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USE OF THIN LAYER CHROMATOGRAPHY IN THE QUALITY ASSESSMENT OF ABHAYARISTAM

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ABSTRACT
Five brands of Abhayaristam were analyzed by HPTLC and thin layer chromatography for ascertaining the consistency of chemical constituents. TLC patterns showed differences which may be due to adulteration or improper proportions of ingredients. The study emphasize on the importance of defining quality parameters for Abhayaristam and for other Ayurvedic preparations in general.
Key words: Ayurvedic medicines, quality assessment, HPTLC and thin layer chromatography

INTRODUCTION

Ayurvedic formulations mainly use plants and plant products as raw materials. The selection of raw materials, method of preparation and preservation etc are important in maintaining the quality of the medicines without which the correct pharmacological effect cannot be expected. The selection of raw materials has special importance in the light of the phytochemical observation of the variation in secondary metabolite profile of plant materials due to changes in geographical conditions, stage of growth, time of collection and period of preservation[1,2]. Efficient, quick and simple analytical procedures are necessary for ascertaining the influence of these parameters on the medicines prepared. One of the existing methods of quality assessment is through the use of marker compounds and co-TLC[3, 4]. Since details of established marker compounds may be streamlined for generating positive results. Direct chemical analysis aimed at identification of the constituents cannot be successful due to the complexity of the systems. It is in this context that we thought of using simple physical methods such as HPTLC and TLC for a comparative study of the composition of Ayurvedic medicines.

Common Ayurvedic medicines such as Aristas and Kashayams exist in the colloidal form. Both have hydrophilic and lipophilic components, with minor amounts of low polar compounds. No single TLC system can reveal the complete compound profile. A minimum of four solvent systems are necessary to study the entire range. First the sample may be partitioned with chloroform and the chloroform fraction may be used to study the nature of low polar components. A moderately polar system like chloroform with 5-10% alcohol may be used for developing TLC. The fraction left after the separation of the chloroform layer need to be studied in at least three systems, Chloroform-20% alcohol, chloroform-60 % alcohol and finally chloroform-alcohol-water or chloroform-alcohol-acetic acid solvent system. The HPTLC studies may conveniently be conducted with the alcohol soluble part of the concentrated medicine.

MATERIALS AND METHODS

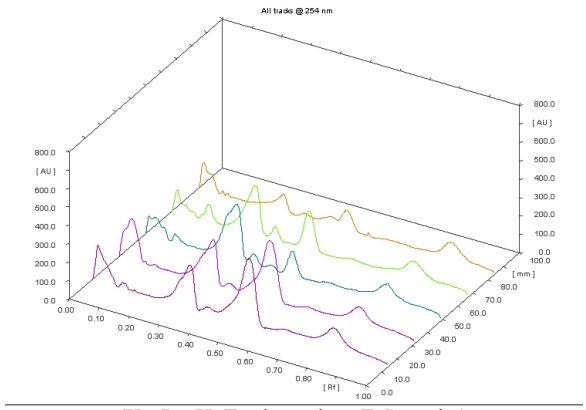
HPTLC were conducted in pre- coated aluminium plates and samples were applied as bands using a micro injector. TLC's were performed on glass plates coated with silica gel. Silica gel G was obtained from NICE chemicals, India. Chloroform and alcohol used as solvents were purchased from Priya Laboratories, Trivandrum, Kerala, India and were of LR grade. Five brands of Abhayaristam used in this study were purchased from commercial sources. Nagarjuna, Batch No. JLTA, Mfd.01/10; Dabur, Batch No SB0003, Mfd. 04/09, Kottakal, Batch No. 503777, Mfd. Jan.2010; Oushadhi, Batch No.04872353550, Mfd. 12/1/10 and Vaidyaratnam, 278, Mfd. Oct. 09 were the samples used in the study.

Development of chromatogram and visualization

Development of HPTLC chromatograms were done in 10% and 50% ethanol-chloroform mixtures and visualized in a UV-Visible chamber and the data was graphically recorded. Thin layer chromatography was done in different concentrations of alcohol, chloroform and acetic acid mixtures and visualization was effected by using iodine vapours.

