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PHARMACEUTICO-ANALYTICAL COMPARISON OF KIRATATIKTHADHI YOGA, TRADITIONAL VERSES SOXHLET EXTRACTION METHOD

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ABSTRACT

The current work focuses on the development of Hydro-alcoholic and Aqueous extracts of *Kiratatikthadhi yoga*, and to compare it with *decoction* of the same using HPTLC finger printing. *Kiratatikthadhi yoga* is a traditional Ayurvedic formulation consisting of five herbal ingredients: *Kiratatiktha, Katurohini, Musta, Parpataka,* and *Amrutha* which is mentioned in the *Charaka Samhita Chikitsa Stana* as a remedy for *Punaravarthaka Jwara,* a specific type of fever. In the light of recent amendments to the Drugs and Cosmetics Rule 158 B clause IV and the guidelines of the Ayurvedic Pharmacopoeia of India, the study explores newly introduced *Kalpana* of Hydro-alcoholic and Aqueous extracts of *Kiratatikthadhi yoga*. To achieve this, a Soxhlet apparatus was used to prepare the extracts and followed contemporary method of *kwatha nirmana* to prepare the traditional *decoction*. The resulting products were converted into a powdered form using a tray drying method after incorporating an appropriate amount of fine powder from the same formulation. The quantity of fine powder required for this drying process was standardized beforehand. Subsequently, a comparative analysis was conducted between the extracts and the *decoction* using High-Performance Thin-Layer Chromatography (HPTLC) fingerprinting. The final results demonstrated that the powdered extracts exhibited higher content of actives than the powdered *kasaya,* as evidenced by a greater number of peaks observed in the HPTLC analysis. Furthermore, the physicochemical analysis of both the extracts and the *decoction* showed similar ranges of values.

Keywords: Hydro-alcoholic extract, Aqueous extract, *kiratatikthadhi yoga*, Soxhlet apparatus, tray drying, High-Performance Thin-Layer Chromatography.

INTRODUCTION

For centuries, the use of Decoction preparations has been integral to Ayurvedic medicine, so much so that Ayurveda is often synonymous with these formulations. Within Ayurveda, the branch known as *Bhaishajya Kalpana* specializes in the art of formulating and preparing the *Pancha Vidha Kasaya Kalpanas,* namely *Swarasa, Kalka, Sritham, Seeta* and *Phantam*. These traditional medicines, with their roots extending back into time immemorial, are valued for their numerous benefits, particularly their lack of adverse side effects. It is noteworthy that while the efficacy of these preparations may require a longer duration for the complete eradication of diseases, they ensure thorough removal from the very root. The distinctive attribute of these remedies lies in their holistic approach, guaranteeing the comprehensive elimination of ailments without leaving lingering traces.

Traditionally, a generic way of *Kashaya* preparation method is strictly adopted by following the classical textbooks. Usually, this method of preparation requires a considerable amount of time and man-effort; And moreover, the shelf life of the decoction prepared via this method is less than 24 hours. An alternative approach to address the Shelf-life issue was introduction of *Pravahi kwatha* – wherein preservatives were added to increase the shelf-life of the decoction. But this adoption of *Pravahi Kwatha* comes at the cost of patient's health due to addition of preservatives. And so, many manufacturers have turned towards extracts of these formulations that has numerous advantages like ease of administration, highly effective, requires low dose usage, and increased Shelf-life etc.

The contemporary trend in Ayurvedic pharmaceuticals strives to optimize dosage forms for enhanced potency, administration convenience, and palatability. This movement is a product of advancing knowledge about the phytochemical composition of source plants and improved methods for evaluating their pharmacological and therapeutic attributes.[1]

Recognizing this trend, the introduction of plant extracts as more effective therapeutic agents gains prominence.[2] Extraction, as the term is used in Pharmacy, involves the separation of medicinally active portion from plant or animal tissues using selective solvents through standard extraction procedures.[3] Extracts are prepared by using an appropriate menstruum, with a view to extraction of active principles or at least

elimination of the inert bulk. Hence, present day ayurvedic drug industries are experimenting with various extraction techniques. By harnessing extraction techniques, the bulk of drug materials can be reduced, while enhancing potency and administration convenience.[4] Also, bulk being less, extracts can be formulated into dosage forms like tablets in appropriate dose, thereby increasing the patient compliance.[4]

As per the latest amendment in the Drugs and Cosmetics Rule 158 B clause IV, and the Ayurvedic Pharmacopoeia of India part 1 volume 8 and 9, Hydro-alcoholic and Aqueous extraction are accepted as newly introduced *kalpanas* of present era.[5] Moreover, further safety studies are not required for Aqueous and Hydro-alcoholic extracts when compared to other extracts.

