International Research Journal of Ayurveda & Yoga
Vol. 5 (8),51-60 , August, 2022
ISSN: 2581-785X;https://irjay.com/
DOI: **10.47223/IRJAY.2022.5807**

Pharmacognostical and Phytochemical Study of Ayurveda Formulation Named *Gandhakadi Yoga* with and without *Pippali*

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Article Info

Article history:
Received on: 17-07-2022
Accepted on: 23-08-2022
Available online: 31-08-2022

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ABSTRACT:

Many medicinal plants have been used in Ayurveda whose oneness depends on synonyms and vernacular names. It is difficult for proper identification of all source plants and comparison of the drugs which is described in Ayurvedic literature. But with the help of botany and its divisions it can be possible. One can get proper information about Adulteration, Quality Control, and Substitution of raw drugs with the help of Pharmacognosy. Present study was undertaken in order to confirm the authenticity of the drugs used in the preparation of both Drug A (*Gandhakadi yoga* with *pippali*) and Drug B (*Gandhakadi yoga* without *pippali*); To analyse the sample of both Drug A and Drug B by utilizing suitable parameters and to distinguish the prepared drugs. The results were compiled and presented in organized manner.

Keywords : *Gandhakadi yoga*, *PIppali*, Quality control, HPTLC

INTRODUCTION

Pharmaceutics is the discipline of pharmacy that deals with the process of turning a new chemical entity (NCE) or old drugs into a medication to be used safely and effectively by patients. There are many chemicals with pharmacological properties, but need special measures to help them achieve therapeutically relevant amounts at their sites of action. Pharmaceutics helps relate the formulation of drugs to their delivery and disposition in the body.[1] Pharmaceutics deals with the formulation of a pure drug substance into a dosage form. In Ayurveda, the analytical techniques have been mentioned in classical books to understand, the quality of the finished product. However, qualitative and quantitative analysis of drugs by using the modern techniques and instruments of the science is also the need of the time. Chemical analysis of drug assures not only chemical constituents but also tells us standards of any formation. It not only gives the standards of the product but indirectly gives advice for further advancement if required. It is an imminent need for a well-coordinated research plan touching physiochemical study of drug in present era.

Pharmacognosy is the study of plants or other natural sources as a possible source of drugs. The American Society of Pharmacognosy defines pharmacognosy as "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources".[2] The practical perspective of pharmacognosy includes quality control (identity, purity, and consistency), efficacy

(therapeutic indications, clinical studies and pharmacological investigations), safety (adverse reactions, drug interactions, contraindications, precautions).Present study was undertaken in order to confirm the authenticity of the drugs used in the preparation of both Drug A (*Gandhakadi yoga* with *pippali*) and Drug B (*Gandhakadi yoga* without *pippali*); To analyze the sample of both Drug A and Drug B by utilizing suitable parameters and to distinguish the prepared drugs. The current study was carried out to evaluate the Physico-chemical parameters of test drug.

MATERIALS AND METHODS

Collection, identification and authentication of raw drugs:

1. Fresh *Bhringaraja* (*Eclipta alba* Hassk.) *Panchanga* (Whole plant), *Agastya* (*Sesbenia grandiflora* Linn.) leaves were compiled from the periphery of Jamnagar in the month of February-March 2020.
2. All drugs were authenticated by the specialist of Pharmacognosy laboratory, I. P. G. T. & R. A., Jamnagar. Then the fine particles of these drugs were used for fine particles microscopic study.
3. The Ayurvedic Pharmacopoeia of India (A.P.I.) standards were used for authentication.[3]
4. Raw *Gandhaka* (Natural Sulphur) and Fruits of *Vidanga* (*Embelia ribes* Burm.f.) were acquired from the Pharmacy of Gujarat Ayurved University, Jamnagar.
5. The in house Pharmacognosy Laboratory of the Institute authenticated all the drugs for quality and purity by employing Ayurvedic Pharmacopoeia of India (A.P.I.) standards.
6. Following Standard Operating Procedures (S.O.P.) prepared *Gandhakadi Yoga Vati* and physico-chemical analysis of the prepared product was carried out in the pharmaceutical laboratory of I.P.G.T. & R.A., G.A.U., Jamnagar.Small quantity of Drug A and Drug B Vati dissolved in distilled water and filtered through filter paper then filtrate dried and placed on slide, first observed in plain water and then blemished with Phluroglucinol and concentrated HCl to study the characters of the drug. The micro-photographs were taken by using Carl-Zeiss Trinocular microscope attached with camera.[4]

The finished product, *Gandhakadi Yoga Vati* was analysed on following parameters.

