WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 15, 1525-1539. Research Article ISSN 2277–7105

STANDARDIZATION AND ANALYTICAL ASSESSMENT OF *SIDDHARTHAKADI AGAD VATI*

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Article Received on
18 June 2025,
Revised on 08 July 2025,
Accepted on 29 July 2025
DOI: 10.20959/wjpr202515-37822

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ABSTRACT

Ayurveda, the ancient system of medicine originating in India over 5,000 years ago, is a holistic science that emphasizes the balance of body, mind, and spirit for maintaining health and treating disease. "*Ayurveda*, officially recognized by WHO as a traditional health system, offers an integrative approach that complements modern medicine while respecting local traditions and biodiversity." By harnessing the power of nature, *Ayurvedic* remedies aim to address the root causes of ailments rather than just alleviating symptoms. Ayurveda formulation is a combination of powerful compounds that work synergistically to detoxify, rejuvenate, and prevent disease, with an emphasis on maintaining long-term health rather than just symptomatic relief.[1] By considering individual constitution (*Prakriti*) and current imbalances (*Vikriti*), *Ayurvedic* formulations offer personalized solutions that not only treat physical ailments but also nurture mental and emotional health. While *Ayurvedic* formulations are highly effective in promoting health and well-being, the standardization of their mechanisms of action, pharmacology, pharmacokinetics, and pharmacovigilance remains largely unexplored. Despite their long-standing use in traditional medicine, scientific validation and comprehensive research on these aspects are still in the early stages, which limits their integration into mainstream healthcare practices.[2] *Siddharthakadi Agad Vati* is one such potent *Ayurvedic* formulation traditionally prescribed for a variety of disorders. However, there is a lack of documented studies on its physicochemical properties and organoleptic characteristics. Therefore, the

present study aims to evaluate the organoleptic features, physicochemical parameters, pesticide residue, heavy metal test and HPTLC profile of *Siddharthakadi Agad Vati.*

KEYWORDS: *Ayurveda, Siddharthakadi Agad Vati*, organoleptic, physicochemical parameters, Pesticide residue, Heavy metal test.

INTRODUCTION

Understanding the physicochemical characteristics of drug substances is crucial in the development of pharmaceutical formulations. It is necessary to evaluate the compatibility between active ingredients, excipients, and finished dosage forms in accordance with established pharmaceutical guidelines. Proper characterization of active pharmaceutical ingredients (APIs) not only ensures the quality of raw materials used in production but also contributes to the effectiveness and safety of the final pharmaceutical products. With the growing demand for traditional and indigenous medicines, ensuring consistent quality standards has become increasingly important. Therefore, the development and implementation of robust standardization protocols are essential. However, the absence of well-defined reference standards has posed significant challenges in the quality assessment of many complex formulations. Classical *Ayurvedic* texts describe numerous *Agad* formulations, which are prescribed for both acute and chronic conditions, including those caused by toxic and non-toxic agents.

***Siddharthakadi Agad*[3]:** The formulation *Siddharthakadi Agad* is a blend of eighteen herbal remedies with *bast mutra*. *Siddharthakadi Agad Vati* is available in *Charak* Chapter 9, *"Unmad Adhyay,*" in *Samhita Chikitsa Sthana*. In chapter 5 of the *Uttar Tantra, Bhootpratished Adhaya*[4], *Acharya Vagbhatta* explains *Siddharthakadi Agad* In *Unmaad Chikitsa, Acharya Yog Ratnakar*[5] explains *Siddharthakadi agad. Siddharthakadi Agad Vati* is the preferred medication for conditions like *Manas Roga, Visham Jwar*, and various skin ailments.[6] The majority of the components in *Siddharthakadi Agad* possess properties that combat skin diseases, alleviate itching, eliminate parasites, reduce inflammation, fight microbes, prevent allergic reactions, relieve itching, and modulate the immune system, thereby helping to reduce inflammation, ease itching, and boost immunity.

Collection of Drugs

Haridra- Rhizome of *Haridra* was collected from Uttarkashi. It was carefully cleaned under running water and allowed to dry in the shade.

Daruharidra- Root of *Daruharidra* was collected from Uttarkashi. It was carefully cleaned under running water and allowed to dry in the shade.