The *Kiratatikthadhi yoga* selected for the current study is described in *Charaka Samhita chikitsa stana* as a treatment adopted in *Punaravarthaka jwara.* It consists of drugs namely *Kiratatiktha, katurohini, Musta, Parpataka* and *Guduchi*. The transformation of *decoction* formulations into extract form holds the potential for substantial benefits, as it facilitates broader usage, demands smaller quantities, and boasts enhanced efficacy within shorter time frames. This adaptation opens avenues for widespread application, making the medicinal properties more accessible and ensuring a more potent impact in a condensed period.

AIMS AND OBJECTIVES

1. To develop Hydro-alcoholic extract, Aqueous extract and decoction form of *Kiratatikthadhi yoga.*
2. To compare analytical parameters and HPTLC finger printing of both extracts and decoction of *Kiratatikthadhi yoga*.

MATERIALS AND METHODS

1. COLLECTION AND PREPARATION OF RAW DRUGS

Raw drugs like *Kiratatiktha, Katurohini, Musta, Parpataka* and *Guduchi* were collected and washed with water thoroughly to remove physical impurities. A part of this collected drug was separated for the macroscopic evaluation and microscopical evaluation.

Remaining part of the collected drug was dried well under shade.

2. EXPERIMENTAL STUDY

Hydro-alcoholic extract:

The ingredients of *Kiratatikthadhi yoga* were made into moderately coarse powder form (ie, all particles should pass through sieve no.22 and not more than 40% of particles pass through sieve no.60). 9.6 gm of each ingredient were weighed and taken, thus making a total of 48 gms. They were then packed in a timble made of filter paper. The timble was then transferred into the Soxhlet apparatus which was connected with a round bottom flask and a condenser.

Here the solvent used was 50% aqueous alcohol, prepared using 400 ml ethanol and 400 ml distilled water. The solvent was added into the Soxhlet apparatus and was heated under reflux at a temperature between 80-85^0 until it becomes colorless. This took around 5 hrs and the solvent passed through the siphon tube for 6 times. The obtained extract was collected and measured.

The extract obtained was then reduced to a concentrated form by heating over water bath. It was having a semi liquid consistency. Fine powder of *Kiratatikthadhi yoga* was added to the extract and was kept for drying under the shade. Thin flakes of Hydro-alcoholic extract of *kiratatikthadhi yoga* was obtained and was packed in an air tight container.

Figure No 1: Hydro-alcoholic extract preparation using Soxhlet apparatus

Figure No 2: various steps involved in drying of hydro-alcoholic extract

Hydro-alcoholic extract → Powdered ingredients → Tray drying

Aqueous extraction:

The ingredients of *Kiratatikthadhi yoga* were made into moderately coarse powder form.

9.6 gm of each ingredient were weighed and taken,thus making a total of 48 gms. They were then packed in a timble made of filter paper. The timble was then transferred into the Soxhlet apparatus which was connected with a round bottom flask and a condenser. Here the solvent used was distilled water. 800ml distilled water was added into the Soxhlet apparatus and was heated under reflux at a temperature between 80-85^0 until it becomes colorless. This took around 6 hrs and the solvent passed through the siphon tube for 6 times. The obtained extract was collected and measured.

The extract obtained was then reduced to a concentrated form by heating over water bath. It was having a semi liquid consistency. Fine powder of *Kiratatikthadhi yoga* was added to the extract and was kept for drying under the shade. Thin flakes of Aqueous extract of *kiratatikthadhi yoga* was obtained and was packed in an air tight container.

Figure No 3: Aqueous extract preparation using Soxhlet apparatus

Figure No 4: various steps involved in drying of Aqueous extract

Aqueous extract	Tray drying	Powdered ingredients

Preparation of *Kiratatikthadhi Decoction* as per Classical Method:

9.6 gm each of *Kiratatiktha, Katurohini, Musta, Parpataka* and *Amrutha* were taken, cleaned well and crushed into small pieces. It was transferred to a stainless-steel vessel into which 96ml of water was added and the level was marked. Later, the remaining 672ml of water was added to it and boiled under mild fire until it gets reduced to 96ml. The mixture was filtered and stored.

Conversion of *decoction* to powder form:

20ml of this *decoction* was taken in a tray and added with 2gm of finely powdered ingredients of the formulation. It was mixed properly to obtain a homogeneous mixture. This was further kept under the shade for drying. 4gm of powder was obtained as the final product after drying. It was then packed in an air tight container.

The obtained extracts and *Decoction* were then subjected to various analytical tests and HPTLC fingerprinting.

Figure No.5 various steps involved in preparation of decoction of *Kiratatikthadhi yoga*

Preparation of kasaya | 96ml of Kasaya | Reduced to 20ml

OBSERVATIONS AND RESULTS

- **Soxhlet extraction:**

Soxhlet extraction was done to prepare Hydro-alcoholic and Aqueous extracts of *Kiratatikthadhi yoga*. 48gm of ingredients were taken and the extract was prepared using 800ml of solvent.

600ml of Hydro-alcoholic extract and 650ml of Aqueous extracts were obtained and it was then concentrated to 100ml by heating over water bath.

