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RESEARCH ARTICLE

SCIENTIFIC PROOF FOR 'SEEING IS BELIEVING' –CHROMATOGRAM BASED EVIDENCE FOR ANTI DANDRUFF OIL

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ABSTRACT

One of the reasons for the poor conversion of Siddha drugs from tall promise to therapeutically effective despite they are being formulated with time tested and laboratory proven herbal ingredients is due to the question of whether the herbs are present in the finished product. The present study has established the fingerprint perseverance of certain herbs on 'as is basis' and in the finished product. We have chosen anti dandruff oil, a scientifically and clinically proven Siddha drug for the present study. Study findings shows that anti dandruff oil speak and does its promise may be because the herbs in the formulation are well preserved in the finished product as established by HPTLC.

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INTRODUCTION

From time immemorial, herbal oils are being used in India for various medicinal purposes viz., enhancing hair growth, treating dandruff, alleviating pain, skin dryness etc [1, 2, 3, 4 & 5]. The traditional Indian system of medicine has rich mention of oils that are used for treating wide variety of diseases including Psoriasis and Vitiligo [5, 6, 7& 8]. Most of the herbal oils are prepared either with single herb or with multiple herbs. The oil miscible constituents of the herbs are eluted and the eluents of the herb (s) in the respective oil is believed to be responsible for various therapeutic benefits. The profile and the fingerprint of respective herb (s) when determined at the individual level through chromatogram, the profile also must reasonably match and detectable in the finished product. Only when such sync is established, the sanctity of product efficacy can be achieved with reasonable conviction. In poly herbal oils, the signature fingerprint of the herbs must be detectable in the finished product. In the present paper we report the confirmation and fingerprint matching of different herbs in the proprietary Siddha medicine- Anti dandruff oil. The findings are discussed in the paper.

MATERIALS AND METHODS

Description: Anti dandruff oil* is a licensed proprietary Siddha medicine of Dr. JRK's Siddha Research and Pharmaceuticals Pvt Ltd Chennai.

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Composition of Anti dandruff

Each 5ml contain extracts of %
Hibiscus rosa-sinensis: 100mg2
Wrightiatinctoria: 100mg2
Cassia alata: 100mg2
Azadirictaindica: 100mg2
Oleumcocosnucifera: Q.S

Collection of the plant materials

All the above 4 herbs were procured from the approved medicinal plant supplier. In brief the leaf/ flower/seed whichever is applicable of the above plants were procured and their quality was then ascertained. The above plant materials were then shade dried to reduce the moisture content to 5 %, wherever applicable.

Extraction process

The leaf/flower/seed materials (after crushing) were individually subjected to extraction using super critical fluid and the final extract was adjusted to 10 % in coconut oil. All the required process such as duration of treatment, continuous stirring etc., were followed to ensure complete extraction of the metabolites.

Preparation of Anti dandruff oil: Anti dandruff oil was prepared with oil extracts of each herb. To achieve 2% of 100 % oil extract of each herb, 20 % of the oil extract of each herb was used and the final volume was made up to 100 with coconut oil.

HPTLC analysis of 10 % extracts of individual herbs

The above 4 oil extracts were treated separately with methanol at 1:1 ratio. 5 micro liter of the methanolic fraction of the oil extract of each herb was loaded separately on HPTLC plate-silica gel 60 F 254 of E. MERCK KGaA and was allowed to run using a mobile phase composed of Hexane: Ethyl acetate at 8: 2 ratio. The plates were scanned through a TLC scanner (CAMAG TLC Scanner 3 "Scanner3_150607" S/N 150607 (1.14.28) at 254nm and the profile was subjected to derivatization using p-Anisaldehydesulphuric acid stain and the derivatized TLC plate was scanned at 366 nm. The profile of each herbal extract was recorded.

HPTLC analysis Anti dandruff oil

The anti dandruff oil was subjected to TLC separation after preparing the methanolic fraction as described above and was scanned at 254 nm. The profile was further derivatized using p-Anisaldehydesulphuric acid stain and was scanned at 366 nm. The Rf values were tabulated.