Devdaru- stemwood was collected from Uttarkashi.

Shirish- Shirish bark was collected from the haridwar campus of Rishikul. After giving it a thorough cleaning under running water, it was left to dry in the shade.

Katabhi (shweta Shirish)- Bark of *shweta Shirish* was collected from haridwar. It was carefully cleaned under running water and allowed to dry in the shade.

Shunthi, Pippali, Marich, Aamlaki, Haritaki, Bibhitaki, Priyangu, Vacha, Hingu, Sarsapa, Karanj, Sweat Aprajita, Manjistha all dried drugs were collected from Herbal automation, Haridwar.

Authentication of The Collected Sample

All samples of the dry herb ingredients were recognized and verified by the distinguished specialists from the *Dravyaguna* Department at Rishikul Campus in Haridwar (UAU).

Reference No. Dg/RC/UAU.- 235 Date...04/10/2024

DRUGS IDENTIFICATION & VERIFICATION CERTIFICATE

Certified that dissertation entitled "Clinical Evaluation of the *Siddharthakadi Agad* in *Dadru*" is the work of Dr. Muskan Rangar who will carry out the research under the supervision of Dr. Ramesh Chandra Tiwari, HOD of P.G. Department of *Agad Tantra Evum Vidhi Vaidyaka*, Rishikul Campus, UAU, Haridwar. The drugs to be used in the thesis are the following:

S.No.	Name of the drug	Latin name	Part used
1.	*Sarshapa*	*Brassica juncea* (Linn.)	Seed
2.	*Karanja*	*Pongamia pinnata* (Pierre.)	Seed
3.	*Manjistha*	*Rubia cordifolia* (Linn.)	Stem pieces
4.	*Shirisha*	*Albizia lebbeck* (Benth.)	Stem bark
5.	*Katabhi twaka (shweta shirisha)*	*Albizia procera* (Benth.)	Stem bark
6.	*Haridra*	*Curcuma longa* (Linn.)	Rhizome
7.	*Daru haridra*	*Berberis aristata* (DC.)	Stem/ root wood
8.	*Sunthi*	*Zingiber officinale* (Rose.)	Rhizome
9.	*Pippali*	*Piper longum* (Linn.)	Fruit
10.	*Maricha*	*Piper nigrum* (Linn.)	Fruit
11.	*Priyangu*	*Callicarpa macrophylla* (Vahl.)	Fruit
12.	*Devdaru*	*Cedrus deodara* (Roxb. Loud)	Stem wood
13.	*Vacha*	*Acorus calamus* (Linn.)	Rhizome
14.	*Aparajita*	*Clitorea tenatea* (Linn.)	Seed
15.	*Hingu*	*Ferula narthex* (Boiss.)	Resin
16.	*Haritaki*	*Terminalia chebula* (Retz.)	Fruit
17.	*Amalaki*	*Emblica officinalis* (Gaertn.)	Fruit
18	*Bibhitaki*	*Terminalia bellerica* (Gaertn.)	Fruit

The drugs are identified and verified by the Department of *Dravyaguna* before the start of the thesis work.

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Haridwar.

Preparation of *Siddharthakadi Agad*

After thorough cleaning, each type of raw herbal drug was weighed at 1kg and subsequently placed in the grinding machine.

In order to purify Hingu, cow's ghee is used for Bharjanan.

The grinding process was repeated 3-4 times, and the raw materials were sifted through three different sieves on three occasions.

The fine powder was finally filtered using a sieve with a mesh size of 80.

At the end of this process, 16 kg of powder was collected after a loss of 2 kg. The collected goat urine was purified by distillation method.

Following that, the infused goat urine was incorporated into the total amount of powder.

Next, a starch binder solution was mixed into the herbal drug powder, then blended thoroughly in a binding machine to create a paste.

A stainless-steel pan was then used to spread out the resultant paste. This pan was then placed in a hot air dryer and left for approximately 3-4 hours. After drying, the paste material was powdered once more and sifted through sieves.

Following that, the material was moved to the tablet compression machine to form tablets.

At the end of the procedure, tablets were coated. To shield the tablets from external moisture and humidity, coating materials were applied.

