



HPTLC AND TLC ANALYSIS OF SIDDHA FORMULATION DEVA CHOORANAM

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Article Received on 22/03/2018

Article Revised on 15/04/2018

Article Accepted on 06/05/2018

ABSTRACT

HIV a well known virus since 1980s, and the main etiological agent of acquired immune deficiency syndrome (AIDS) is considered to be the most important diseases among the sexually transmitted infection in the recent decade, and is believed to be evolved in 1980s. Globally 40 million people are living with the AIDS and pandemic in nearly 150 countries. The traditional form of medicine is widely accepted in the community especially in South India. This study was aimed to find the chemical composition of the herbal formulation of the siddha drug *Deva Chooranam* that has been reported to have the combinations of herbs *Cedrus deodara*, *Alpinia galangal*, *Cinnamomum tamala* which have significant immunomodulatory property. The TLC and HPTLC were performed for the *Deva Chooranam*. The HPTLC fingerprint revealed the presence of seven prominent peaks showing the presence of versatile components. The peak four occupied the major area of 29.43% with an R_f value of 0.60 and it denoted the abundant existence of this compound followed by peak 3 and peak 5 occupying the area of 19.32 and 15.33%. This study had provided a detailed analysis of the phytochemicals present in the *Deva Chooranam* with a fingerprint analysis of HPTLC depicting respective peaks.

KEYWORDS: HIV, AIDS, *Deva Chooranam*, HPTLC analysis.

INTRODUCTION

Siddha is the traditional system of medicine originated and followed in Tamil Nadu and it believes that any imbalance in the human body lead to illness. Traditional Indian Medicine has a long history and is well established in the India. The Mineral or metallic component in combination with drug is administered in very small quantities, along with adjuvants (such as honey, ghee, milk, betel leaf juice and hot water). It is believed that it would modify the potency, toxicity and efficacy of the drugs.^[1] The current estimate suggest that over 2.4 million people in India live with HIV/AIDS (Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome) and the retroviral therapy had resulted in the significant increase in the morbidity, mortality, prolonged life and quality of life of AIDS patients.^[2-6] HIV is considered to be the major contributor the global burden of disease. HIV is considered to be the leading cause of disability adjusted life years worldwide for people aged 30–44 years during 2010, and the fifth leading cause of death in all ages. AIDS related deaths peaked at 2.3 million in 2005, and the same has found decreased to 1.6 million by 2012 globally.^[7,8]

The data suggest that nearly 70 to 80% of the patient uses non allopathic mode of treatment like Indian System of Medicine which includes Ayurveda, Siddha, Unani, naturopathy, homeopathy and yoga.^[9] There are many studies around the globe on HIV and few medicinal plants is believed to be effective and followed in the treatment regimen of siddha namely (*Rasaghandam Mezhu*, *Amukkara Chooranam* and *Nellikai Lehyam*).^[10] Similarly a Siddha drug *Deva Chooranam* with combination of three herbs and is mentioned in Agathiyar gunavagadam of classical *Siddha* literature stating the effectiveness against Chronic fever, diarrhea, dysentery, oral ulcers, respiratory ailments, skin diseases and tumours which are the associated symptoms in HIV infected individuals.^[11]

The life expectancy of HIV infected population has improved significantly in the last two decade due to effective retroviral therapy. In HIV infected individual the immunity get depleted and would result in cardiovascular diseases, chronic obstructive pulmonary disease, cancers, arthritis, osteoporosis, and liver disease.^[12,13] Reports suggest that if the infected patients engage themselves in regular physical activity would

help them to be with physical fitness and mental health.^[14,15]

In our previous study we have highlighted the importance of *Deva Chooranam* and showing the importance of three major plants and its various properties^[16] and in continuation to this in this study an attempt has been made to study the major compounds present in *Deva Chooranam* using TLC and HPTLC assay.

1. MATERIALS AND METHODS

1.1. Preparation of trial drug *Deva Chooranam*

The herbal drugs namely *Cedrus deodara* (Devadaru), *Alpinia galanga* (Arathai), *Cinnamomum tamala* (Lavanga pathiri) were shade dried grind into fine powder and all the three herbal plants were weighed in equal quantity and filtered using a mesh cloth and the content were stored in a sterile container.

1.2. Preliminary phytochemical analysis of *Deva Chooranam*: Preliminary phyto-chemical analysis of prepared *Deva Chooranam* was performed employing standard protocols to determine the different phytochemical constituent in it.