RESULTS

The HPTLC chromatogram has revealed the distinct signature fingerprints of all the four herbs present in the Anti-Dandruff oil such as *Hibiscus rosa-sinensis*, *Azadirictaindica*, *Cassia alata* and *Wrightiatinctoria* (Table 1) when scanned at 254 nm and after derivatization at 366 nm.

Table 1. HPTLC profile of various herbal extracts of Anti dandruff Oil scanned at 254nm

Rf Value- Chromatogram of anti dandruff oil vis-à-vis its herbs					
CO	HR	AI	CA	WT	Dano
0.21	0.11	0.2	0.2	0.03	0.03
0.27	0.2	0.42	0.32	0.06	0.07
0.45	0.41	0.61	0.38	0.17	0.18
0.61	0.62	0.67	0.45	0.24	0.22
0.67	0.67	0.78	0.67	0.31	0.47
0.78	0.78		0.78	0.44	0.67
0.85				0.60	0.76
0.87				0.67	
0.94				0.77	

Table- 2. Derivatization of various herbal Extracts of Antidandruff oil scanned at 366 nm

Rf Value- Chromatogram of anti dandruff oil vis-à-vis its herbs					
CO	HR	AI	CA	WT	Dano
0.42	0.13	0.19	0.19	0.04	0.19
0.49	0.19	0.22	0.39	0.08	0.22
0.59	0.23	0.34	0.46	0.17	0.29
0.63	0.31	0.36	0.62	0.28	0.32
0.65	0.35	0.47	0.64	0.3	0.35
0.81	0.47	0.53	0.69	0.47	0.48
0.83	0.56	0.61	0.81	0.64	0.53
0.9	0.61	0.64	0.82	0.66	0.60
-	0.63	0.69	0.84	0.69	0.67
-	0.7	0.8	-	0.8	0.68
-	0.83	0.82	-	-	0.77
-	-	0.86	-	-	0.91
-	-	-	-	-	0.95

Figures 1 to Figure 14

The individual profiles of each herb could be seen in Anti-Dandruff oil as well. The signature fingerprint of the individual herb could be distinctly identified in Antidandruff oil through chromatogram. Further the signature fingerprint of each herb was quite unique and different from coconut oil (Table 2)