Following the normal procedures of the Ayurvedic Pharmacopoeia of India (API) 16-21 guidelines, the *Vati's* chemical analysis, physicochemical analysis, and tablet parameters—such as hardness, friability, average weight, and dissolution time—were carried out once it was prepared.

Analytical Study Results Of *Siddharthakadi Agad Vati*

The following tests and processes were conducted following the preparation of *Siddharthakadi Agad Vati* in order to further authenticate it through analytical studies.

ORGANOLEPTIC ANALYSIS

Using a pharmacognostical method, the manufactured medication was assessed organoleptically with the naked eye, noting its look, colour, Odor, taste, touch, and texture. The following are the findings of *Siddharthakadi Agad Vati's* organoleptic parameter.

Appearance - coated tablet of round shaped Smell - Pungent

Colour - brownish yellow coloured Touch - Hard and smooth texture

Taste - Bitter, Pungent, Astringent, (*Katu, Tikta, Kashaya rasa)*

PHYSICO-CHEMICAL ANALYSIS

Hardness

A tablet's breaking point, structural integrity, and how it changes "under conditions of storage, transportation, packaging, and handling before usage" are all determined by the pharmaceutical industry using a laboratory procedure called tablet hardness testing. The hardness of *Siddharthakadi Agad Vati* was determined to be 5.0 kg/cm^2 in this test.

Weight Uniformity

Tablet weight is most affected by factors such compression machine tooling, which is influenced by head pressure, machine speed, and powder flow characteristics. Weight variation when compressed is frequently caused by irregular particle size distribution and powder or granulate density. One in-process test metric used to ensure dosage unit consistency during compression is weight uniformity. The average diameter and width of the *Siddharthakadi Agad Vati* were 11.23 mm and 5.75 mm, respectively.

Disintegration Time

This test ascertains if, when submerged in a liquid medium under experimental conditions, dosage forms such as tablets, capsules, boluses, pessaries, and suppositories dissolve within a given amount of time (disintegration time). Disintegration occurs when the unit being tested either leaves no residue on the apparatus's screen or, if it does, it is composed of fragments of the tablet's broken component parts, like the insoluble tablet coating or capsule shells. According to this test, *Siddharthakadi Agad Vati's* disintegration time (in minutes) was between three and four minutes.

Ash Value

When assessing the quality and purity of crude pharmaceuticals, ash readings can be helpful. It is the most used method for detecting adulterated inorganic materials and is more important for quality control and standardization. Ash including carbonates, phosphates, salt, potassium,

calcium, and magnesium silicates is commonly produced when crude pharmaceuticals are burned. The amount of total ash shows how many minerals are naturally present in medicinal plants and how many foreign substances were added during handling or processing throughout manufacture. The more inorganic components there are, the higher the ash value. *Siddharthakadi Agad vati's* total ash value was 6.52% w/w.

Loss on Drying

The purpose of this test was to ascertain *Siddharthakadi Agad's* moisture content. The mass loss on drying is shown as w/w. The medicine will be better if the LOD is as low as possible. It was discovered that *Siddharthakadi Agad* has a LOD of 8.25%w/w. The medicine may deteriorate as a result of excessive dampness, which may encourage the growth of germs. When water and other volatile chemicals evaporate under specific conditions, the weight loss is expressed as a percentage w/w and is called "loss on drying."

Acid-insoluble ash

The presence of fine soil and sand particles is indicated by the concentration of acid-insoluble ash. In tests, acid-insoluble ash (AIA) is frequently employed as a digestibility metric. Three variations of the original gravimetric approach are commonly employed to ascertain AIA contents.

- burning the sample's organic detritus by ashing, then boiling it in hydrochloric acid and re-ashing.
- *Siddharthakadi Agad's* acid insoluble ash value was determined to be 0.92%.w/w.