Physical tests:

Following Physical parameters of both *Gandhakadi Yoga Vati* were analyzed[5] and presented in table no 1.

1. Shape
2. Hardness
3. Uniformity
4. Disintegration time

Physico-chemical analysis:

Following Physico-chemical parameters of both groups were analyzed. Details are given in Table no. 2.

1. Determination of pH value[6]
2. Determination of Loss on drying at 110°C[7]
3. Determination of Ash value[8]
4. Determination of acid insoluble ash[9]
5. Determination of Water-soluble extractives
6. Determination of Methanol soluble extractives
7. Solubility in Carbon disulphide

Methods of various physico-chemical analyses are described below:

pH Value:

Each 5 gm of test drug sample were weighed and taken in a conical flask. Then added 50 ml precisely measured water and stirred well for few minutes; kept those solutions for some time and then filtered it through filter paper and the filtered solutions were taken in a beaker. Standardized by pH meter and electrodes with buffer solution of known pH i.e. 7 pH. Electrode rinsed with distilled water was introduced into the test solution contained in a small beaker and the pH value of solution was read.

Loss on drying (LOD):

2 gm of drug samples of each group were taken in a pre-weighed dried Petri dish. Each sample was dried in an oven at 110ºC until reaching a constant weight. The Petri dish were taken out, self-cooled and weighed promptly. The weight loss was counted and expressed as % w/w.

Ash value:

2 gm precisely weighed samples of each group were taken in a pre-weighed dried-crucibles. It was incinerated in a muffle furnace up to 450°C, until they were freed from carbon. The crucibles were taken out, self-cooled and weighed promptly. From the weight of the ash, the ash value was derived with reference to the air-dried drug. It was counted and expressed as % w/w.

Water soluble extractive:

2.5 gm of the samples of each group were weighed precisely. 50 ml of distilled water was added to it and kept covered overnight. Next day, it was filtered. 20 ml of the filtrate was precisely measured with a pipette and transferred to the already weighed evaporating dish. The evaporating dish of both samples was placed on a water

bath for evaporation of the water. After evaporation of the water and dried in an oven, allowed cooling and weighed promptly. From the weight of the residue obtained, the percentage of water-soluble extractive were counted and expressed as % w/w.

Methanol soluble extractive:

2.5 gm test drug samples of each group was weighed precisely. 50 ml methanol was added to both sample and kept covered overnight. Remaining procedure was same as water soluble extract method. From the weight of the residue acquired, the percentage of alcohol soluble extractive was counted and expressed as % w/w.

High Performance Thin Layer Chromatography (HPTLC):[10]

Chromatography is the separation of a mixture into individual components using a stationary phase and mobile phase. Thin Layer Chromatography is a method based on adsorption chromatography. The adsorbent such as silica gel-G F-254 is coated to a thickness at 0.3mm or clean TLC plates using commercial spreader, the plates are activated at 105°C for 30 minutes and used.

HPTLC is a sophisticated and automated form of TLC. HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis there by dramatically reducing analytical time. With HPTLC, the analysis can be viewed using different wave lengths of light thereby providing a more complete picture of the plant than is typically observed with more specific types of analyses.

Principle of HPTLC:

Principle remains the same as of TLC i.e. adsorption. One or more compounds are spotted on a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action (against gravitational force). The component with more affinity towards stationary phase travels faster. Thus, the components are separated on a thin layer chromatographic plate based on the affinity of the components towards the stationary phase.

The R_f values (it is the ratio of distance travelled by the solute to that of solvent front) may vary depending upon the purity of the solvent; nature of the substance to be resolved; composition of solvent; presence of impurities; adsorbent used; polarity of the solvent, substance and adsorbent etc.

Steps involved in HPTLC:

- Selection of chromatographic layer.
- Sample and standard preparation.
- Layer pre-washing, Layer pre-conditioning.
- Application of sample and standard.
- Chromatographic development.
- Detection of spots.
- Scanning
- Documentation of chromatic plate

RESULTS

- **Organoleptic characters:** Organoleptic characters Drug A and Drug B Vati of like color, touch, odor and taste are described in the Table no 3. **Microscopic characters:** Pharmacognostical characteristics of Drug A and Drug B powder under the microscope showed that:

  Drug A: Microscopic photos prepared (Plate 1)

  Drug B: Microscopic photos prepared (Plate 2)

  Solvent system – Toluene: Ethyl acetate: Acetic Acid (7:2:1)

  Results of HPTLC of Drug A and Drug B

  Drug 1: Drug A found result in short UV (254nm) and long UV (366nm)

  Drug 2: Drug B drug found result in short UV (254nm) and long UV (366nm)

  Plate of Drug 1: Densitogram of HPTLC of *Gandhakadi Yoga* with *Pippali*

  Plate of Drug 2: Densitogram of HPTLC of *Gandhakadi Yoga* without *Pippali*

  In-Drug A High-Performance Thin Layer Chromatography (HPTLC) at 254 nm and 366 nm resulted in 5 and 3 spots while in Drug B High Performance Thin Layer Chromatography (HPTLC) at 254nm and 366nm resulted in 7 and 2 spots respectively. This is helpful for the easy separation of the constituents.

