissolved absorbing substance. Radiant energy waves range from 200 nm to about 400 nm in the UV region and from 400 nm to about 750 nm in the visible region. The UV or visible spectrum of a molecule is the result of change in energy of a molecule as a whole or rather than of a particular band. The UV and visible spectrum of a substance generally do not have a high degree of specificity but they are suitable for quantitative assays for many substances and useful as additional means of identification. Hence, the UV spectrum of the drug was selected as one of the parameter.

The UV spectra were recorded in a Systronics double beam UV visible recording spectrophotometer (Model 2201). Bilvadi Ashchyotana and Bilvadi eye drops were used as samples for scanning and the details of the U.V. visible spectra obtained were recorded.

(9) Thin Layer Chromatographic Study (TLC):[1,2]

In the present study TLC has been adopted as a separation technique. Thin layer chromatography is a technique where a solute distributes between two phases.

(i) Stationary phase (adsorbent layer): In the form of a thin layer of adsorbent Silica Gel GF 254 on a glass plate or aluminium plate

(ii) Mobile phase (solvent system): In the form of a liquid (pure solvent or mixtures). By this we can separate individual compound from a mixture. By observing the intensity and R_f value of separated spots we can identify different compounds present in it. In 1938, Izmailer and Schraiber introduced this technique for the first time at the Ukramian institute for experimental pharmacy but it was not accepted until late 1950. When Stable publicized the method, developed a kit of basic equipments and made then available. Since then, the TLC has become an important tool for both qualitative and quantitative analysis. Thin layer chromatographic study of the Bilvadi Ashchyotana and Bilvadi eye drops samples were carried out by using the following conditions:

Adsorbent layer - Silica gel GF254

Solvent system - Toluene: Ethyl acetate (85:15)

Detection: i) Day light, ii) Exposure to U.V. light, iii) Exposure to Iodine vapour, and iv) Spraying with 200mg of Vanillin + Conc. Sulphuric acid reagent followed by heating the plate at 110^{o} C for 10 minutes.

To find out the presence of organic matter in Bilvadi Ashchyotana and Bilvadi eye drops -10ml spray reagent followed by heating the plate at 110^{o} C for 10 minutes. The methanol extracts of Bilvadi Ashchyotana and Bilvadi eye drops were spotted on a precoated TLC plate in two separate bands and the chromatogram was developed by using equal quantity of Diethyl ether & Hexane as the solvent system. The chromatograms obtained after using each detection system were observed carefully, and the details like number and colour of the spots and their R_f values were recorded.

(10) High Performance Thin Layer Chromatography

Introduction: [3,4] Detection and identification of a compound from the group of the compounds efficiently in the presence of pure reference compounds, otherwise, efficient separation and establishing the marker compounds is the hall mark of High performance thin layer chromatography (HPTLC) technique is used as a tool for qualitative and quantitative analytical parameters in the fields of medicine, pharmaceuticals, chemistry, bio-chemistry, food analysis, etc.

Principle of HPTLC: Adsorption is the principle which is same in both HPTLC and TLC. One or more compounds are spotted in a thin layer of adsorbent

coated on a chromatographic plate. The mobile phase solvent flows through the capillary action against gravitational force. Components with more affinity towards stationary phase travel slower and components with lesser affinity towards stationary phase travel faster. Thus the components are separated on a thin layer chromatographic plate based on the affinity of the components towards the stationary phase.

HPTLC was done using pre-coated 10 x 10 cm high performance silica gel GF 254 plates (Merck, KgaA, Germany). Samples were applied as 6mm wide bands, 6mm apart, by the spray-on technique using CAMAG linomat-5 sample applicator fitted with a 100-µL syringe (Hamilton, BonaduZ, Switzerland). A constant application rate of 0.1 µL s^{-1} was used. Totally 3 following bands were sprayed and later developed into tracks.