Table No 1: Soxhlet extraction of Hydro-alcoholic (HA) and Aqueous(A) extracts

	Quantity of ingredients	Amount of solvent	Amount of extract obtained	Concentrated to
HA Extract	48gm	400ml ethanol and 400ml distilled water	600ml	100ml
A extract	48gm	800ml distilled water	650ml	100ml

- **Convertion of extracts into powdered form:**

1gm of powdered ingredients of the formulation was added to 10 ml of this extract and

was kept for drying under the shade. 1.08 gm of powdered Hydro-alcoholic extract and 1.4 gm of powdered Aqueous extracts were obtained after the process of drying. They were then packed in air tight containers.

- **Preparation of *Kiratatikthadhi Decoction:***

96 ml of *Kiratatikthadhi decoction* was obtained from 48gm of coarsely powdered ingredients. It was then further reduced to 20ml by heating over water bath. This was then poured over a plastic sheet placed over a tray and was kept for drying under the shade after adding 2 gm powdered ingredients of *Kiratatikthadhi yoga.* 4gm of dry extract was obtained which was ground into fine powder and stored in an air tight container.

- **Organoleptic characters of Hydro-alcoholic, Aqueous extracts and *Kiratatikthadhi decoction***

Table No 2: Organoleptic of Hydro-alcoholic, Aqueous extracts and *decoction* of *Kiratatikthadi yoga.*

Parameters	Hydro-alcoholic extract	Aqueous extract	*Decoction*
Colour	Blackish	Brownish black	Brownish black
Odour	Characteristic	Characteristic	Characteristic
State	Solid powdery	Solid powdery	Powdery
Taste	Bitter	Bitter	Bitter

- **Physico-chemical characters of Hydro-alcoholic, Aqueous extracts and *Decoction* of *Kiratatikthadhi yoga***

Table No 3: Physico-chemical characters of Hydro-alcoholic, Aqueous extracts and *decoction* of *Kiratatikthadhi yoga.*

Parameters	Hydro-alcoholic extract	Aqueous extract	*Decoction*
P^H (5% solution)	4.3	5.3	4.9
Loss on drying at 105^0	0%	0%	1%
Total ash	9%w/w	9.4%w/w	10%
Acid insoluble ash	0.02%w/w	1.1%w/w	1%

- **HPTLC finger printing**:

HPTLC finger printing was done for Hydro-alcoholic extract, Aqueous extract and

decoction of *Kiratatikthadhi yoga.* It was done at three different wavelengths, 254nm, 366nm and 550nm.

1. HPTLC finger printing profile of Hydro-alcoholic, Aqueous extracts and *decoction* of *Kiratatikthadhi yoga* at 254nm

At 254nm, the Aqueous extract have shown 10 peak, Hydro-alcoholic extract have shown 14 peaks and there were 4 peaks for the *kiratatikthadhi decoction*. Rf Maximum of 0.29 was obtained for HA extract and Aqueous extract and of 0.96 for *Decoction* of *Kiratatikthadi yoga* at 254nm.