DISCUSSION AND CONCLUSION

Authentication and Identification of *Bhringaraja* (*Eclipta alba* Hassk.), *Vidanga* (*Embelia ribes* Burm.f.) *Agastya* (*Sesbenia grandiflora* Linn.) were carried out in this section. The morphological and microscopic characters of prepared drugs are authenticated in pharmacognosy laboratory, I.P.G.T. & R.A., Jamnagar and compared with API references. The plant selected for *Gandhaka Shodhana* in this particular study was *E. Alba* Hassk.There are two varieties of *Agastya* (*Sesbenia. grandiflora* Linn.) are

recognized one with white and other with red flowers. Among these, white flower variety was taken for current study. There are three varieties of Moringa explained in Ayurvedic text books.1. *Shyama* (black variety). 2. *Shveta* (white variety) and 3. *Rakta* (red variety). Among these, white flower variety was taken for current study. They have their own marvellous medicinal qualities. As *Agastya* is a popular drug from medicinal point of view, a detailed evaluation should be carried out about different parts of the plant i.e. bark, leaves, flowers, roots which can be helpful in identification of authentic drug and adulterants. One study has been carried out on pharmacognostical and physicochemical evaluation of *Agastya* leaves[11] and also one article has been published on the micro-morphological and micrometric evaluation of *Agastya* (*Sesbenia*. *grandiflora* Linn.) flower.[12] In spite of pharmacognostical study and microscopic study of *Gandhakadi Yoga* with and without *Pippali Vati* has been carried out to standardize the finished product. Microscopy of *Gandhakadi Yoga Vati* showed the striking characters of all individual four drugs in each trial drug's final product. It confirms the ingredients current in the finished product.[13] Analytical study of Drug A and Drug B *Vati* was conducted to estimates the organoleptic, physical, physico-chemical parameters and to evolve suitable chromatography (TLC) pattern of the drug. The organoleptic parameters showed the following: Texture of both *Group Vati* was smooth indicating the surface uniformity without cracks. This is the primary character to assess the quality of *Vati*. In both drugs Colour was Dark buff, Taste was sore follower by Astringent and Odour was Slightly Aromatic and Touch was Hard which is characteristics due to the distinct properties of the various ingredients. These characteristics make them no distinctive and no identifiable with the active ingredients that they contain. The physical parameters demonstrated the following: Drug A weight of single *Vati* was found to be tablet 248 mg. Hardness was found to be 3.33 kg/cm. Hardness parameters give a clue to the intrinsic strength of powdered materials. Friability was found to be 1.6%. Friability suggests strength of the *Vati*. Disintegration time is 4 min. Drug B average weight of single *Vati* was found to be tablet 250 mg. Hardness was found to be 2.33 kg/cm. Hardness parameters give a clue to the intrinsic strength of powdered materials. Friability was found to be 1.6%. Friability suggests strength of the tablet. Disintegration time is 4 min. Disintegration times of the both *Vati* are maximum 4 min, which means that the disintegration time of both Drug *Vati* is fairly within the acceptable limits. It ensures that the *Vati* disintegrates while present in stomach and advance for further metabolism.The physico-chemical parameters demonstrated the following: The pH value of both Drug A and Drug B is 6.5 and 7.00 respectively. This indicated slightly Alkali nature of the formulation. The degree of ionization and lipoid solubility of a drug are two vital factors that determine the rate of absorption of drugs from GI tract, and indeed their passage through cellular membranes easily. Drugs that are weak organic acids are mostly in unionized form are soluble in lipids and absorbed rapidly through cellular membranes in comparison to strong acids or bases. Loss on drying 0.001%w/w and 0.002% w/w indicating the percentage of moisture present in the finished product. Total ashes are designed to measure the total amount of material remaining after ignition. Total ash values of test drugs are 7.5%w/w and 7.9%w/w. This may be probably due to the presence of some inorganic contents consolidated from *Gandhaka*, during *Shodhana* or *Bhavana* and from various constituent ingredients. Water soluble extract are 12.5%w/w and 12.9 %w/w. methanol soluble extract is 5.7% w/w and 7.5%w/w, which are almost identical for water and alcohol. Solubility in Carbon disulphide estimated to be 12.7 %w/w and 10.7 %w/w. It may suggest the amount of free sulphur, present in unbound form in the *Vati* and rest of sulphur may have formed a compound, more precisely organo-mineral compound. In-Drug A High-Performance Thin Layer Chromatography (HPTLC) at 254 nm and 366 nm resulted in 5 and 3 spots while in drug B High Performance Thin Layer Chromatography (HPTLC) at 254nm and 366nm resulted in 7 and 2 spots respectively. This is helpful for the easy separation of the constituents.

