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Standardization of a classical Ayurveda formulation Pathyashadangam Kwath by thin layer chromatography

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Abstract
Ayurvedic physicians of older days used to collect plant materials themselves for preparing drugs under their supervision. So there were no chances for substitution or adulteration and the medicines were sure to have the desired therapeutic effect. The ever increasing demand for ayurvedic medicines, multitude of diseases, scarcity of plant materials and commercialization of drugs have forced physicians of modern times to rely on available market preparations. Hence standardization of these preparations has become a need of the hour to ensure genuineness and therapeutic value. Thin layer chromatography can be used as a simple and reliable tool for standardization of finished products. Pathyashadangam kashayam a classical ayurvedic formulation was subjected to TLC along with raw materials and standards. The presence of all the ingredients in the formulation was confirmed by similarity in bands and R_F values. From the study it was inferred that TLC can be used as a reliable tool for standardization of ayurvedic formulations.

Keywords: Standardization, TLC, Pathyashadangam kwath, Ayurveda

1. Introduction
Ayurveda is an age old person-centered Indian system of medicine which incorporates traditional values and emphasizes on prevention rather than treatment of diseases [1]. Being a system of medicine based on the curative potential of medicinal plants, Ayurveda is still practiced on a large scale in India, a country with a store house of more than 15000 medicinal plants. The concept of polyherbalism where multiple herbs are combined in a particular proportion to enhance therapeutic potential and minimize toxicity is widely used in Ayurveda [2]. Kwath is such a polyherbal preparation where several herbs are boiled together with 4, 8 or 16 times water under constant flame till the volume is reduced to one fourth [3]. Pathyashadangam kwath is a formulation prepared from seven ingredients *Terminalia chebula* Retz. , *Terminalia bellirica* (Gaertn.) Roxb., *Phyllanthus emblica* L., *Andrographis paniculata* (Burm. f.) Wall. exNees., *Curcuma longa* L., *Azadirachta indica* A. Juss. and *Tinospora cordifolia* (Willd.) Miers, used for migraine, cluster head ache and other infections.
Confirming the authenticity of each raw material and standardization of its preparation is key to ensure therapeutic effect of every drug. Deliberate or unknowing addition of a different raw material or failure of addition of desired raw material can result in a formulation having harmful health effects or lacking desirable properties. Hence it is essential to ensure the presence of each raw material in the finished product to ascertain its therapeutic potential. Achieving this goal without using sophisticated instruments is critical as it will be the method of choice in manufacturing units. Thin layer chromatography with its simplicity, ease, sensitivity and rapidness can serve as a reliable method to ensure the presence of each raw material in the finished product. It serves as a preliminary method of choice for qualitative analysis of pharmaceutical products [4]. Owing to its simplicity and cost effectiveness TLC is frequently used for separation and identification of drugs [5]. With the objective of standardizing Pathyashadangam kwath, the methanol extracts of the formulation and its individual kwaths were subjected to thin layer chromatographic analysis.

2. Materials and methods
The methanol extract of Pathyashadangam kwath and individual kwaths were prepared by refluxing 5 ml of each kwath with methanol. The extract was filtered and used for TLC. Silica gel 60 F_{254} (Merck) plates were used. 3-5 microliters of the extracts were applied as bands of about 6-8 mm length, 7 mm apart and 10 mm from the base of the plate. The TLC chamber was saturated with mobile phase for 30 minutes and the plates were developed to a distance of80 mm from the application line. Then the plates were dried and observed under visible and long UV light. The plates were sprayed with appropriate derivatizing reagent, heated and then visualized. R_F value was calculated by the formula

RF = Distance travelled by the solute/distance travelled by the solvent front

For thin layer chromatography of Pathyashadangam kwath, *Terminalia chebula, Terminalia bellirica* and *Phyllanthus emblica* the mobile phase used was Acetone: Chloroform: Formic acid: Toluene: Methanol in the ratio 2:2.5:0.5:3:2. Gallic acid was used as standard [6]. Plates were derivatized with 0.1% aqueous $FeCl_3$. 0.1% aqueous $FeCl_3$ was prepared by dissolving 0.1 g $FeCl_3$in 100 ml water [7].

Pathyashadangam kwath and *Curcuma longa* - The mobile phase used was Chloroform: Ethanol: Formic acid in the ratio 80:4:0.8(v/v/v) [8]. Curcumin was used as standard.