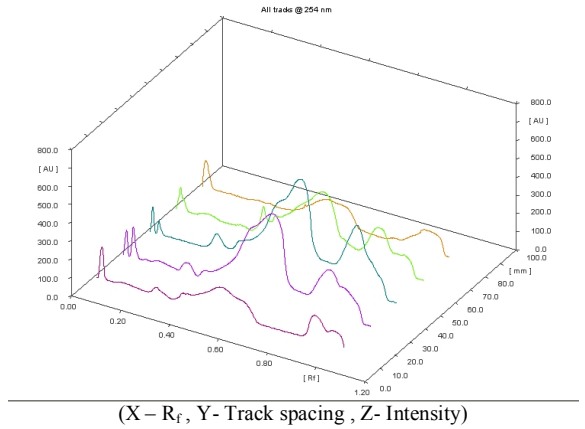
RESULTS

Five brands of Abhayaristam namely Nagarjuna, Dabur, Kottakal, Oushadhi and Vaidyaratnam used in the study are popular brands supported by strong commercial infrastructure. If all brands confine to quality standards prescribed in authoritative literature each should give the same TLC for the entire range of polarities. The graph of HPTLC analysis of Nagarjuna, Dabur, Kottakal, Oushadhi and Vaidyaratnam aristas conducted with the two solvent systems are shown in figures 1 and 2 respectively.



(X – R_f , Y- Track spacing , Z- Intensity)

Figure 1: HPTLC analysis of all aristams in 10% ethanol-chloroform mixture



(X – R_f , Y- Track spacing , Z- Intensity)

Figure 2: HPTLC analysis of all aristams in 50% ethanol-chloroform mixture

The R_f values of the constituents were compared and observed differences are shown in Tables 1 and 2 respectively.

Table 1: R_f values of all brands of aristams in 10% ethanol-chloroform

Peak No	AN	AD	AK	AO	AV
1	0.02	0.04	0.03	0.02	0.02
2	0.10	0.12	0.10	0.07	0.08
3	0.33	0.32	0.31	0.12	0.09
4	0.39	0.37	0.36	0.28	0.28
5	0.53	0.51	0.43	0.34	0.36
6	0.66	0.64	0.49	0.46	0.45
7	0.81	0.81	0.62	0.61	0.50
8	0.94	0.93	0.81	0.81	0.57
9			0.94	0.96	0.63
10					0.85
11					0.93

Table 2: R_f values of all brands of aristams in 50% ethanol-chloroform

Peak No	AN	AD	AK	AO	AV
1	0.03	0.02	0.02	0.03	0.02
2	0.13	0.05	0.05	0.14	0.29
3	0.25	0.16	0.29	0.27	0.53
4	0.36	0.27	0.63	0.37	0.81
5	0.53	0.35	0.86	0.42	0.92
6	0.90	0.62		0.61	0.94
7	0.98	0.86		0.85	
8		0.91		0.94	

(AN – Nagarjuna, AD – Dabur, AK – Kottakkal, AO – Oushadhi, AV – Vaidyaratnam)

Thin layer chromatographic analyses of all aristams were conducted in different polarities such as 30%, 40%, 50% ethanol-chloroform mixtures and 40% butanol- acetic acid as given in the figures (Plate1, Plate 2, Plate 3, and Plate 4).

Plate 1- 30% ethanol-chloroform

Plate 2- 40% ethanol-chloroform

Plate 3- 50% ethanol-chloroform

Plate 4- 40% acetic acid-butanol

DISCUSSION

There are differences in the HPTLC results. The first two samples show similarities in the lower polarity but they have differences in the higher polarity. The other three samples are different in the lower as well as higher polarities. In thin layer chromatographic analysis plate 1 (30 % Ethanol in chloroform) show composition difference for sample 1 while samples 1,4 and 5 are showing differences in plate2 (40% Ethanol). Plate 3 (50 % Ethanol) shows similar behaviour for all samples except 5 while plate 4 (Butanol- acetic acid) show differences with sample 3. It may be noted that no two samples have the same behaviour over the entire range. The study clearly shows that the same medicine marketed by different agencies have got different chemical profiles. Naturally their pharmacological effects will also be dissimilar. Standardization of chemical and pharmacological profiles is absolutely essential. The study points to the possibility of using HPTLC or TLC profiles as a tool for standardization.

CONCLUSION

The chemical profiles of the same medicine marketed by different agencies are showing differences. This may be due to the differences in the raw materials involved or differences in the method of preparation and marketing. The study does not reveal the impact of these differences in the pharmacological activity and in no way indicate the superiority of any one brand over the others. However it is desirable that all medicines show similar chemical

profiles and that matches with that of a standard. HPTLC and TLC in the specified systems may be used for ascertaining this agreement.

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