Acknowledgements - Nil
Conflict of interest - None
Source of finance & support – Nil

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How to cite this article: Meena U, Meena S, Kori VK " Pharmacognostical And Phytochemical Study Of Ayurveda Formulation Named *Gandhakadi Yoga* With And Without *Pippali*" IRJAY.[online]2022;5(8); 51-60.
Available from: https://irjay.com
DOI link- https://doi.org/10.47223/IRJAY.2022.5807

Table no 1 : Showing Organoleptic characters

No.	Characters	Drug A	Drug B
1.	Colour	Dark buff	Dark buff
2.	Touch	Fine Powder	Fine Powder
3.	Odour	Sulphur	Sulphur
4.	Taste	Astringent	Astringent

Table no. 2 : Showing the Physico-chemical analysis of Drug A and Drug B

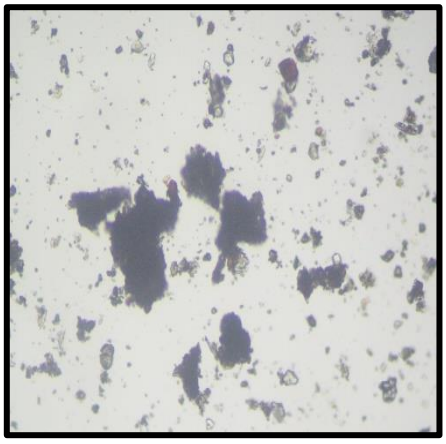
Sr. No.	Parameters	Drug A	Drug B
1.	PH	6.5	7.00
2.	Loss on drying (at 110°C)	0.001 %w/w	0.002 %w/w
3.	Ash value	7.5 %w/w	7.9 %w/w
4.	Water soluble extractive	12.5 %w/w	12.9 %w/w
5.	Methanol soluble extractive	5.7 %w/w	7.5%w/w

Table no 3 : Showing the Physical analysis of Drug A and Drug B

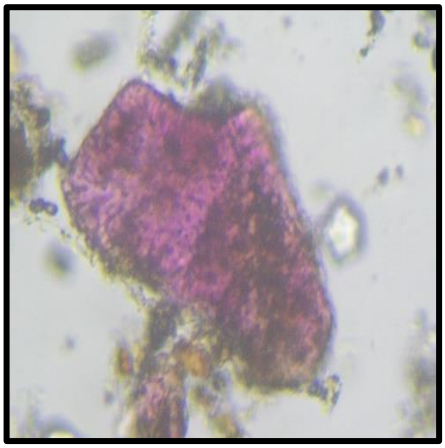
Sr. No.	Parameters		Drug A	Drug B
	Shape		Round	Round
	Hardness		3.33 kg/cm^2	2.33 kg/cm^2
	Uniformity	Max. (mg) wt.	248mg	250 mg
		Min. (mg) wt.	211mg	210mg
		Avg. (mg) wt.	243 mg	245mg
4.	Disintegration time		4min	4min

Plate: 1 Microphotographs of *Gandhakadi Yoga* with *Pippali* prepared with *Agastya Patra Swarasa Bhavana*