Plates were developed to a distance of 9cms, with toluene- ethyle acetate- chloroform 9:1:0.5 (v/v) as mobile phase. Volume of mobile phase was 15ml. Before development the chamber was saturated with mobile phase for 30 minutes at room temperature. Then the plate was developed in twin through chamber, development time was approximately 30 minutes. After development the mobile phase was evaporated from the plate by drying in a fume-hood for 10 minutes. Then densitometry scanning was performed with a Camag TLC scanner III in reflectance-absorbance mode at 254nm and 366nm, under control of CATS software (V 3.15, Camag). The slit dimensions were 5mm x 0.45mm and the scanning speed was 10mm s^{-1}. The peaks obtained were observed and recorded, individual R_f values were also noted.

Chromatographic conditions:

Adsorbent Layer: Precoated TLC aluminium sheets of Silica gel 60 F_{254} GLP plates (Merk KGaA, Germany)

Sample Application: By Auto-sampler CAMAG Linomat V

Mobile Phase: 1- Dichloromethane

Detection: 1-Viewing the TLC chromatogram at 254 nm under UV. 2-Viewing the TLC chromatogram at 366 nm under UV

Procedure: Analysis were performed on 10 cm and 20 cm high performance silica gel GF 60_{254} plates (Merck) of 0.5 mm thickness. The plates were precleaned by development to the top with HPLC grad methanol and dried in a fume-hood before use and sample solutions were applied to the plate by means of CAMAG Linomate V automated spray on band application equipped with a 100 µL syringe then the plates were developed in different systems as mobile phase in vapour-equlibrated Camag twin-through chamber containing a saturation pad. The development time was approx. 30 minutes after development the mobile phase was evaporated from the plate by drying in a fume hood for 10 min and detection was done under 254 and 366 nm UV.

Result:

Organoleptic Parameters: The organoleptic characters of the Bilvadi Ashchyotana and Bilvadi Eye Drops samples are given in following table

Table - 1

	Parameters	Bilvadi Ashchyotana	Bilvadi Eye Drops
01	Texture	Watery	Watery
02	Colour	Light Brown	Clear / watery
03	Taste	Bitter	Mild bitter
04	Odour	Characteristic	Characteristic

Physio-Chemical parameters: The analytical data of common Physicochemical parameters of the samples are given in table.

Table 2

Sr.	Parameters / Samples	Results	
		Bilvadi Ashchyotana	Bilvadi Eye Drops
01	Specific gravity	1.0023	1.0004
02	Refractive index	1.3410 (at room temprature)	1.3450
03	pH	8.41	7.16
04	Total solid content	0.25% w/v	0.16 % w/v
05	Tannin matter	0.033% w/v	-
06	Alkaloids matter	0.01 % w/v	0.02 % w/v
08	Volatile matter	-	-

Table-3 showing number of spots obtained under 254nm & 366nm

Track name	Visualization UV 254nm		Visualization UV 366nm	
	No. of spots	R values	No. of spots	R values
Bilvadi Ashchyotana	07	0.03, 0.27, 0.55, 0.68 , 0.75, 0.85, 0.92	05	0.01, 0.28, 0.55, 0.69, 0.72
Bilvadi Eye Drops	08	0.02, 0.05, 0.45, 0.55, 0.65, 0.72, 0.80, 0.93	04	0.01, 0.55, 0.67, 0.73

Some of the following observations can be made from above table: For sample Bilvadi Aschyotana chromogram shows 07 spots and for Bilvadi Eye Drops 08 spots under UV 254nm while under UV 366nm Bilvadi Aschyotana had 05 spots and Bilvadi Eye Drops had 04 spots thay indicating that higher suceptivity was observed at UV 254nm.

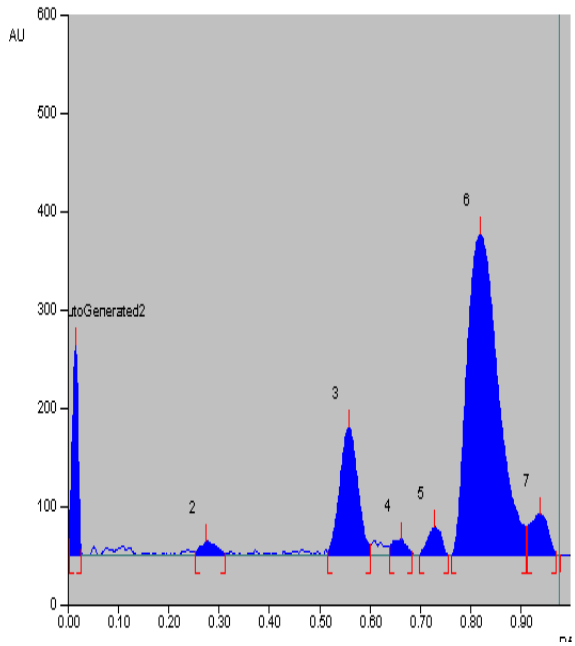
The point with maximum concentration of separated materials occupies maximum percentage of area when displayed graphically at respective R_f value. Some of the observations of different tracks are as follows.

