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A COMPARATIVE QUALITATIVE PHYTOCHEMICAL ANALYSIS OF IN-HOUSE AND COMMERCIAL POLYHERBAL FORMULATIONS USING THIN LAYER CHROMATOGRAPHY

Sanket Charola[1*], Ravinayak Patlavath[1], Mitika Desai[2], Lency Panchal[1], Pujan Kansara[2], Afsha Ansari[1], Charmi Patel[1]
[1]Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India.
[2]Dr. Vikram Sarabhai Institute of Cell and Molecular Biology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India.
**Author for Correspondence: sanket.charola-botany@msubaroda.ac.in*

ABSTRACT
Phytochemicals play an important role in protecting cells, reducing inflammation, preventing cancer growth, balancing hormones, and improving gut health. Many polyherbal commercial products in the market claims health benefits based on the ingredients. To validate the claim of one such polyherbal product, an in-house plant mixture was generated and tested for the phytoconstituents using Thin Layer Chromatography. A commercial product was purchased in powder form, and an in-house mixture of similar form was prepared using the same ingredients. With the maceration method, a methanolic extract was prepared and the TLC was standardized using various solvent systems. The plates were developed with iodine to visualize the bands. It was found that the commercial product showed eight bands on TLC in comparison to ten bands in in-house sample. The result indicates that polyherbal composition in commercial mixture is different from the in-house sample.

Keywords: *TLC, Polyherbal Mixture, Phytochemicals, Herbal Product*

INTRODUCTION
Plants are an important resource for medicinal purposes. Phytochemicals, being the bioactive compounds, have garnered significant attention for many years due to their diverse therapeutic properties, as economically cheaper option, and easy accessibility (Manzoor *et al.*, 2021). These compounds, namely flavonoids, alkaloids, terpenoids, and phenolics, play a pivotal role in mitigating oxidative stress, modulating immune response, and promoting overall health (Pandey and Rizvi, 2009; Zhang *et al*., 2015). The growing interest in this field has led to the development of numerous polyherbal formulations, which combine multiple plants to enhance therapeutic efficacy through synergistic effects (Patwardhan and Gautam, 2005).
Polyherbal products are widely marketed for their health benefits, including improved gut health, reduced inflammation, and cancer prevention (Gupta and Birdi, 2017). However, the efficacy and safety of these products depend on the quality and consistency of their phytochemical composition. Many marketed products claim on-pack for various clinical efficacy without even proper scientific validation. Despite the widespread availability of commercial polyherbal products, there is a lack of rigorous scientific validation of their phytoconstituents, which raises concerns about their authenticity and therapeutic claims (Sahoo *et al.*, 2010). Thin Layer Chromatography (TLC) is a widely used analytical technique for the qualitative and semi-quantitative analysis of phytochemicals, offering a cost-effective and reliable method for fingerprinting complex herbal mixtures (Wagner and Bladt, 1996). Recent studies have highlighted discrepancies between the claimed and actual phytochemical composition of commercial herbal products. Numerous studies have investigated polyherbal mixtures and discovered significant variations in their ingredient compositions, despite being marketed under the same name (Kumar *et al.*, 2022). During

COVID-19 outbreak, many products were marketed with claim as immunity boosters (Kalal and Charola, 2021) . However, there were significant lacunas of scientific validation. A study has revealed significant variations in the phytochemical profiles of commercially available polyherbal formulations compared to their in-house prepared counterparts (Kumar et al., 2022). Such inconsistencies underscore the need for standardized methods to validate the composition and quality of polyherbal products.
In this study, we aimed to evaluate the phytochemical composition of a commercially available polyherbal product and compare it with an in-house prepared mixture of the same ingredients. Using TLC, we standardized the extraction and analysis protocols to identify and compare the phytoconstituents in both samples. Our findings provide critical insights into the quality and consistency of commercial polyherbal products, highlighting the importance of rigorous scientific validation to ensure their safety and efficacy.

MATERIALS AND METHODS

Plant Materials

A commercially available polyherbal product was purchased from the market and its batch number, date of manufacture, date of expiry, and plant names were recorded. For in-house preparation of the same, dried plant herbs were purchased individually from certified herbs dealer. The samples were identified using taxonomic characters and their voucher specimens were prepared.

Preparation of plant extracts

Collected commercial polyherbal product (CS) was in coarse powder form. The in-house preparation of the plant mixture (GS) was prepared after air drying the herbs at room temperature for 24 hours. Using grinder, all herbs were blended in equal amounts to a course powder and were stored in an air-tight container. Both the samples, CS and GS were subjected to maceration using methanol for 48 hours at 10% w/v. The extracts were then filtered using Whatman filter paper 1. The filtrates were concentrated using rotary evaporator and % yield was noted. The extracts were stored at 4 ºC until further use.