Figure No 6: graph of HPTLC result of Hydro-alcoholic extract at 254nm

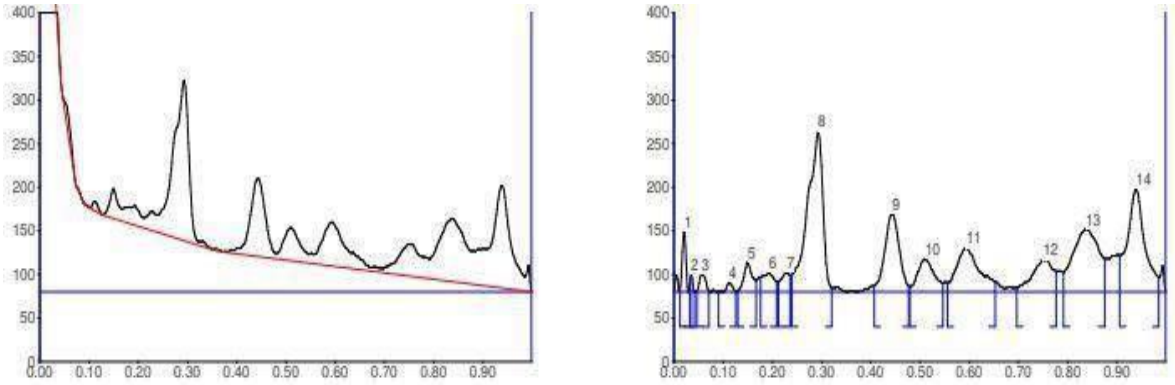


Figure No 7:graph of HPTLC result of Aqueous extract at 254nm

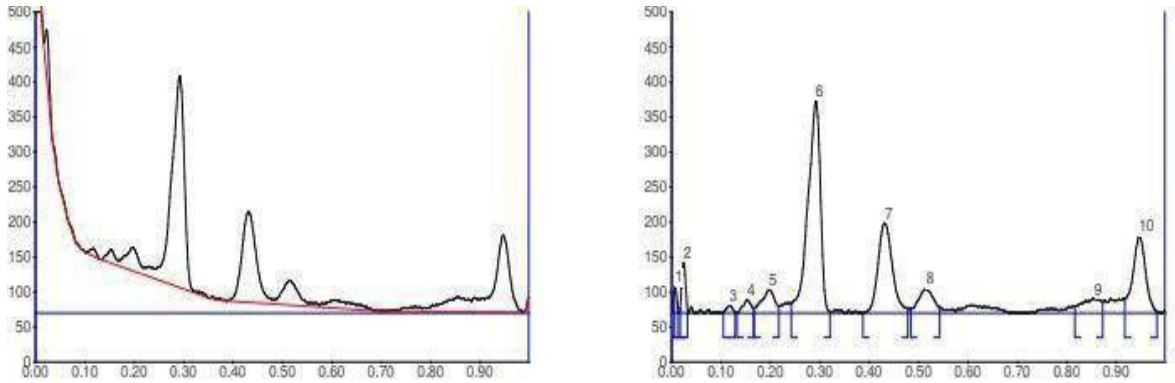


Figure No 8: graph of HPTLC result of *Kiratatikthadhi decoction* at 254nm

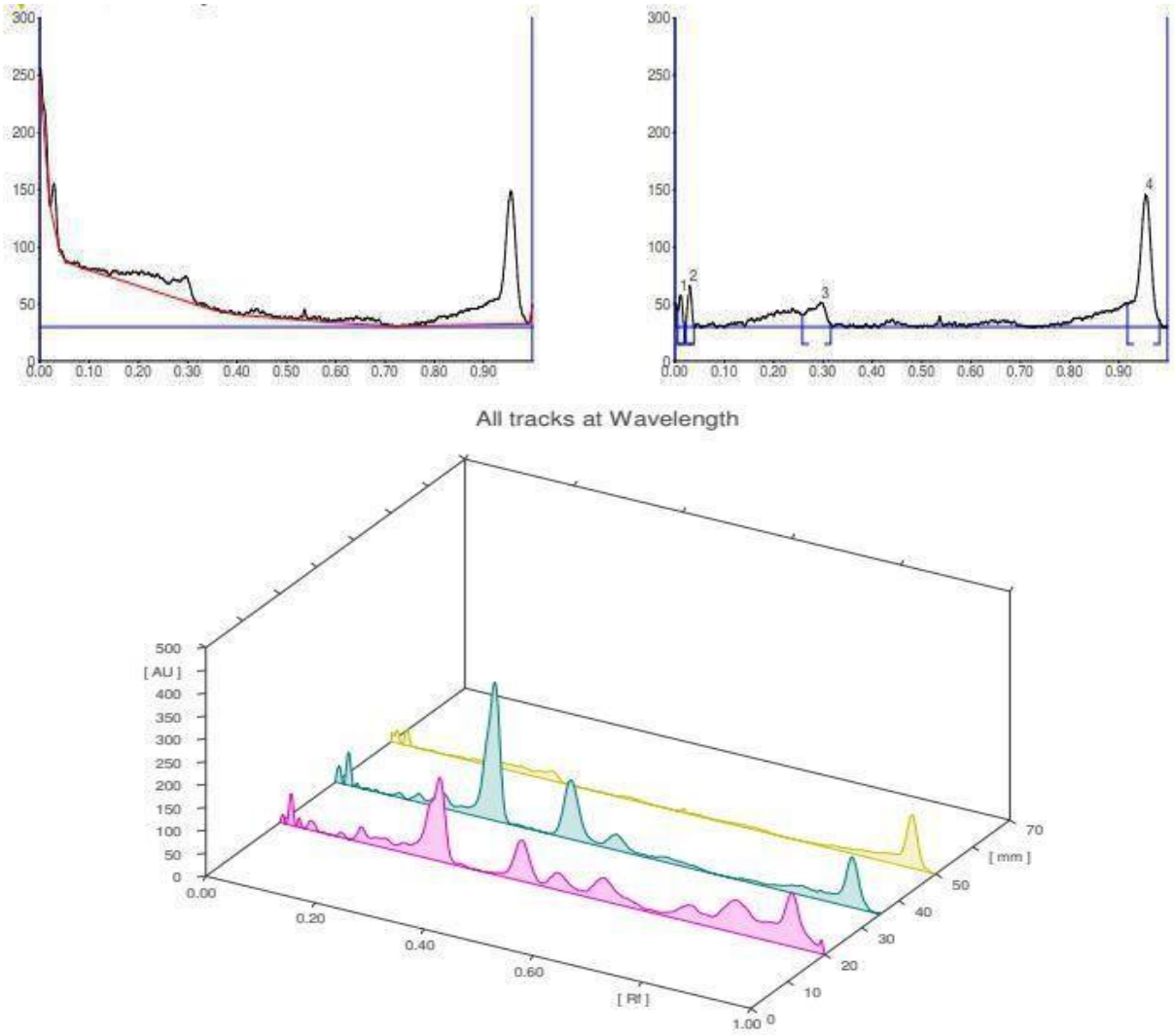


Figure No 9: HPTLC analysis of Hydro-alcoholic extract, Aqueous extract and *Decoction* form of *Kiratatikthadhi yoga* at 254nm.