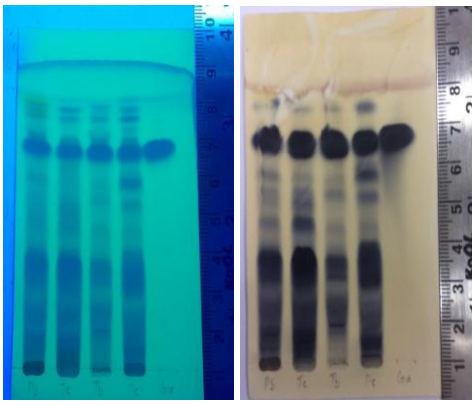
Pathyashadangam kwath and *Andrographis paniculata* – Mobile phase used was Chloroform: Methanol in the ratio The plates were derivatized using 20% methanolic sulphuric acid [9].

Pathyashadangam kwath and *Tinospora cordifolia* – Mobile phase used was Chloroform: Ethanol in the ratio 9.5:0.5 and derivatized with 20% methanolic sulphuric acid (v/v) [10].

Pathyashadangam kwath and *Azadirachta indica* – Mobile phase used was Chloroform: Ethyl acetate: Formic acid in the ratio 5:4:1.The plates were derivatized with methanolic sulphuric acid [11].

3. Results

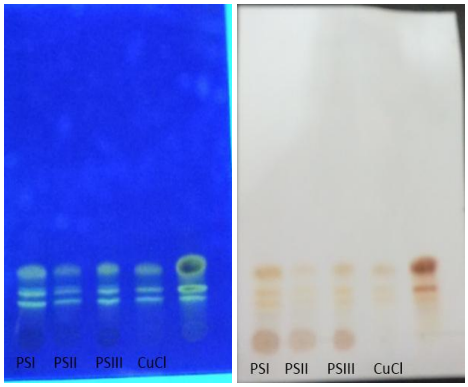
TLC of methanol extracts of Pathyashadangam kwath (PS), individual kwaths of *Terminalia chebula* (Tc), *Terminalia bellirica* (Tb) and *Phyllanthus emblica* (Pe) along with Gallic acid, when observed under long UV showed a number of blue bands at similar R_F values. After derivatization with 0.1% aqueous $FeCl_3$ all these bands appeared black in colour confirming their phenolic nature. A prominent band was observed for PS, Tc, Tb and Peat $R_F$value 0.725 corresponding to gallic acid. From this it can be concluded that gallic acid is a main constituent in Pathyashadangam kwath and also in *Terminalia chebula, Terminalia bellirica* and *Phyllanthus emblica*. Bands were observed for Pathyashadangam kwath and all the individual kwaths at R_F 0.82, 0.612 and 0.56. For PS, Tc and Pe bands were also observed at R_F 0.85. Band at R_F 0.875 was observed for PS and Tb. Bands were observed for PS and Tc at R_F 0.5 and 0.46, for Pb and Pe at R_F0.437. From this it can be understood that Acetone: Chloroform: Formic acid: Toluene: Methanol in the ratio 2:2.5:0.5:3:2 is a suitable mobile phase for chromatographic analysis of Pathyashadangam kwath and its ingredients *Terminalia chebula, Terminalia bellirica* and *Phyllanthus emblica*.



(a) (b)

Fig 1: Pathyashadangam kwath (PS), kwaths of Terminalia chebula (Tc), Terminalia bellirica (Tb), Phyllanthus emblica (Pe) and Gallic acid (Ga). (a) Plate observed under long UV and (b) plate after derivatization with 0.1% aqueous FeCl3.

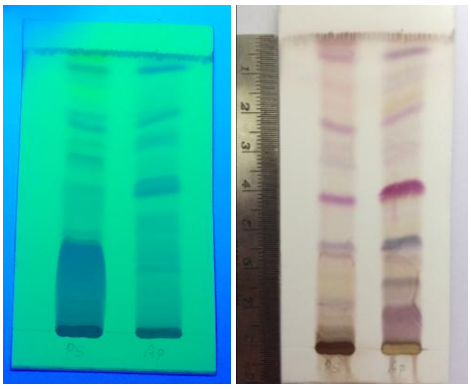
Thin layer chromatography of methanol extracts of three batches of Pathyashadangam kwath (PS), Curcuminoidsstandard (Cu) and *Curcuma longa* (Cl) kwath using Chloroform: Ethanol: Formic acid in the ratio 80:4:0.8(v/v/v) mobile phase showed three bands corresponding to curcuminoids. The bands were clearly observed under visible and long UV light showing that curcuminoids are present in kwath, even though reports on extraction of curcumin in water are rare.