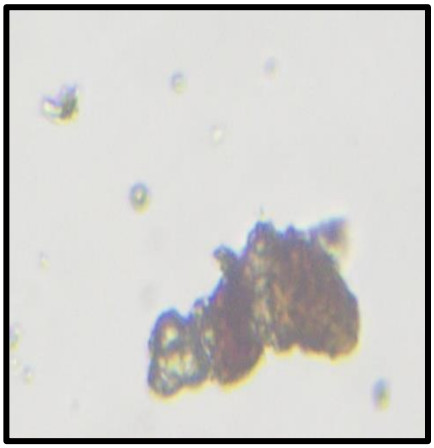
1-Black debries of *Gandhak*



2-Lignified stone cells of *Vidanga*



3-Olioresin content of *Pippali*



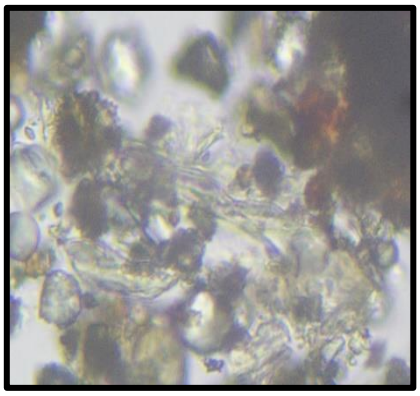
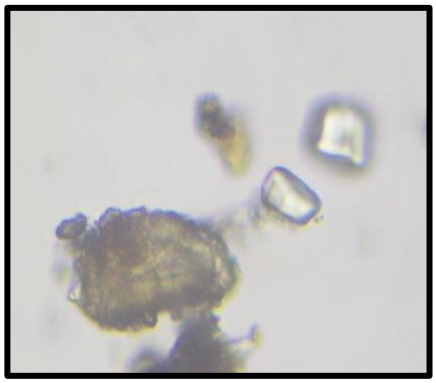
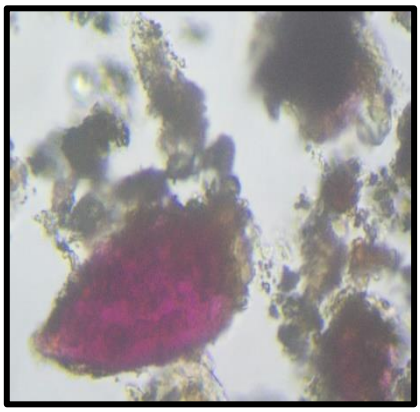
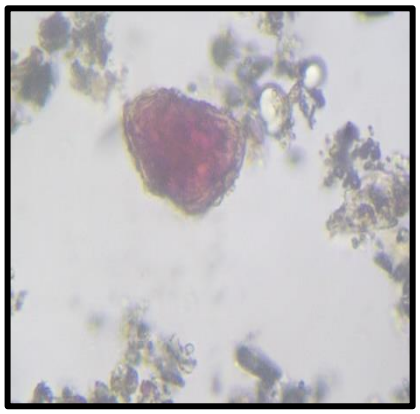
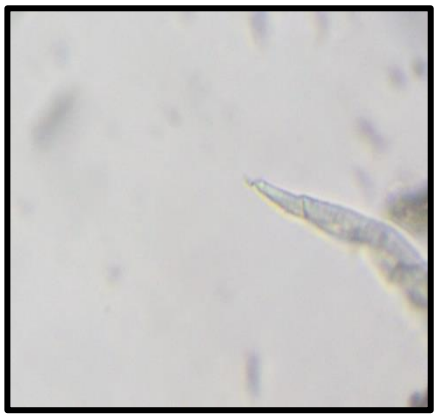
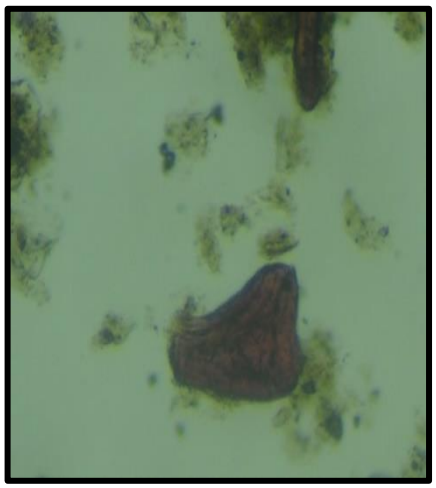
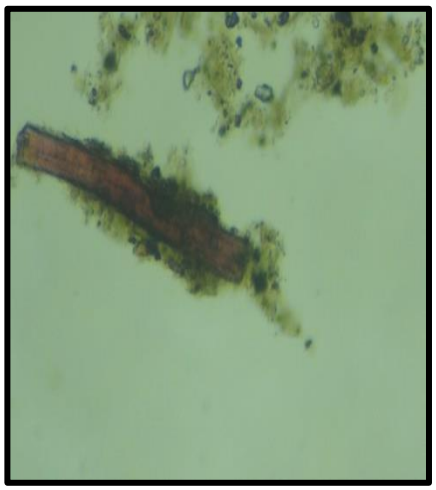
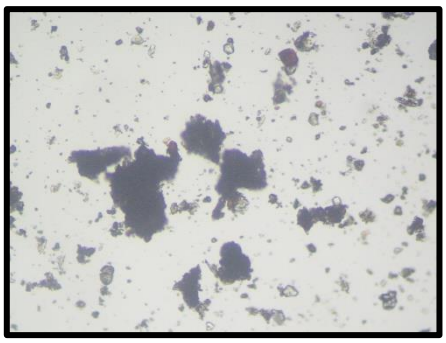
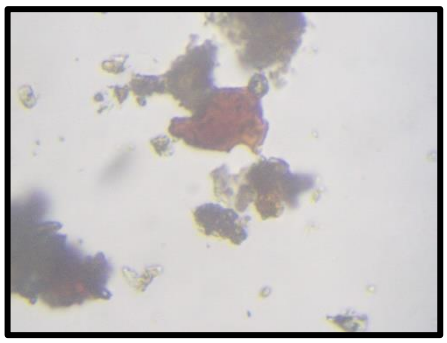
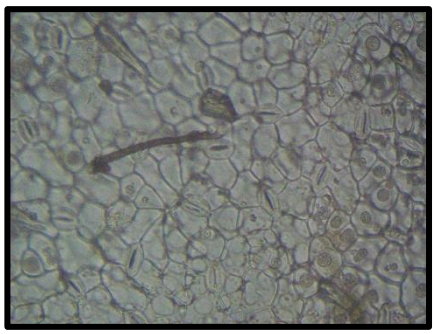
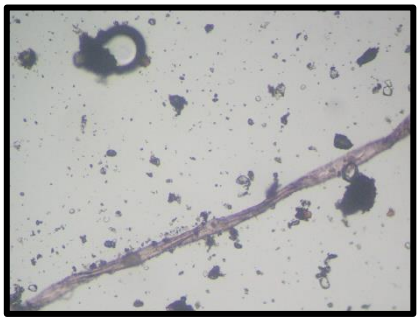
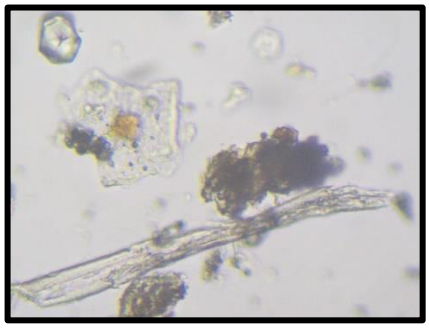