Alcohol soluble extractive value

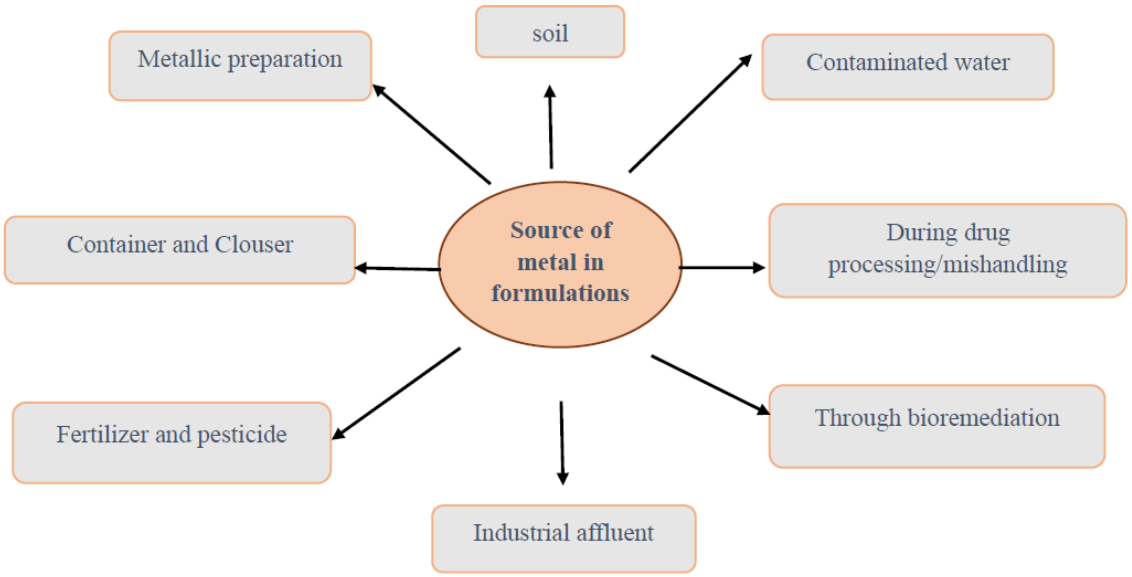
The water-soluble extractive value and the alcohol-soluble extractive value served the same function. A lower extractive value suggests inadequate processing during formulation, drying, or storage, adulteration, or the usage of exhausted material. *Siddharthakadi Agad's* alcohol-soluble extractive value was determined to be 16.58%w/w.

Water-soluble extractive value

Water-soluble extractive value is essential when evaluating crude medicines. A reduced extractive value suggests adulteration, the presence of contaminated material, or incorrect drying, storing, or formulation procedures. *Siddharthakadi Agad's* water-soluble ash value was determined to be 20.84%w/w.

Heavy metal detection

The earth's crust naturally contains heavy metals, but negligent human activity has had a substantial impact on their geochemical cycles and biochemical equilibrium. As a result, metals build up in plant parts and secondary metabolites, which causes a specific pharmacological activity and, when present in excess, a poisonous impact.



1. Sample Preparation

- Weigh 1–2 grams of powdered *Siddharthakadi Agad Vati*.
- Digest the sample using acid digestion (typically a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) or hydrofluoric acid (HF)).
- Heat until a clear solution is obtained.
- Cool and filter the solution.
- Make up the volume with deionized water to a known volume (usually 50 or 100 mL).

2. Instrumental Analysis

Use one of the following techniques

a. AAS (Atomic Absorption Spectroscopy)

- Suitable for detecting metals like lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd).
- Calibrate with standard metal solutions.
- Analyze digested sample for metal concentration.

b. ICP-OES / ICP-MS (Inductively Coupled Plasma)

- Better suited for multi-element detection and more sensitive.
- Follow manufacturer's protocol for calibration and sample introduction.
- Record concentrations of relevant heavy metals.

3. Interpretation

- Compare results with permissible limits set by AYUSH/WHO/USP standards.

Microbial contamination(pathogens)

The substances used in drug manufacturing and the local air conditions affect the microbial composition during contamination, which varies over time. During the current investigation, the microbiological load (quantification and identification of microorganisms) of the final *Siddharthakadi Agad's* Vati product was assessed. Tests were conducted for particular pathogens.

The following microbial strains.

Pathogens:-
Pseudomonas aeruginosa
Staphylococcus aureus
Escherichia coli
Salmonella species

Sample Preparation

- Crush the *Siddharthakadi Agad Vati* into a fine powder using a sterile mortar and pestle.
- Weigh a specific amount (usually 10 g) of the powdered sample.
- Suspend in 90 mL of sterile buffered saline or peptone water to make a 1:10 dilution.