(a) (b)

Fig 2: Three batches of Pathyashadangam kwath (PS I, PS II, PS III), Curcuminoids and Curcuma longa kwath. (a) Plate observed under long UV and (b) visible light.

TLC of methanol extract of pathyashadangam kwath and Andrographis kwath (Ap) when developed with Chloroform: Methanol 7:1 showed blue coloured bands at R_F 0.94, 0.71 and 0.49. On derivatization using 20% v/v methanolic sulphuric acid all these bands appeared violet in colour. After derivatization, ash coloured bands were observed at R_F 0.34 and 0.2.Similarity in R_F and colour of bands in both the individual kwath and pathyashadangam kwath confirms the presence of Andrographis in the formulation. Hence TLC using mobile phase Chloroform: methanol in the ratio 7:1 can be used to confirm the presence of *Andrographis paniculata* in polyherbal formulations.

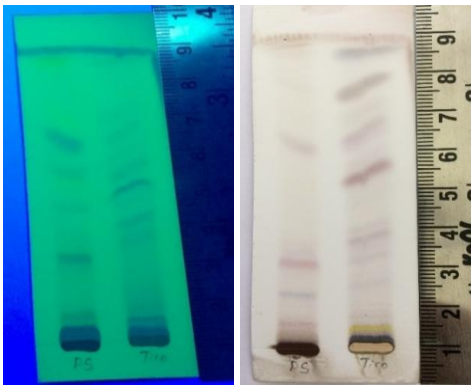


(a) (b)

Fig 3: Pathyashadangam kwath (PS) and Andrographis kwath (Ap) (a) Plate observed under long UV and (b) plate after derivatization with 20% v/v methanolic sulphuric acid.

When methanol extract of PS and *Tinospora cordifolia* (Tico) were spotted on TLC silica gel 60 F_{254} plates, developed with mobile phase Chloroform: Ethanol (9.5:0.5)and observed under long UV light blue bands were observed at R_F values 0.44,0.58 and 0.69. After derivatization with methanolic sulphuric acid, violet coloured bands were observed at R_F values 0.16, 0.25, 0.69 and 0.825. The presence of similar

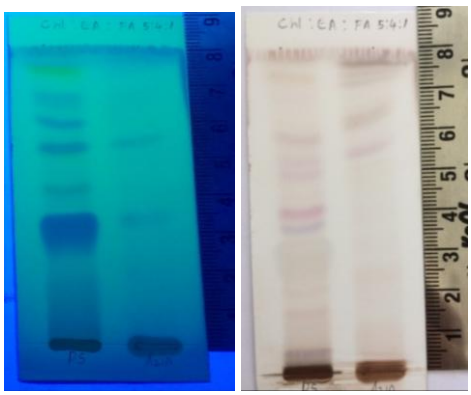
bands in the individual kwath and the formulation, confirms its presence in the formulation. Hence in order to confirm the presence of *Tinospora cordifolia* in polyherbal formulations TLC using Chloroform: Ethanol (9.5:0.5) mobile phase can be employed.



(a) (b)

Fig 4: Pathyashadangam kwath (PS) and Tinospora cordifolia (Tico) (a) Plate observed under long UV and (b) plate after derivatization with methanolic sulphuric acid.

TLC of PS and *Azadirachta indica* kwath (Azin) using Chloroform: Ethyl acetate: Formic acid (5:4:1) mobile phase revealed two blue bands at R_F value 0.43 and 0.69 under long UV for both the formulation and individual kwath. After derivatization with methanolic sulphuric acid violet coloured bands were observed at R_F values 0.66 and 0.75 in both the cases confirming the presence of *Azadirachta indica* in the formulation. Hence these R_F values can be used for confirming the presence of *Azadirachta indica* in polyherbal formulations on TLC analysis using the said mobile phase. Pathyashadangam kwath showed additional blue bands at R_F values 0.53, 0.75, 0.81 and a yellow coloured band at R_F 0.93. After derivatization PS showed additional blue band at R_F 0.44 and violet bands at 0.5 and 0.6 and Azin showed additional violet bands at R_F 0.89, 0.93 and 0.98.