2. HPTLC finger printing profile of Hydro-alcoholic, Aqueous extracts and decoction of Kiratatikthadhi yoga at 366 nm

At 366 nm, the Aqueous extract have shown 5 peak, Hydro-alcoholic extract have shown 6 peaks and there only 1 peak for the *kiratatikthadhi decoction*. Rf maximum of 0.26 for HA extract, 0.20 for A extract and 0.15 for *Kiratatikthadhi decoction* was obtained.

Figure No 10: Graph of HPTLC result of Hydro-alcoholic extract at 366nm

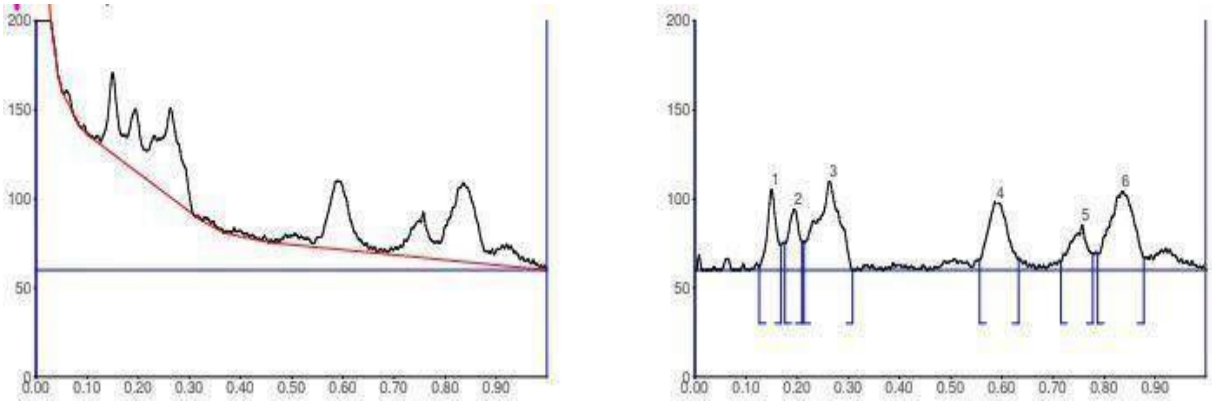


Figure No.11 :graph of HPTLC result of Aqueous extract at 366nm

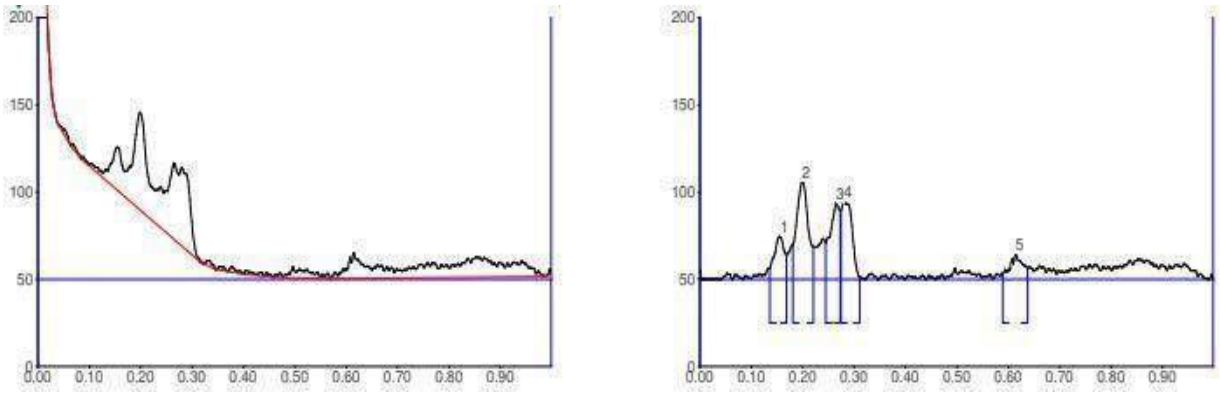
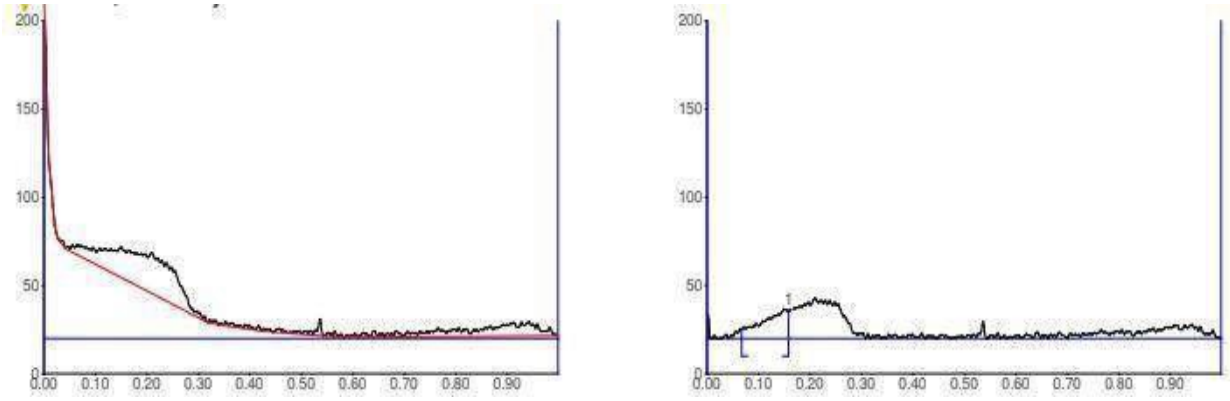


