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TLC AS A TOOL FOR STANDARDISATION OF AYURVEDIC FORMULATIONS WITH SPECIAL REFERENCE TO *KUTAJARISHTA*

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ABSTRACT: *In ancient days, Physicians having the comprehensive knowledge of Bhaishajya Kalpana, used to prepare the drugs themselves to treat their patients. So there was no doubt in obtaining genuine drug with desired therapeutic effect. But in recent years, the growing population and their life style, industrialization etc have forced physicians to depend on market preparations. As such we find the necessity of standardization of these preparations. The quality assessments of a drug, which is a chemical irrespective of the system is possible by 'Thin Layer Chromatographic technique' using known Chemical constituents as reference standards. A herbal preparation 'Kutajarishta', has been standardized by using this technique and the significance of the findings is discussed.*

INTRODUCTION

In Ayurveda great emphasis is given to the complete knowledge of drugs including identification, procurement, processing and application under a separate branch of learning called "Bhaishajya – Kalpana". In ancient days physicians used to prepare drugs themselves according to the need to treat the patients. They were well qualified in identifying the drugs and trained in various processes of formulation of compound drugs. So, it was never a problem to obtain quality drugs was never questioned. But the situation has changed very fast with time. The growing population and their life style, industrialization etc, have forced a physician to depend largely on market preparations and he is not certain about the quality of these drugs or of the genuineness of the ingredients used in the preparations.

The only way left fro him is to depend blindly on these products with the hope of obtaining desired effects or to switch over new products of the same drug of different make (if desired results are not forthcoming). The situation is far from satisfactory and there is a need to access the standard of Ayurvedic drugs. To standardize Ayurvedic preparations, "The central council for research in Ayurveda and Siddha", has described certain methods (organolptic & physicochemical tests) which are inadequate to ensure the quality or therapeutic efficacy of the finished products or even to ascertain whether the correct raw materials are used or not. Quality assessment of compound herbal preparations is rather a tricky job but is relatively simple for preparations containing one or a few plant drugs as the major raw materials. Quality assessment of these drugs can be accomplished by "Thin layer Chromatography" technique, using known chemical constituents as reference standards (markers).

A drug is a 'Chemical', regardless of the system and in a herbal preparation these Chemicals are supplied by the herbs used in the preparations. The knowledge of the essential Chemical are supplied by the herbs used in the preparations. The knowledge of the essential Chemical constituents is a medicinal plant and detection of their presence in a preparation can be readily ascertained by TLC. Even a Semi-quantitative assessment of the Chemical constituents of the preparation is possible by TLC. It is really a competent tool to standardize a drug and more relevant than mere estimation of ash content, determination of specific – gravity etc which may conform to the prescribed values even if an essential medicinal plant is absent in a preparation. No doubt, more sophisticated techniques (H.P.L.C, GLC, Etc) have been developed but TLC is relatively simple, handy, easier, quick, convenient, efficient and inexpensive method for quick assessment of the quality of most of the herbal preparations.

MATERIALS AND METHODS

Thin layer Chromatography is a technique where in a solute distributes between two phases (I) Stationary phase in the form of a thin layer of adsorbent on a glass plate (II) Mobile phase in the form of a liquid (pure solvent or mixtures). In 1938 first time this technique was introduced by Izmailor and Schrabier at the Ukranian institute for experimental pharmacy. But its acceptance was not achieved until late 1950, when stall publicized the method, developed a kit of basic equipments and made them available. Since then TLC has become an important tool for both qualitative and quantitative analysis.

Materials

The essential equipment required for TLC study are :

1) Flat uniform glass plates.

2) An aligning tray on which the plates can be place while applying the substance.

3) The coating substance consists of finely divided absorbent may contain fluorescing material to help in visualizing spots that absorb ultraviolet light.

4) A Spreader is essential to spread the absorbent over the entire surface of the plate, uniformly of desired thickness.

5) A storage race to support the plates during drying and transportation.

6) Graduate micro-pipettes to deliver the solutions of substance to be studied, on plates.

7) A developing Chamber with a lid which can accommodate one or more plates.

8) A sprayer which can emit a fine spray of reagents for visualization of spots.

9) An ultra-violet lamp suitable for observation at short (254 mm) and long (366 mm) ultra-violet wave lengths.