(a) (b)

Fig 5: Pathyashadangam kwath (PS) and Azadirachta indica (Azin) (a) Plate observed under long UV and (b) plate after derivatization with methanolic sulphuric acid.

4. Discussion

TLC is a method of choice for analysis of herbal products on account of minimum preparation requirements, low cost, repeatability, possibility of detecting compounds absorbing UV light, and ease of documentation [12]. Different pharmacopoeias like AHP, IHP, IP and API recommend TLC as a method for evaluation of phytochemical constituents of herbal drugs. It is a salient parameter for standardization of herbal drugs and has been employed for quality evaluation and establishment of batch to batch consistency of several churnas [13]. Being a handy, decent, cost effective and simple method, it has been used for the standardization of Ayurvedic formulations like *Kutajarishta* [14]. It has also been used for the standardization of *Aswagandharishta*, wherein Benzene: Ethyl Acetate (9:1) mobile phase was employed [15]. TLC of ethanol extract using Chloroform (9): Ether (1) mobile phase and silica gel GF_{254} plates has been proved to be a reliable and useful tool for quality control and routine analysis of *Haridrakhanda* [16]. Toluene: Ethyl acetate: Formic acid: Glacial acetic acid in the proportion of 5:5:1:1 has been employed for TLC analysis of a polyherbal Ayurveda formulation for obesity, *Vara Asanadikwatha* [17]. A comparative analysis of *Argwadhadikwatham* and individual kwathams was carried out by TLC wherein the disappearance of two bands found in individual kwaths, in the formulation was inferred to be due to the formation of a new compound, accounting for its increased therapeutic potential [18].Even though Pathyashadangam kwath is widely used for the treatment of head ache and nervine disorders, there are no reports on standardization of this formulation by TLC. Several methods for thin layer chromatographic standardization of individual ingredients of the kwath are available in standard reference books. The suitability of these mobile phases for chromatographic analysis of the formulation was studied. From the study it was found that mobile phases Acetone: Chloroform: Formic acid: Toluene: Methanol in the ratio 2:2.5:0.5:3:2, Chloroform: Ethanol: Formic acid in the ratio 80:4:0.8, Chloroform: Methanol (7:1), Chloroform: Ethanol (9.5:0.5) and Chloroform: Ethyl acetate: Formic acid (5:4:1) were suitable for standardization of the kwath along with individual ingredients. Thin layer chromatographic analysis using these mobile phases revealed that some of the bands of individual kwaths are found at the same R_F in the polyherbal formulation, which can be employed for quality evaluation. Some of the bands of individual kwaths are missing in the formulation, the reason for which may be masking of certain chemicals by other ingredients orcomplex formation due to continuous heating during the preparation of the formulation resulting in synergism, accounting for the enhanced therapeutic activity of the formulation over individual ingredients. It has been reported that the therapeutic effects of herbal medicines are enhanced when compatible herbal ingredients are formulated together [19]. Further studies are required to unravel the mystery behind the same.

5. Conclusion

Pathyashadangam kwath, a polyherbal formulation prepared from seven ingredients was subjected to TLC analysis along with individual ingredients using different mobile phases with an objective to standardize the formulation. Depending on the individual ingredient, appropriate mobile phase was chosen so as to get clear bands. Accordingly Acetone: Chloroform: Formic acid: Toluene: Methanol in the ratio 2:2.5:0.5:3:2 was found to be a suitable mobile phase to confirm the presence of *Phyllanthus emblica*, *Terminalia bellirica* and *Terminalia chebula*, Chloroform: Ethanol: Formic acid in the ratio 80:4:0.8(v/v/v) for *Curcuma longa*, Chloroform: Methanol in the ratio 7:1 for *Andrographis paniculata*, Chloroform: Ethanol (9.5:0.5) for *Tinospora cordifolia* and Chloroform: Ethyl acetate: Formic acid (5:4:1) for assuring the presence of *Azadirachta indica*in formulations. It was also found that the presence of each ingredient in Pathyashadangam Kwath could

be confirmed by TLC which is a simple, non-laborious, rapid and less costly mode of analysis which can be performed without the need of sophisticated instruments. Hence it can be concluded that TLC can be adopted as the method of choice for quality evaluation of polyherbal formulations like Pathyashadangam kwath.

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