1.3. TLC Analysis

The powdered test sample were subjected to thin layer chromatography (TLC) using conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck). The readymade gel was cut with scissor and used for separation. Plates were marked with a soft pencil and used a fine Micro pipette to spot the sample on the TLC plate. The 10-micro liter of the sample was pipette and placed on the TLC plate at a distance of 1 cm. In the twin trough chamber different solvent system Toulene: Ethyl Acetate: Acetic Acid (1.5:1:0.5) were added and the spotted TLC plate was placed in the chamber. After the run plates were dried and were observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm.

1.4. High Performance Thin Layer Chromatography Analysis: HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. In addition it is a reliable method for the quantification at nanograms level of samples hence it is conveniently adopted for routine quality control analysis providing a chromatographic fingerprint to identify the purity of the phytocompounds in the raw materials.

1.5. Chromatogram Development: It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the

component to be analysed. After elution, plates were taken out of the chamber and dried.

1.6. Scanning: Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each extract and Rf values were tabulated.

2. RESULTS AND DISCUSSION

The HPTLC study on the small sample of one of the drug formulation widely used in siddha the *Deva Chooranam* showed detailed finger printing analysis of drug with the different component or the phytochemical constituents present in it. It is a known fact that this drug formulation contains the medicinal herbs *Cedrus deodara* (Devadaru), *Alpinia galanga* (Arathai), *Cinnamomum tamala* (Lavanga pathiri) but the phytocompounds present is unknown.

The preliminary phytochemical analysis has revealed the presence of various components the details are given in (Table 1).

Table. 1. Phytochemical composition of *Deva Chooranam*.

S. No.	Components	<i>Deva Chooranam</i>
1	Phenolic compound	++
2	Alcohol	+++
3	Aldehydes	++
4	Esters	+++
5	Saponins	+
6	Flavonoids	++
8	Tannin	+++
9	Fixed oil and fats	++++
10	Alkaloids	++
11	Terpinoids	+

++++ = appreciable amount, ++ = average amount, + = trace amount

Later the HPTLC analysis was performed and this study we have shed a light in the active constituents present in the *Deva Chooranam* which is depicted by the presence of 7 prominent peaks and in which each peak corresponds to seven versatile components present within it. The HPTLC results is interpreted based on the area coverage of the peak, height of the peak, number of peak and the Rf value of the peaks. It was noted that the 7 components occupied the Rf value ranging from 0.11 to 0.82. Further the peak 4 occupies the major percentage of area of 29.43 % which denotes the abundant existence of this compound followed by this peak 3 and 5 occupies the percentage area of 19.32 and 15.33 %. The graphical and the tabulated output is given in the (Table 2 & Figure 1).

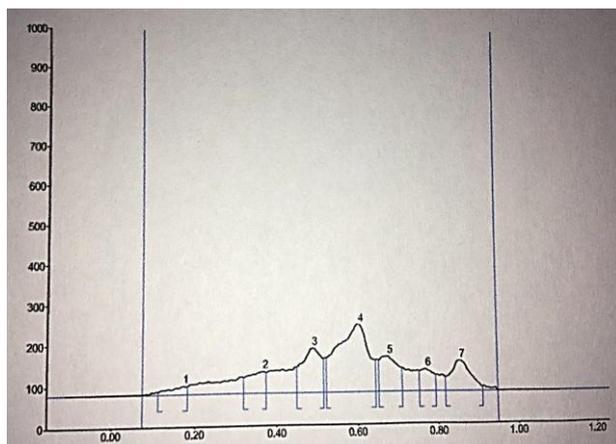


Figure. 1: Graphical output of HPTLC of *Deva Chooraman*.

Table. 2: HPLC fingerprint of *Deva Chooraman*.

Peak	Start Rf	End Rf	Area %
1	0.11	0.18	2.79
2	0.32	0.37	7.52
3	0.45	0.52	16.82
4	0.52	0.64	42.11
5	0.65	0.71	12.80
6	0.71	0.80	5.90
7	0.80	0.91	12.06

CONCLUSION

Through this study, the preliminary phytoconstituents of the drug *Deva Chooranam* has been analysed using TLC and HPTLC assay. Further the phytoconstituents have to be analysed quantitatively and its preclinical and clinical studies have to be performed for its therapeutic potential against HIV.

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