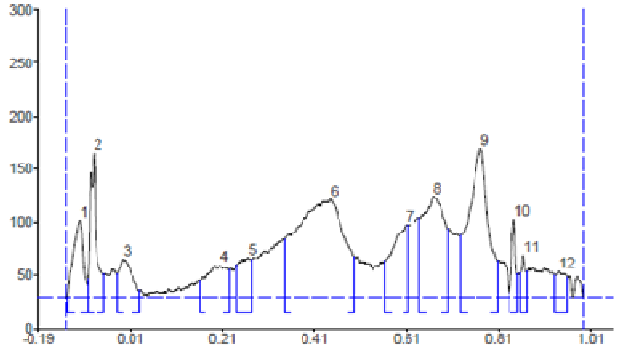

Fig. 1. HPTLC Chromatogram of Coconut oil at 254 nm

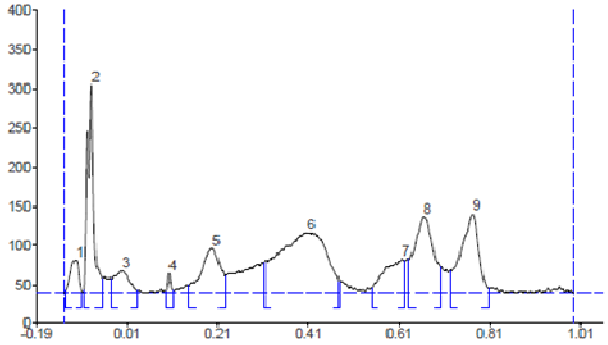

Fig.2. HPTLC chromatogram of oil extract of*Hibiscus rosasinensis* at 254 nm

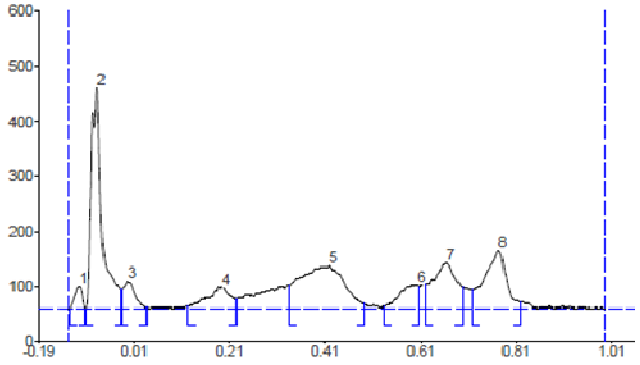

Fig. 3. HPTLC chromatogram of oil extract of*Azadirachtaindica* at 254 nm

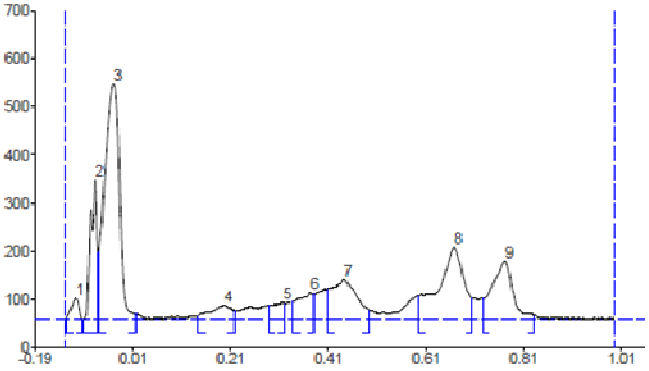

Fig. 4. HPTLC chromatogram of oil extract of *Cassia alata* at 254 nm

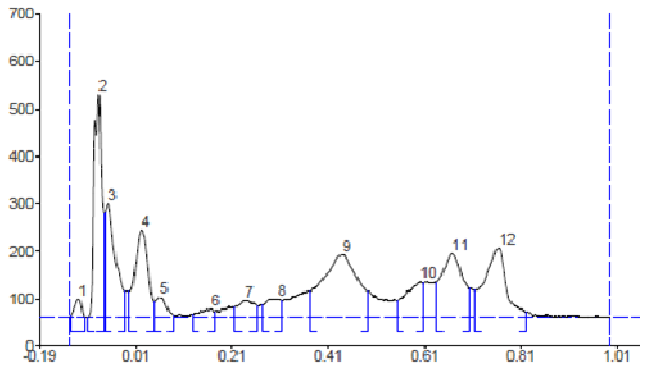

Fig. 5. HPTLC chromatogram of oil extract of *Wrightiatinctoria* at 254 nm

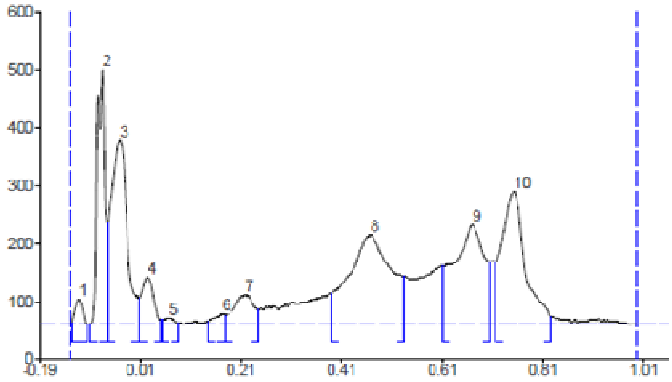

Fig. 6. HPTLC chromatogram of Dano-Anti Dandruff oil at 254 nm

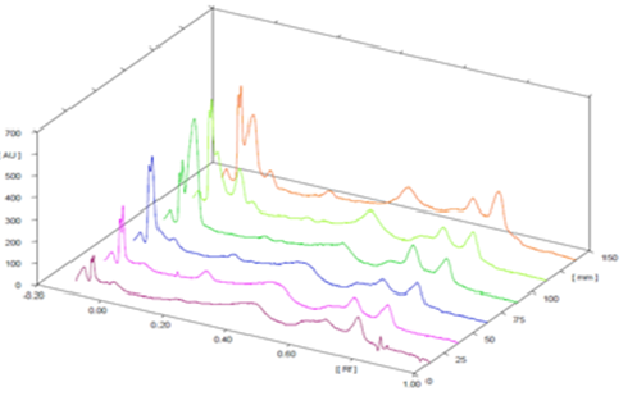

Fig. 7. Densitometric profile of CO and oil extracts of HR, AI, CA, WT and Dano at 254 nm