Figure No.12:graph of HPTLC result of *Kiratatikthadhi decoction* at 366nm



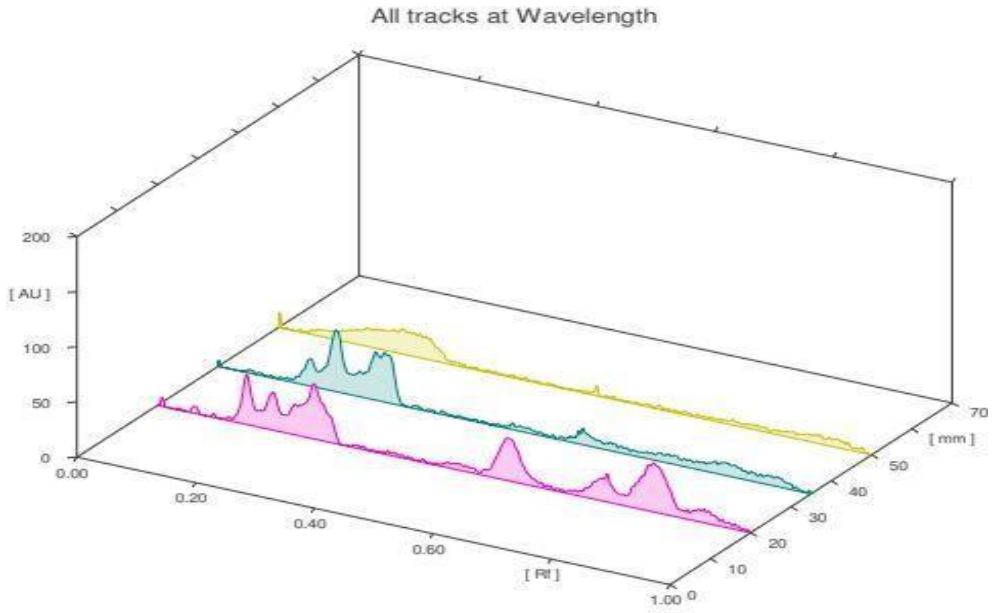


Figure No.13 : HPTLC analysis of Hydro-alcoholic extract, Aqueous extract and *Decoction* form of *Kiratatikthadhi yoga* at 366nm

3. HPTLC finger printing profile of Hydro-alcoholic, Aqueous extracts and decoction of Kiratatikthadhi yoga at 550nm

At 550nm, the Aqueous extract have shown 9 peaks, Hydro-alcoholic extract have shown 12 peaks and for *Kiratatikthadhi decoction* there were 6 peaks

The Rf Maximum values obtained were 0.06, 0.07 and 0.26 for HA extract, A extract and *Decoction* of *Kiratatikthadhi yoga*.