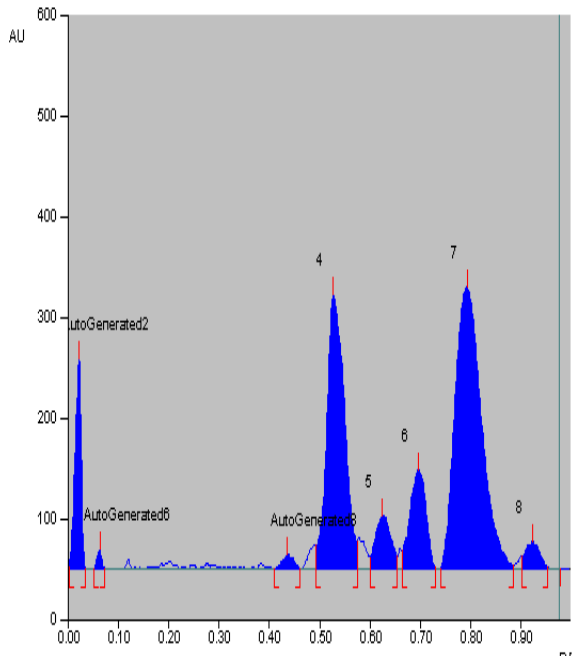
Observations at 254nm: Highest area covered 20 % in all the three tracks, indicates presence of same compound at its highest peak. Separation screen design could not be done because of less yield hence identification of the compound was not possible in this study.

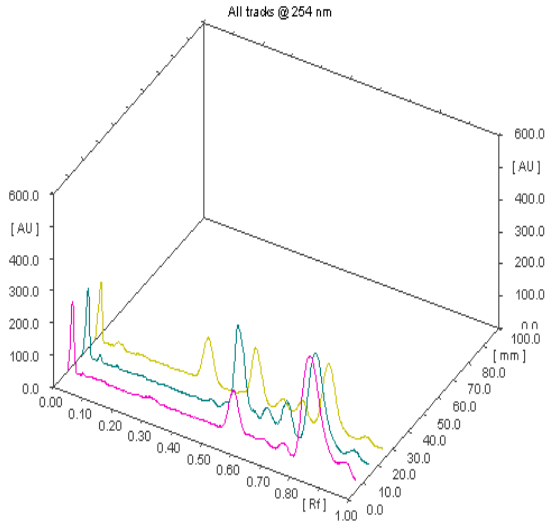
For identification of a compound after isolation we need to adopt bulk handling facility for which we should posses more yield. In this study as very less yield was there other scheme has to be designed to increase the yield then we can further identify the unsaturated compounds which are present in this compound preparation.

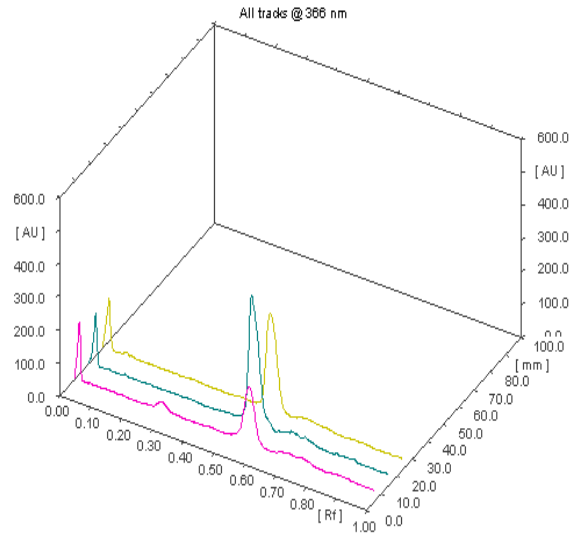
Densitometry scanning was performed with a Camag T.L.C. scanner III in reflectance absorbance mode at 254 nm and 366 nm under control of CATS software (V 3.15 Camag). The slit dimensions were 6 mm x 0.45 mm and the scanning speed was 10 mm^{-1}