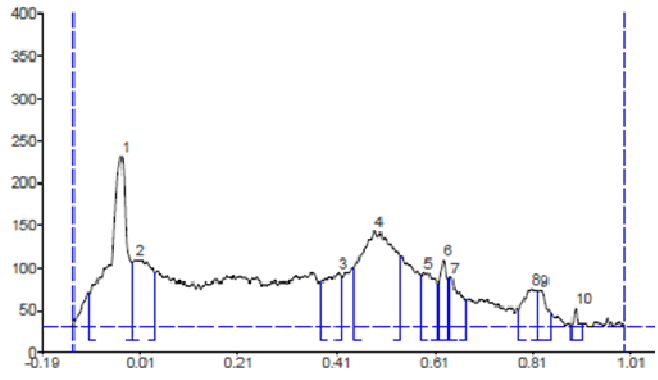

Fig. 8. HPTLC chromatogram of Coconut oil at 366 nm

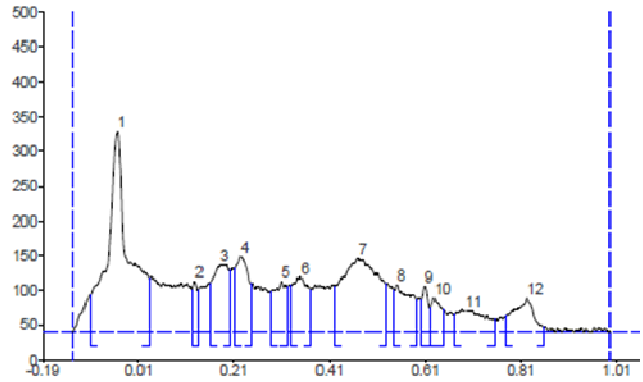

Fig. 9. HPTLC chromatogram of oil extract of*Hibiscus rosasinensis* at 366 nm

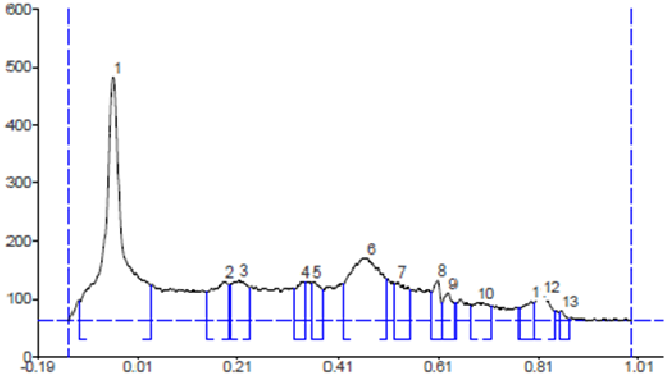

Fig.10. HPTLC chromatogram of oil extractof *Azadirachtaindica* at 366 nm

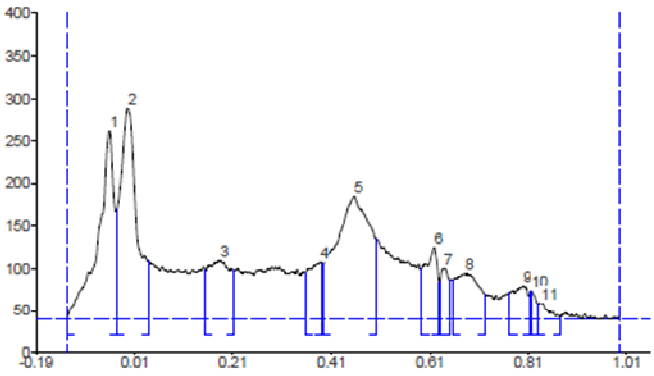

Fig. 11. HPTLC chromatogram of oil extract of*Cassia alata* at 366 nm

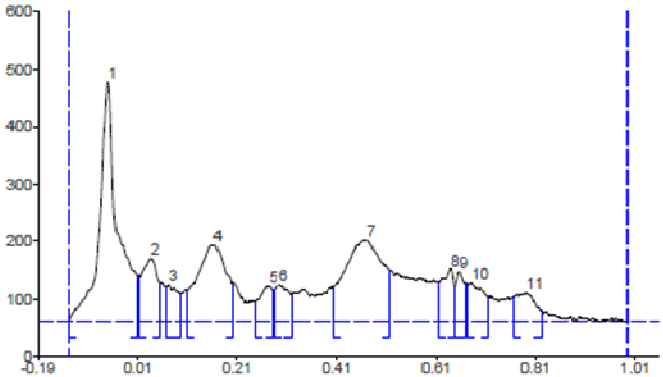

Fig.12. HPTLC chromatogram of oil extract of *Wrightiatinctoria* at 366 nm

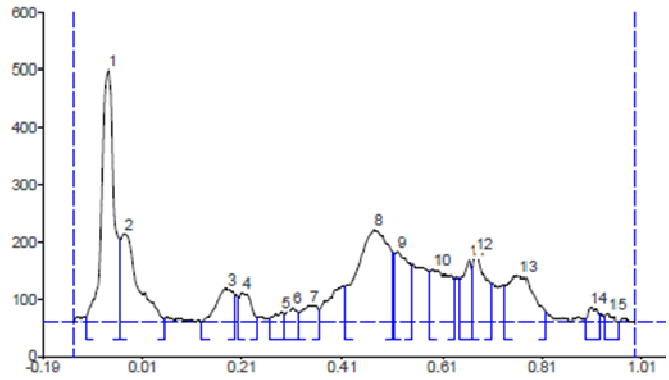

Fig.13. HPTLC chromatogram of Dano oil at 366 nm

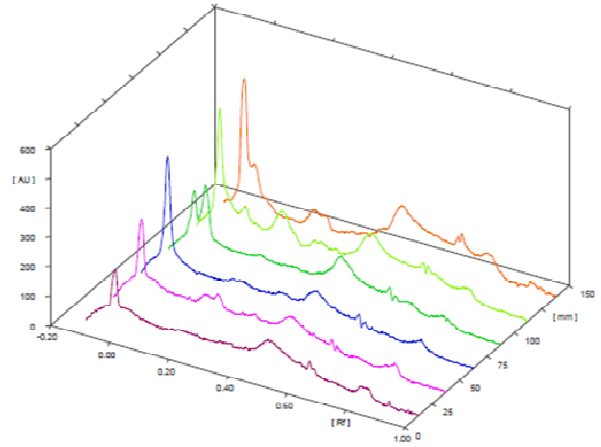

Fig. 14. Densitometric profile of CO and oil extracts of HR, AI, CA, WT and Dano at 366 nm

DISCUSSION

Despite the use of herbs with proven efficacy at laboratory level, when traditional medicine are made with such proven herbs, the clinical efficacy remains doubted and questioned. The question on the adequate presence of individual herbs in the finished product is the mandatory prerequisite for the therapeutic benefits. Whether this fundamental tenet of drug making is followed in traditional systems of medicine is unclear. The use of various herbs and by regressively following the traditional process may not need to a drug with a proven efficacy. To annul the above disconnect and to establish the credence between the product and its promise, we have evaluated the HPTLC chromatogram of Anti dandruff oil a proprietary Siddha drug. Findings of the study have clearly shown that Anti dandruff oil is effective purely because it has followed all the essentials of drug development. The herbs used in the formulation are equally effective at laboratory test as well as in the product because the presence and concentration of each herb could be established in the finished product. The presence of unique fingerprint of each herb in anti dandruff oil clearly suggests the least interaction between different herbs in Dano resulting in either their modification or degradation. This proves that each herb individually and collectively exhibit their best therapeutic effect thus making Dano the most effective anti- dandruff oil from siddha system of medicine. Further the above science makes dano an unparalleled drug even amongst many allopathic drugs. The broad spectrum anti-fungal activity, cell proliferation inhibition and hair growth promotion are some of the distinct benefits of different herbs in Anti-Dandruff oil. The distinct characteristics of all the herbs appeared to be well preserved in Anti- Dandruff oil, thus resulting in its superior therapeutics. Nevertheless the findings of the present study highlights the integration of an advanced science and scientific techniques by Dr. JRK's Siddha Research and Pharmaceuticals in developing Siddha drugs and revitalizing the Siddha system of medicine.

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