Serial Dilution

- Perform serial dilutions (up to 10^{-6}) using sterile diluents to reduce microbial load for enumeration.

Plating and Incubation

- Total Aerobic Microbial Count (TAMC): Pour or spread plate method on Plate Count Agar; incubate at 30–35°C for 48–72 hours.
- Total Yeast and Mold Count (TYMC): Use Sabouraud Dextrose Agar; incubate at 20–25°C for 5–7 days.

Pathogen Detection (as per pharmacopeial guidelines)

- *E. coli*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*: Inoculate appropriate selective/enrichment media followed by confirmatory tests.

Result Interpretation

- Compare colony-forming units (CFU) with pharmacopeial limits (e.g., as per WHO or Ayurvedic Pharmacopoeia standards).
- Ensure absence of specified pathogens.

Documentation

- Record all observations, colony counts, and test results for regulatory compliance and product safety.

PHYSICO-CHEMICAL ANALYSIS

S.NO	TEST PARAMETERS	RESULTS	Permissible limits according to API[7]
01	Uniformity of weight	+3.4%, -2.1%	6.0%
02	Disintegration time	3 minutes	2.5 to 10 min
03	Hardness	5.0 kg/cm^2	4 to 10kg/cm^2
04	Loss on drying	8.25%w/w	<10%w/w
05	Total ash	6.52%w/w	<10%w/w
06	Acid insoluble ash	0.92%w/w	<2%w/w
07	Water soluble ash	1.86%w/w	Not less then 50% of total ash
08	Alcohol soluble extractive	16.58%w/w	Not<10%w/w
09	Water soluble extractive	20.84%w/w	Not<10%w/w
10	Heavy metals	-	
	Lead (Pd)	3.16ppm	10ppm
	Cadmium (Cd)	0.25ppm	0.3ppm
	Arsenic (As)	<0.50ppm	3ppm
	Mercury (Hg)	<0.13ppm	1ppm
11	Microbial contamination	-	-
	Pathogens:-	-	-
	Pseudomonas aeruginosa	Absent /g	-
	Staphylococcus aureus	Absent /g	-
	Escherichia coli	Absent /g	-
	Salmonella species	Absent/g	-

ANALYSIS BY HPTLC

An improved version of Thin Layer Chromatography (TLC) is called High Performance Thin Layer Chromatography (HPTLC). Using scanning and UV vision, the assay integrates the separation and measurement of the analyses on silica gel HPTLC plates. The alkaloid content of several drug components, *Siddharthakadi Agad vati*, has been ascertained using this

technique for the three studies conducted at various dates. Based on slight changes in Rf values, HPTLC of *Siddharthakadi Agad Vati* is a preliminary quantitative analysis that displays the number of components contained in the sample precisely and accurately. It also shows how pure the medication is.

Preparation of Test solution: Weigh accurately 1 g of sample in a 250 mL reflux flask Add 10 millilitres of methanol to it, then let it reflux in a water bath for half an hour. On completion of time, remove the reflux flask from the water bath and allow to cool Use Whatman filter paper number one to filter. Utilize the resulting Test Solution for HPTLC fingerprinting.

Preparation of Spray reagent [Anisaldehyde – sulphuric acid reagent]: The mixture consists of 5 mL sulfuric acid (98%) 0.5 mL anisaldehyde, 10 mL glacial acetic acid, and 85 mL methanol.