Method

1) The plates are prepared in the following manner to spot the solutions. The coating suspension is prepared in accordance with the instructions of the supplier and spreaded (0.25 to 0.30 thick) over the plates (20 cms long), using a spreader, designed for the purpose. The coated plates are dried in

air and then heated at 100 – 150^{0}C. On cooling, the plates are sorted in desiccating chamber to protect from moisture.

2) The TLC Chamber is prepared by lining the walls with sheets of filter paper and pouring the developing the solvent to saturate the filter paper. The chamber is closed with the lid and allowed to stand for one hour at room temperature.

3) The solutions being examined are applied in the form of circular spots about 2 to 6 mm in diameter, on a line parallel with and 20 mm from one end of the plate and not nearer than 20mm to the sides; the sports should be 15mm apart. The solvent is allowed to dry and then the Chromatoplate is place in the chamber containing the developing solvent in a vertical position. The Chamber is closed and the mobile phase is allowed to ascend upto the marked line. The plate is then removed, dried and visualized by spraying the appropriate reagent evenly; with the help of a sprayer.

In the standardization of Kutajarishta, prepared by following different methods this technique was successfully employed along with other tests and analysis. 'Kutaja' (*Holarrhena antidysente – nica*) is the main medicinal plant in 'Kutajarishta' and it is known to be a rich source of alkaloids. Further alkaloids of Kurchi bark are known to be the active constituents, so and Ayurvedic formulation containing Kutaja bark should contain the Kurchi alkaloids. Five samples of Kuntajarishta, were prepared following different methods described below:

i) Decoction prepared out of the ingredients of Kutajarishta.

ii) Kutajarishta, prepared as per classical 'Arishta' method.

iii) Kutajarishta, prepared as per Arishta method where in decoction along with its residue was added in the fermenting media.

iv) Kutajarishta, prepared as per 'Asava' method.

v) Kutajarishta, prepared as per Arishta method where in decoction process was repeated thrice with the same ingredients, before fermentation.

The total alkaloids (non-quaternary) of all the above five samples were extracted following official method (I.P.C.) and were used for the study.

The solvent systems employed for resolution of kurchi alkaloids on thin layer Chromatograms are :

i) Benzene – Diethyl ether – aqueous ammonia solution (16 : 16 : 0.1)

ii) Ethanol – Acetic acid (3 : 1)

iii) Ethyl acetate – Pyridine – water (20 : 10 : 30) upper phase.

iv) Chloroform – Methanol – Ammonia solution (16:8:0.1)

v) Chloroform – Acetone (3:1)

vi) Chloroform – Methanol (95:5)

Among these seven solvent systems, solvent IV was found to be suitable for better resolution with Dragendorff's reagent as an indicator. The total alkaloids isolated from different samples were dissolved in 50ml of

methanol and 0.1 ml was used for spotting. TLC study was carried out in this solvent system and the following observation were made.

OBSERVATION AND DISCUSSION

a) All the samples showed five distinct Dragendorff staining spots, three of which were particularly intense indicating the presence of three major alkaloids.

b) Co-Chromatography with conessine, the well known kurchi – alkaloid, revealed its occurrence in all samples as the major constituent.

c) Even though the sample (ii) showed the same number of spots as the other samples, the spots were rather feeble which indicated a lower percentage of total alkaloids in this sample. This was corroborated by quantitative estimation of total alkaloids present in it.

d) The low alkaloid concentration in sample (ii) may be due to

   i) Low extraction of total alkaloids.

   ii) Aireal or microbial decomposition of already extracted alkaloids.

   iii) Changing of alkaloids into some other conjugate or complex forms by some plant acids, present in the ingredients of the preparation.

CONCLUSION:

Thus, TLC is a competent tool to standardize Ayurvedic herbal preparations.

It is relatively a simple, handy, easier, convenient, efficient and inexpensive technique for quick assessment of the quality of herbal preparations.

The semi quantitative assessment of the chemical constituents of a preparation is also possible with this technique.

The genuine kutajarishta sample should contain kurchi alkaloids and the alkaloids isolated from the sample should exhibit at least five Dragendorff's staining spots on a Chromatogram when developed with appropriate solvent system.

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