Figure No.14 : graph of HPTLC result of Hydro-alcoholic extract at 550nm

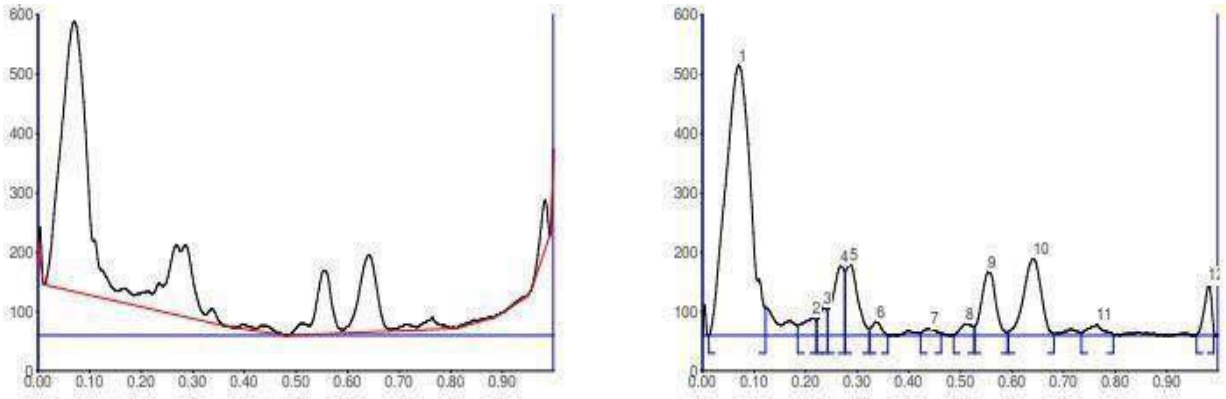


Figure No.15: graph of HPTLC result of Aqueous extract at 550nm

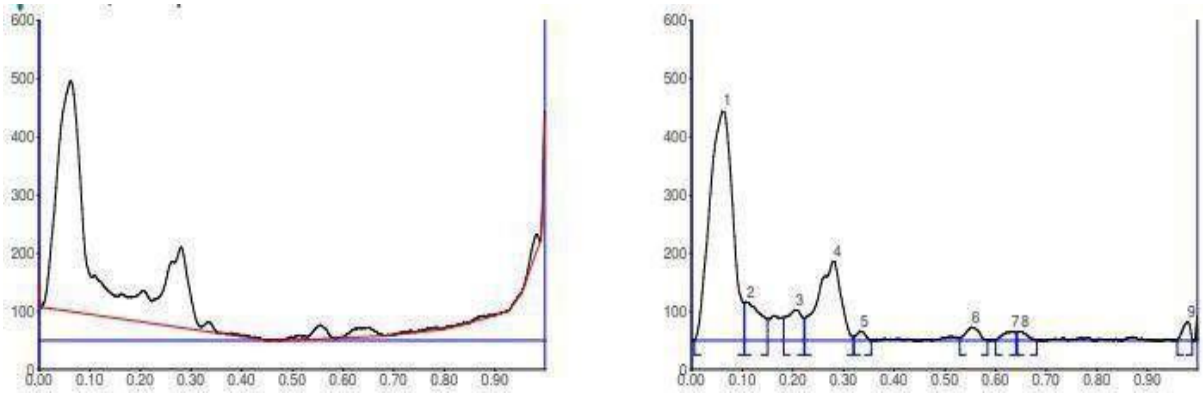


Figure No.16: graph of HPTLC result of *Decoction* at 550nm

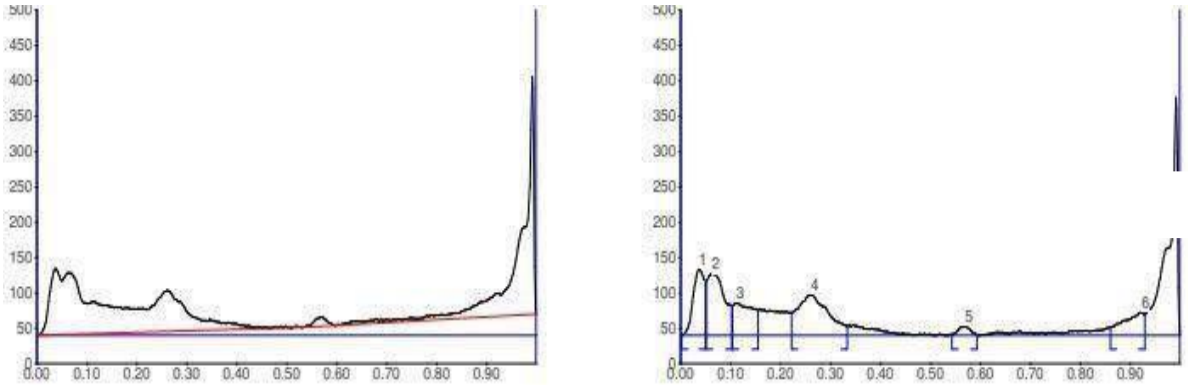
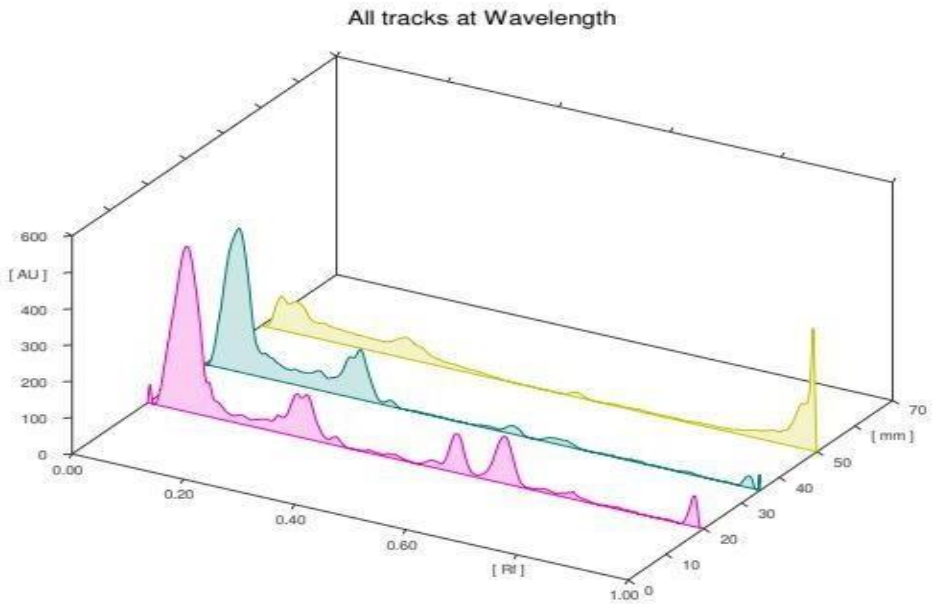