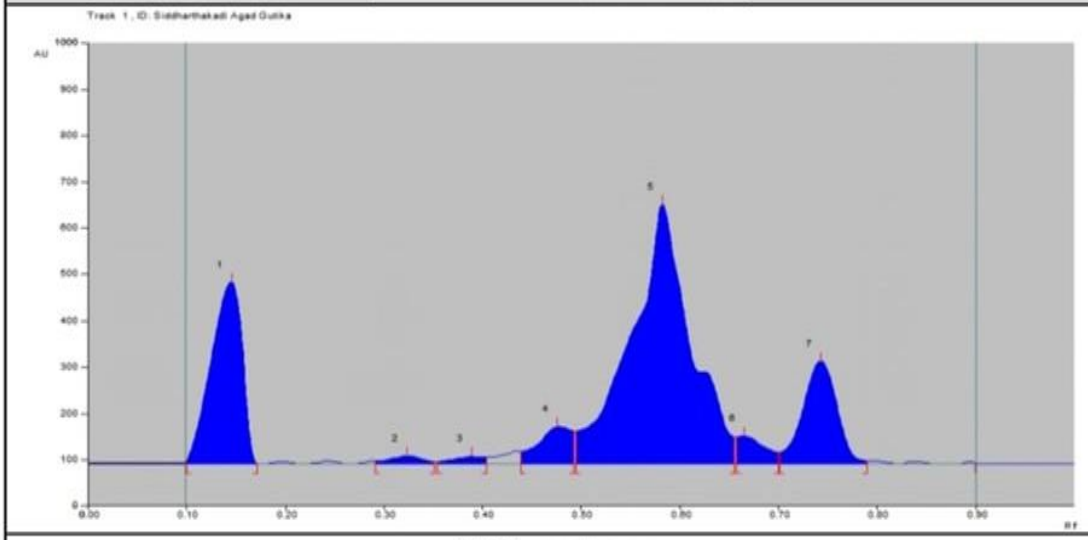
Chromatographic Conditions

Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminum sheets
Application (Y axis) Start Position	10 mm
Development End Position	80 mm from plate base
Sample Application Volume	5.0 µL
Band Length	8 mm
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Toluene : Ethyl acetate : Acetic acid (7 : 2 : 1 v/v)
Visualization	@ 254 nm, @ 366 nm and @ 540 nm (after derivatization)
Spray reagent	Anisaldehyde - Sulphuric acid reagent
Derivatization mode	CAMAG – Dip tank for about 1 minute
Drying Mode, Temp. & Time	TLC Plate Heater Preheated at 100± 50C for 3 minutes

RF value – On performing HPTLC procedure total RF spots obtained are as follows:

- On visualization at 254 nm 7 RF spots were obtained: 0.15, 0.32, 0.39, 0.48, 0.58, 0.67, 0.74.
- On visualization at 366 nm 6 RF spots were obtained: 0.15, 0.39, 0.53, 0.71, 0.79, 0.84.
- On visualization at 540 nm 6 RF spots were obtained: 0.32, 0.43, 0.58, 0.62, 0.71, 0.74.