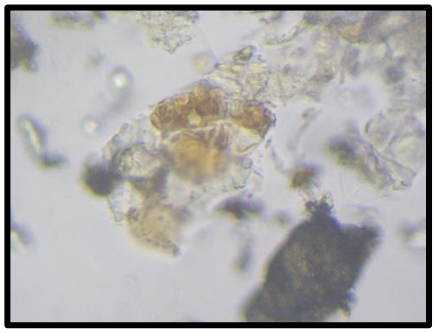
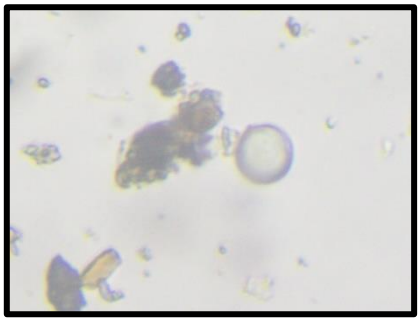
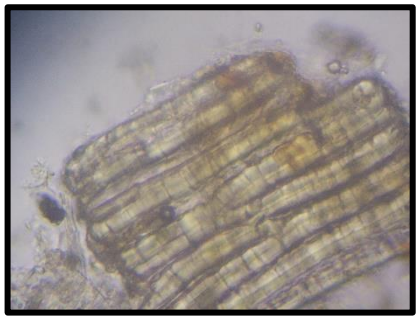
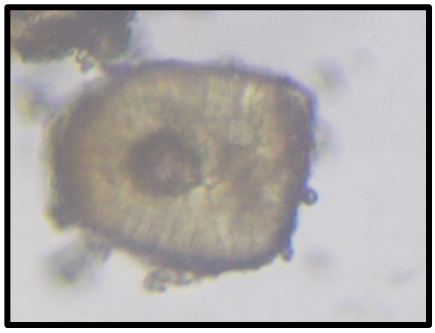
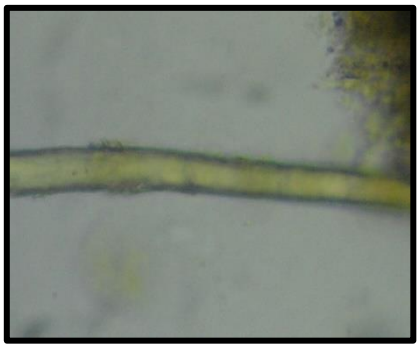
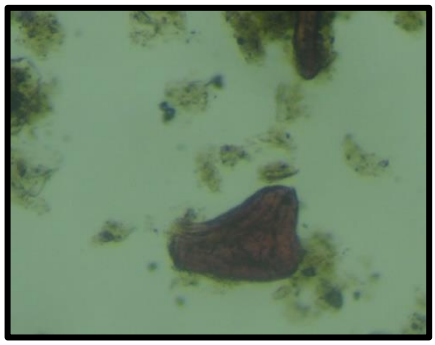
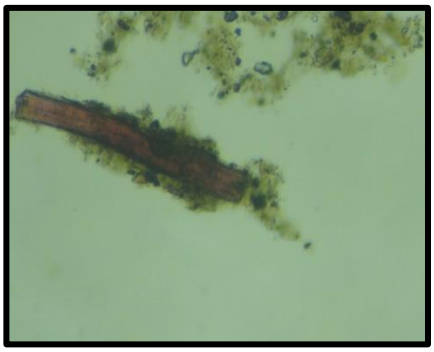
4-Olioresin content of *Vidanga* 	**5-Sclariform vessel of *Agastya*** 	**6-Starch grains of *Agastya*** 
7-Stone cells of *Vidanga* 	**8-Stone cells of *Pippali*** 	**9-Scleroids of *Agastya*** 
10. Lignified stones of Haritaki 	**11. Lignified scleroids of Amalaki** 	**12. Lignified stone cell of Bibhitaki** 