Phytochemical analysis using Thin Layer Chromatography

For phytochemical analysis, TLC plates were purchased from Merck as Silica gel 60 F254 with dimensions of 7 cm x 6 cm. The plates were cut with scissors to suit the size of the experiment using nitrile gloves. Plate marking was done with the soft pencil for start-point and spotting sites around 2 cm apart. Using glass capillaries, 1 µl of sample was spotted at designated places as CS and GS. After air-drying the spots, plates were kept in the TLC chamber for about 15 minutes. Various mobile phase combinations were used like Hexane : Acetic acid (9:3), Hexane : Acetic acid (7.5:4), Hexane : Ethyl acetate : Formic acid (9:1:0.5), and Petroleum ether : Acetic acid (7.5:2.5). After the completion of the run, TLC plates were removed from the chamber and were sprayed with freshly prepared iodine reagent. The plates were photographed in visible light, 366nm UV light, and 254nm UV light. The movement of the analytes was marked with pencil and expressed by its retention factor (Rf). Following formula was used to calculate the Rf value for each band.

$$Rf = \frac{Distance\ traveled\ by\ the\ solute}{Distance\ traveled\ by\ the\ solvent\ front\ on\ TLC\ plate}$$

RESULTS

Plant details of the polyherbal product

The commercial product purchased from the market was analyzed for the on-pack information. The plants listed on the pack are Ganthoda, Dry ginger, Gokharu, Poppy seeds, Aasan, Satavari, Ekhara, Cinnamon, Long pepper, Kamarkas, Mace, White koucha, Balbij, Tejbal, Vaskapoor, White pepper, Cardamom, Black pepper, Salampanja, Nagkesar, Salamdana, Clove, and White Musali. All these twenty-three herbal products were individually purchased from the market and in-house polyherbal mixture was also prepared for further analysis.

Extractive values of the formulations

As shown in Table 1, both the extracts had moderately reddish-brown color. With 10gm of samples, the yield obtained for CS was 208mg, and for GS was 292mg. The % yield (w/w) for the samples were 2.08% and 2.92% respectively.

Table 1: Extractive values of polyherbal products

Sr No	Name of the extract	Color	Yield (gm)	% yield (w/w)
1	CS	Reddish-brown	0.208	2.08
2	GS	Reddish-brown	0.292	2.92

Phytochemical profile using TLC

As shown in Figure 1, the TLC plates were developed using iodine and were visualized with visible light, and UV light at 366nm and 254nm. The CS and GS samples being polyherbal in character showed various bands at TLC plate indicating rich phytochemicals. However, the positions of the bands (Rf values) and the band intensity indicated varied phytochemical composition in both the samples. As shown in Table 2, the plates with Hexane : Acetic acid at ratio of 9:3 as mobile phase showed 8 bands for CS while 10 bands for GS sample. As indicated by Rf values, 0.95, 0.73, 0.47, 0.43, and 0.28 were common bands in both the samples. However, the rest of the bands were unique for CS and GS samples indicating varied composition of the samples. Similarly, Hexane : Acetic acid mobile phase at ratio of 7.5 : 4 developed 5 bands for CS while 8 bands for GS.

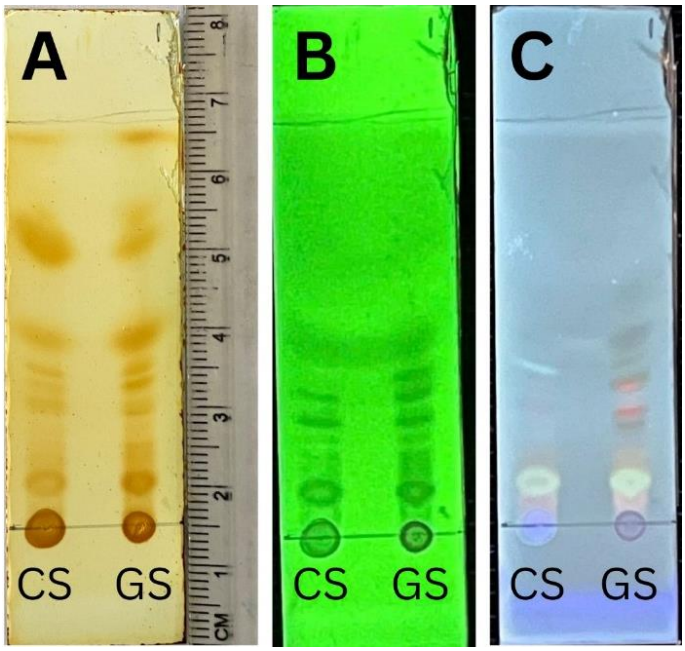

Figure 1: TLC plates developed with Iodine: (A) Visible light, (B) 366nm UV light, (C) 254nm UV light.