HPTLC Chromatogram @ 254 nm



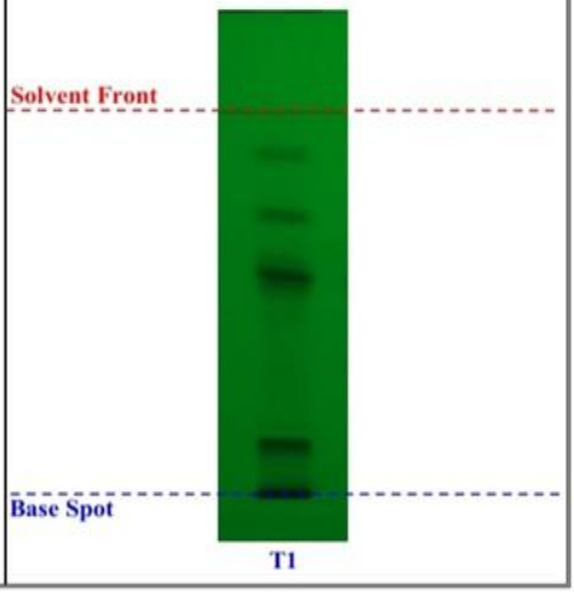
2D Chromatogram

R_f value

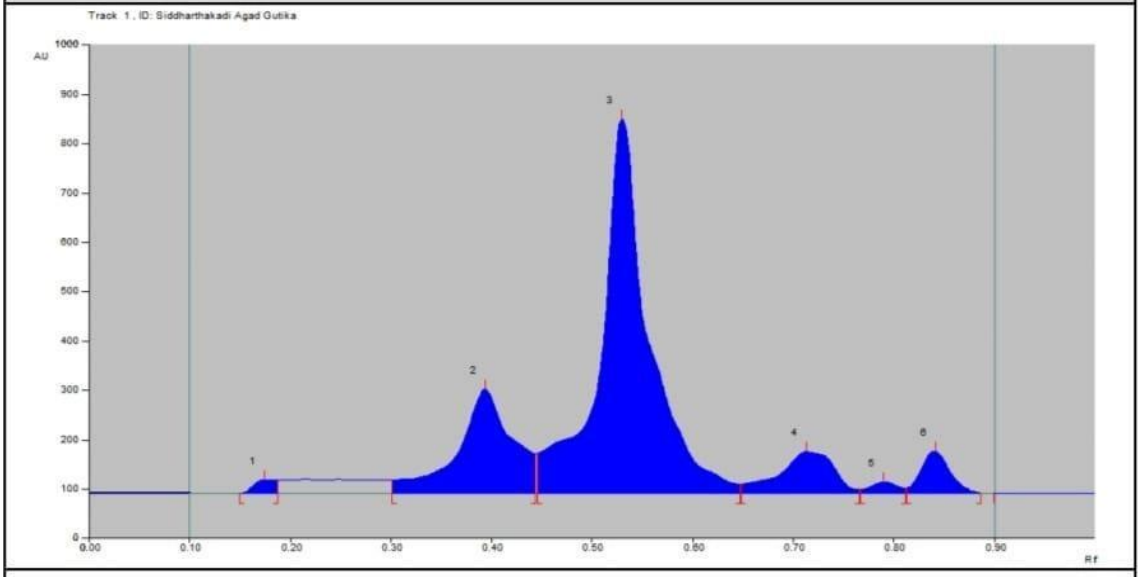
Spot No.	Track T1
1	0.15
2	0.32
3	0.39
4	0.48
5	0.58
6	0.67
7	0.74

Track T1: Siddharthakadi Agad Gutika

HPTLC Plate



HPTLC Chromatogram @ 366 nm



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R_f value

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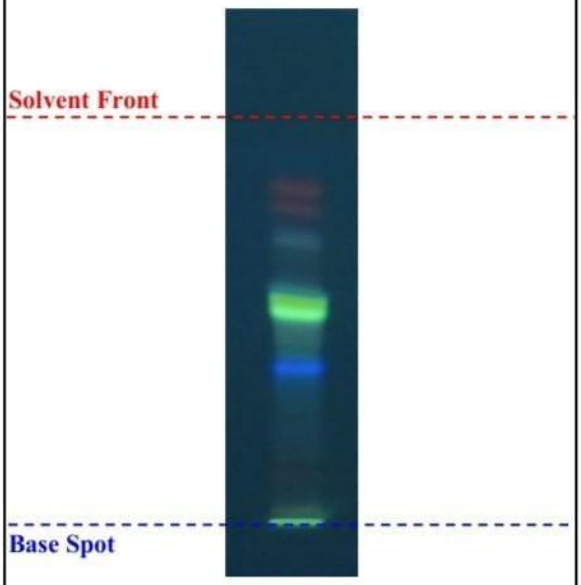
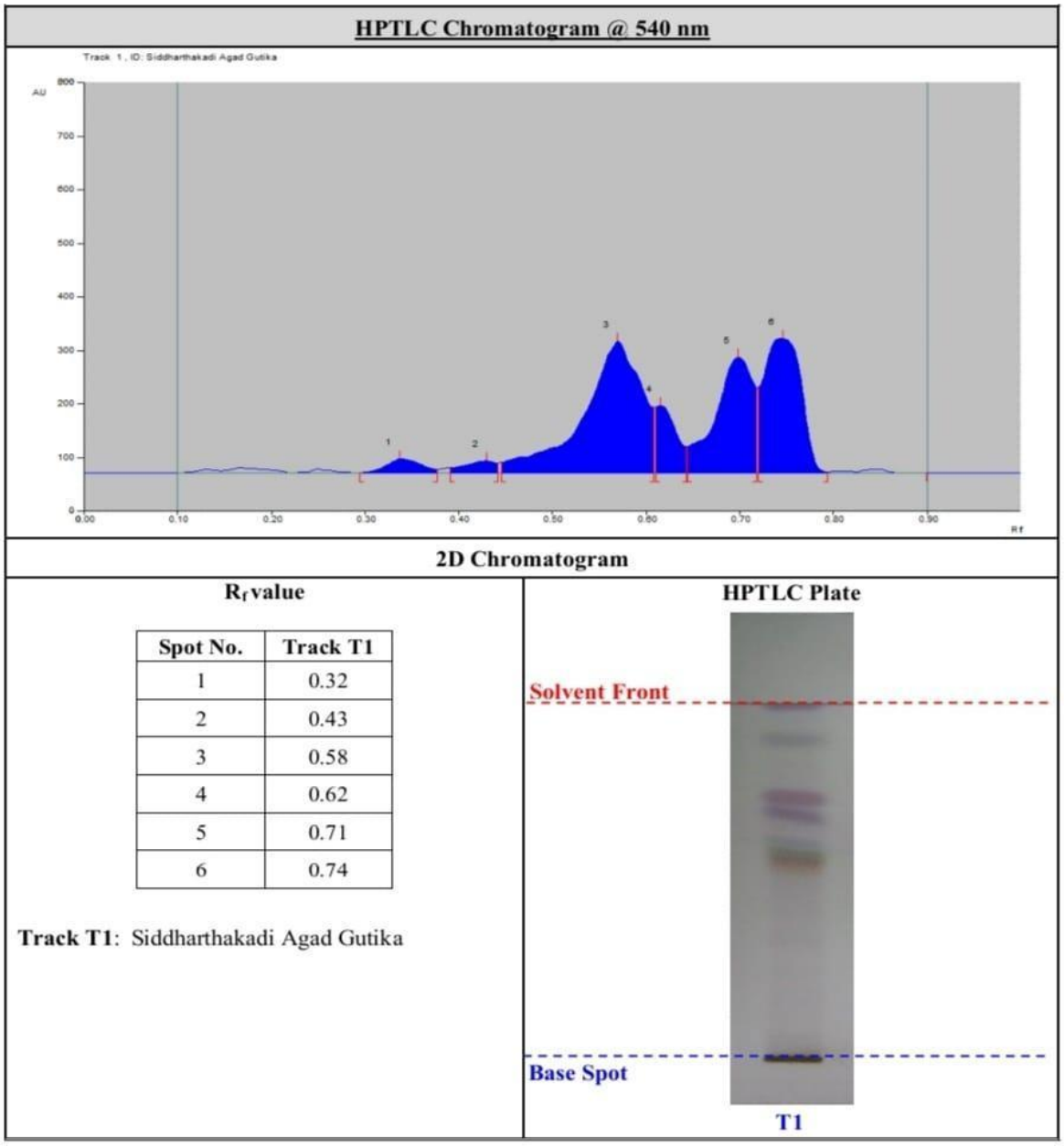
Track T1: Siddharthakadi Agad Gutika