Plate: 2 Microphotographs of *Gandhakadi Yoga* without *Pippali* prepared with *Agastya Patra Swarasa Bhavana*

1-Black debries of *Gandhak* 	**2-Brown content of *Vidanga*** 	**3-Epidermal cells of *Agastya*** 
4-Fibres of *Agastya* 	**5-Brown content of *Vidanga*** 	**6-Mesocarp cells of *Vidanga*** 
7-Oil globule of *Vidanga* 	**8-Scleroids of *Vidanga*** 	**9-Scleroids of *Vidanga*** 

10. Lignified stones of Haritaki	**11. Lignified scleroids of Amalaki**	**12. Lignified stone cell of Bibhitaki**
		

Solvent system – Toluene: Ethyl acetate: Acetic Acid (7:2:1)

Plate of Drug 1: Densitogram of HPTLC of *Gandhakadi Yoga* with *Pippali*

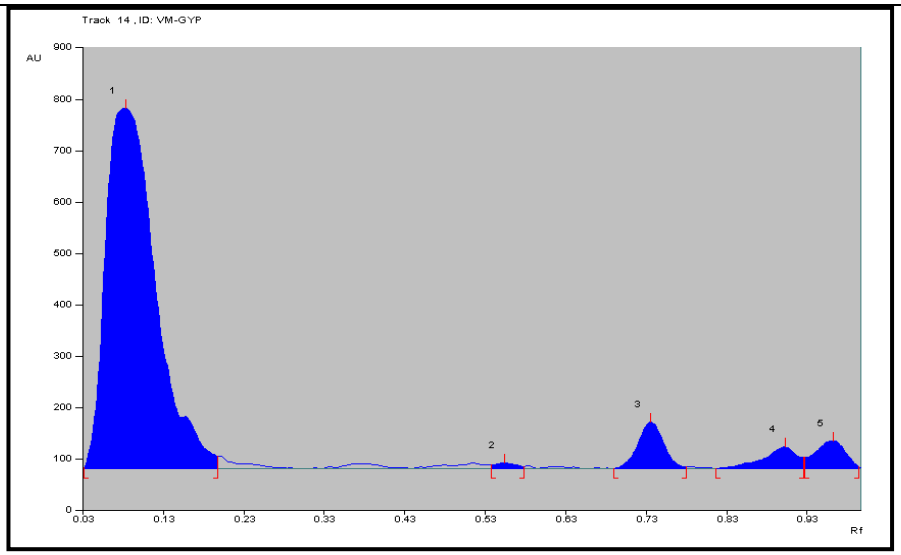
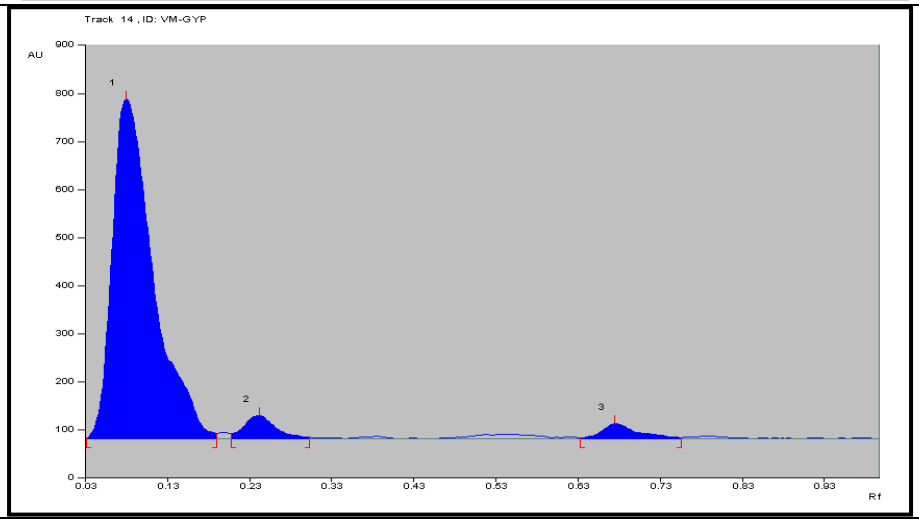
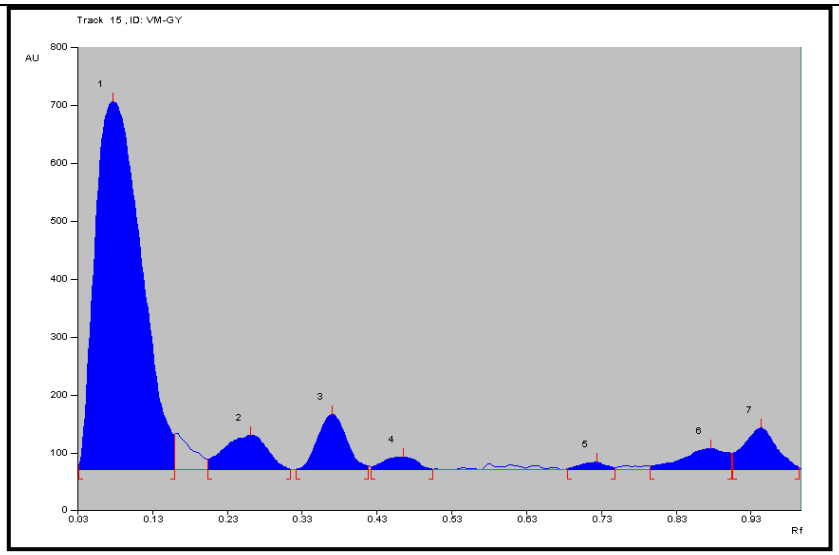
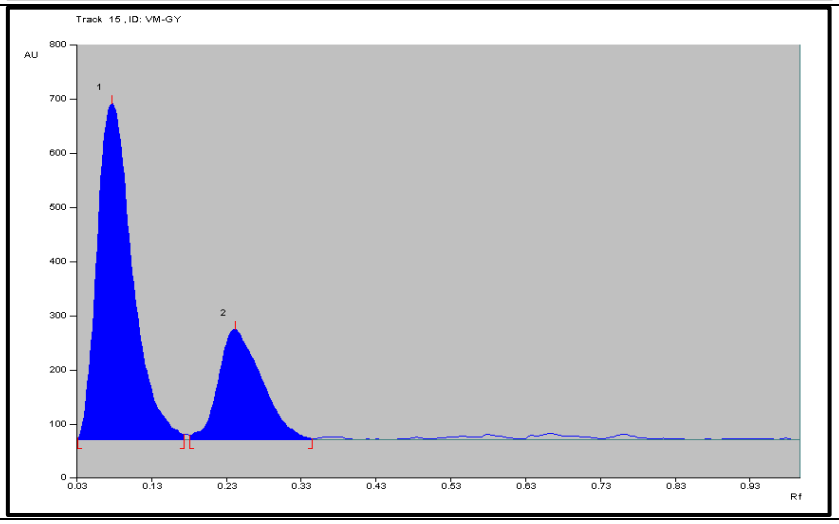
At Short UV (254 nm)	
At Long UV (366 nm)	

Plate of Drug 2: Densitogram of HPTLC of *Gandhakadi Yoga* without *Pippali*

At Short UV (254 nm)	
At Long UV (366 nm)	

SAMPLE	**Short UV (254 nm)**		**Long UV (366 nm)**	
	No. of spots	**Max. R_f Value**	**No. of Spots**	**Max. R_f Value**
No.1	5	0.09, 0.56, 0.74, 0.9, 0.96,	3	3.97, 6.14, 89.89
No.2	7	0.08, 0.26, 0.37, 0.47, 0.73, 0.88, 0.95	2	24.77, 75.23