Figure No.17: HPTLC analysis of Hydro-alcoholic extract, Aqueous extract and *Decoction* form of *Kiratatikthadhi yoga* at 550nm.



From HPTLC results it was observed that, both Hydro-alcoholic and Aqueous extracts of *Kiratatikthadhi yoga* showed almost the same peaks. But the *decoction* prepared out of

Kiratatikthadhi yoga showed only very weak peaks. This may be due to the loss of volatile oil and heat liable constituents from *decoction* at the time of preparation.

DISCUSSION

Herbal drugs, such as those in *kiratatikthadhi yoga* mentioned in *charaka Samhita chikitsa stana*, offer a promising avenue for fever management with a safer profile. And, the incorporation of Hydro-alcoholic and Aqueous extracts into medical practice presents a remarkable advantage. Utilizing extracts necessitates only minute quantities while preserving the potency of the treatment. Notably, research studies have explored the antipyretic potential of individual herbal components and their extracts. However, the spotlight on formulations containing extracts remains relatively limited.

Discussion on Extraction procedure

Hydro-alcoholic and Aqueous extracts of *Kiratatikthadhi yoga* were prepared using a 400 ml Borosilicate glass Soxhlet apparatus and the preparations were done at analytical lab, Department of *Rasasastra and Bhaishajya Kalpana, Government Ayurveda college, Tripunithura.* The detailed procedure for Soxhlet extraction of both hydro-alcoholic and aqueous extracts is outlined in API Part 1 Volume 8 and 9. As per the API guidelines, the prescribed solvent amount for Soxhlet extraction should be three times the quantity of the raw drug placed in the thimble. However, this quantity is exceptionally small and insufficient even for dampening the raw drugs. After multiple trial runs, it was determined that the amount of solvent to be used should match the quantity needed to pass through the siphoning tube three times.

Subsequent extraction steps were carried out using this measurement, continuing until the entire solution lost its color. The ultimate result yielded an extract with a semi-liquid consistency. To transform this extract into a fine powder, a tray drying procedure was applied. Through multiple experiments, it was deduced that optimal dry flakes of the desired extract could be achieved by subjecting the semi-liquid extract to shade drying, augmented by the addition of fine powder composed of the same ingredients as those found in the formulation.

The standardized ratio of fine powder added was determined to be 1gm per 10ml of extract. This choice was informed by the fact that a dry powdered extract could be

obtained in a comparatively shorter duration when compared to the ratio of 500mg per10ml.

Identification using HPTLC

Moreover, to determine the probable number of compounds present, HPTLC finger printing was done at *Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal*.

The number of peaks obtained with *decoction* form of *Kiratatikthadhi yoga* was comparatively less when compared to that obtained using both extracts. The maximum Rf value obtained was

0.29 with both the extracts at 254nm. Whereas for *Decoction* it was 0.96.

Under 336nm, Hydro-alcoholic extract had 6 peaks, Aqueous extract had 5 peaks and only 1 peak was obtained for *Kiratatikthadhi decoction*. Maximum area was covered by the peak with Rf value 0.15 for the *decoction*.

Under 550nm also *Kiratatikthadhi decoction* had a smaller number of peaks when compared to the extracts.

Many of the heat liable constituents and volatile oils may be lost at the time of *decoction* preparation. whereas in case of Soxhlet extraction, a closed and controlled environment prevents the exit of such chemical constituents.

CONCLUSION

- Extracts are regarded as an advantageous form of medication due to their small dosage, rapid and effective action, and ease of transportability.
- When compared to traditional *decoction* preparation, extracts require a considerably smaller quantity of raw herbal materials.
- The end product of extraction of *Kiratatikthadhi yoga* obtained is in a semi-liquid form, which can be transformed into a powder by tray drying in the shade after incorporating s fine powder of the same ingredients in a ratio of 1gm:10ml.
- Powdered extracts exhibited higher content of actives than the powdered *kasaya,* as evidenced by a greater number of peaks observed in the HPTLC analysis.

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