HPTLC Plate





DISCUSSION

With the use of new technologies, the current study examined the characteristics and physicochemical analysis of *Siddharthakadi Agad Vati*. By assisting in the identification of contaminants and confirming the makeup of active components, physicochemical analysis guarantees the uniformity and quality of medication formulations. Predicting a drug's efficacy and safety requires knowledge of its physicochemical characteristics, which influence how it is absorbed, distributed, and metabolized. Both analyses are frequently necessary for regulatory submissions, guaranteeing that medications meet safety and efficacy standards before going on sale. HPTLC is a useful analytical technique for separating and identifying compounds in complex mixtures, aiding in the development of new drugs and formulations. It also helps

establish standards for herbal and other natural products, ensuring that they contain the desired active compounds in consistent amounts.

CONCLUSION

One of the key Ayurvedic formulations that is advised for a number of medical conditions is *Siddharthakadi Agad Vati*. Heavy metal detection, microbial contamination, and HPTLC are preliminary quantitative and qualitative analysis techniques that precisely identify the quantity of components in a sample by analyzing minute changes in Rf values. The drug's purity is confirmed by this analysis. *Siddharthakadi Agad Vati* was made for this study using conventional Ayurvedic techniques, and it satisfies the requirements specified in the API for Vati preparation. All things considered, this microbiological and heavy metal testing guarantees that Ayurvedic products are safe to consume and accepted worldwide, enhancing their reputation in both local and foreign markets. As a result, the physicochemical, organoleptic, and HPTLC profiles mentioned above will serve as a benchmark going forward and aid in determining the drug's efficacy, safety, stability, and quality.

Funding

This research received no external funding.

Disclosure of conflict of interest

The authors declare no conflict of interest.

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