Further, the Hexane : Ethyl acetate : Formic acid at ratio of 9:1:0.5 developed 6 bands for CS and 5 bands for GS. The numbers of developed bands for GS in this case were lesser than that of CS. Additionally, the Petroleum ether : Acetic acid (7.5:2.5) as mobile phase presented 8 bands for CS and 10 bands for GS samples. The presence of the bands in this mobile phase solvent combination was more over common, except 4 unique bands in both the samples.

Table 2: TLC profile with different solvent systems

Solvent systems	Rf values for TLC developed with Iodine	
	CS	GS
Hexane : Acetic acid (9:3)	0.95, 0.73, 0.67, 0.50, 0.47, 0.45, 0.43,0.28	0.95, 0.79, 0.73, 0.55, 0.51, 0.47, 0.43, 0.37, 0.34, 0.28
Hexane : Acetic acid (7.5:4)	0.88, 0.68, 0.61, 0.52, 0.34	0.94, 0.89, 0.73, 0.62, 0.52, 0.47, 0.44, 0.34
Hexane : Ethyl acetate : Formic acid (9:1:0.5)	0.98, 0.79, 0.72, 0.63, 0.45, 0.37	0.98, 0.76, 0.64, 0.44, 0.37
Petroleum ether : Acetic acid (7.5:2.5)	0.97, 0.94, 0.76, 0.68, 0.57, 0.48, 0.42, 0.33	0.97, 0.94, 0.77, 0.66, 0.65, 0.57, 0.51, 0.45, 0.36, 0.26

DISCUSSION

The present study aimed to evaluate the phytochemical composition of a commercially available polyherbal product (CS) and compare it with an in-house prepared polyherbal mixture (GS) using Thin Layer Chromatography (TLC). The findings revealed significant differences in the phytochemical profiles of the two samples, highlighting variations in the composition and quality of the commercial product compared to the in-house formulation.

Phytochemical Diversity and Composition

The TLC analysis demonstrated that both CS and GS samples contained a rich array of phytochemicals, as evidenced by the multiple bands observed under visible and UV light. However, the number of bands and their Rf values varied significantly between the two samples. For instance, the Hexane: Acetic acid (9:3) solvent system revealed 8 bands for CS and 10 bands for GS, indicating a higher phytochemical diversity in the in-house mixture. This discrepancy could be attributed to differences in the quality, concentration, or processing methods of the raw materials used in the commercial product. Similar findings have been reported as variations in the phytochemical profiles of commercial polyherbal formulations compared to their in-house counterparts, emphasizing the need for stringent quality control measures in the herbal industry (Kumar *et al*., 2022). The presence of common bands (e.g., Rf values 0.95, 0.73, 0.47, 0.43, and 0.28) in both samples suggest that certain phytochemicals were consistently present, likely due to the shared ingredients listed on the product label. However, the unique bands in each sample indicate the presence of additional compounds that may arise from variations in plant sourcing, storage conditions, or extraction methods. These findings align with previous studies highlighting the impact of processing techniques on the phytochemical composition of herbal products (Pandey and Mishra, 2020).

Extractive Values and Yield

The extractive values of the two samples further underscored the differences in their composition. The in-house mixture (GS) yielded a higher percentage (2.92% w/w) compared to the commercial product (CS) (2.08% w/w). This difference in yield could be attributed to variations in the efficiency of the extraction process or the quality of the raw materials used. A study has reported that the extractive value of herbal formulations is influenced by factors such as particle size, solvent polarity, and extraction duration, which may explain the observed differences in yield (Gupta *et al*., 2021).

Implications for Quality Control

The variations in phytochemical profiles and extractive values between the commercial and in-house samples highlight the need for standardized quality control protocols in the production of polyherbal formulations. The absence of certain phytochemicals in the commercial product, as indicated by the fewer bands in TLC analysis, raises concerns about its therapeutic efficacy and consistency. This is particularly relevant given the growing consumer reliance on herbal products for health benefits. A recent study has emphasized the importance of adopting advanced analytical techniques, such as High-Performance Thin

Layer Chromatography (HPTLC) and Liquid Chromatography-Mass Spectrometry (LC-MS), to ensure the authenticity and quality of herbal products (Sahoo *et al*., 2023).

CONCLUSION

The findings of this study demonstrate significant differences in the phytochemical composition of a commercial polyherbal product and an in-house prepared mixture. These variations underscore the importance of rigorous quality control measures to ensure the consistency, safety, and efficacy of herbal formulations. The use of standardized analytical methods, coupled with advanced techniques, can help bridge the gap between traditional knowledge and modern scientific validation, ultimately benefiting consumers and the herbal industry alike.

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