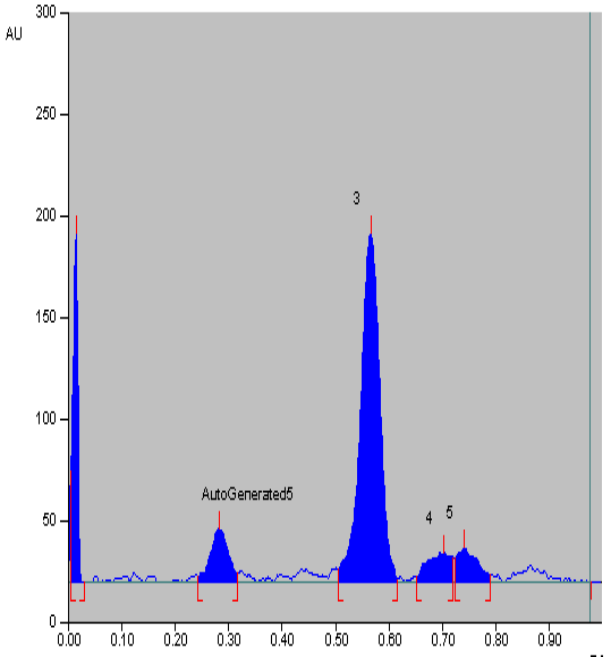
Densitogram of Bilvadi Ashchyotana and Bilvadi Eye Drops with R_f values and 3D chart. (254nm wave length and 366nm wave length):

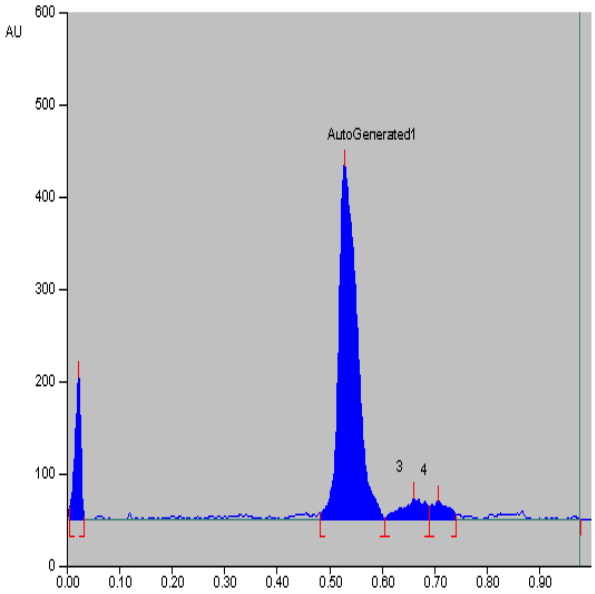












Discussion: HPTLC of Bilvadi Ashchyotana and Bilvadi eye drops shows 7 spots and 8 spots under 254nm wave length and 5 spots and 4 spots under 366nm wave length respectively, indicating the presence of compounds, means either carbon double bond or non-bonded compound of oxygen or nitrogen may be present. The compound which is separated has given more response under short UV.

The point with maximum concentration of separated materials has occupied maximum percentage of area when displayed graphically at respective R_f value

Conclusion: pH of the Bilvadi Ashchyotana and Bilvadi Eye Drops are comes under the crieteria of the pH (7.1) according to modern ophthalmology pharmaceutical.

In HPTLC of the Bilvadi Ashchyotana and Bilvadi Eye Drops spots were detected indicating the presence of compounds and it has given more response under short UV.

For identification of a compound, after isolation we need to adopt bulk handling facility. In this study as very less yield was there other scheme has to be designed to increase the yield, then only we can further identify the unsaturated compounds which are present in this compound preparation.

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