**ANALYTICAL STUDY OF *GUDUCHYADI RASAYANA* -AN AYURVEDIC FORMULATION FOR ACADEMIC STRESS**

**Abstract –**

*Guduchyadi Rasayana*, a traditional Ayurvedic formulation, is gaining attention for its potential to reduce stress and promote mental well-being, particularly in children. The formulation is composed of eight herbal ingredients: *Guduchi* (Tinospora cordifolia), *Apamarga* (Achyranthes aspera), *Shankhpushpi* (Convolvulus pluricaulis), *Vacha* (Acorus calamus), *Haritaki* (Terminalia chebula), *Shatavari* (Asparagus racemosus), *Vayvihdanga* (Embelia ribes), and *Kushtha* (Saussurea lappa). This study investigates and validates the pharmacognostic and analytical properties of *Guduchyadi Rasayana*, with a specific focus on its efficacy in stress reduction for children. Prepared in compliance with Good Manufacturing Practices (GMP), the formulation underwent various analyses, including organoleptic, physicochemical, and thin-layer chromatography (TLC). Organoleptic testing revealed a greenish-yellow color and a pleasant fragrance. Physicochemical results indicated an alcohol-soluble extractive value of 29.94%, total ash content of 9.54%, and water-soluble extractive of 22.07%. TLC fingerprinting provided distinct Rf values (0.48, 0.51, 0.73, 0.83, 0.90), identifying several active phytochemicals that may contribute to its stress-relieving effects. The study’s findings underscore the therapeutic potential of *Guduchyadi Rasayana* in managing stress and enhancing cognitive health in children, supporting its standardization for clinical use in paediatric stress reduction and overall mental well-being.

**Keywords-** *Guduchyadi Rasayana,* Reduce stress, Organoleptic Characters, Physicochemical Parameters, and TLC

**Introduction: -** Herbal drugs possess various properties, including *Rasa, Guna, Virya, Vipaka,* and *Prabhava*, and the importance of a drug is based on these properties and its actions[[1]](#endnote-1). The *Sushruta Samhita* elaborates on these aspects in detail[[2]](#endnote-2). The action of a drug is closely linked to its chemical structure, especially the predominance of certain proto-elements[[3]](#endnote-3). In *Ayurveda, Rasayana* drugs play a crucial role in managing age-related disorders due to their antioxidant properties. These properties help prevent free radical damage, delay aging, and rejuvenate the mind and body[[4]](#endnote-4). Furthermore, *Medhya Rasayana* consists of eight herbal drugs, which are particularly effective in enhancing mental functions and intellect. There is a growing demand for plant-based pharmaceuticals. However, some drug preparations lack formal standards. This study aims to demonstrate the pharmacognostic and analytical potential of *Guduchyadi Medhya Rasayana*, highlighting its beneficial effects, particularly on mental health and cognitive abilities. *Guduchyadi Medhya Rasayana* is described in authentic Ayurvedic texts such as *Chakradatta[[5]](#endnote-5)* and *Yogaratnakara[[6]](#endnote-6)*, where it is listed under the *Rasayanadikara.* This formulation, which includes eight herbal drugs, has gained attention due to its therapeutic properties. Despite the increasing demand for plant-based pharmaceuticals, many preparations still lack proper standards. Therefore, this study seeks to explore and validate the pharmacognostical and analytical aspects of *Guduchyadi Medhya Rasayana*.

The analytical study of *Guduchyadi Rasayana* was conducted using the following parameters: organoleptic properties (appearance, color, odor, taste), physicochemical parameters (loss on drying, total ash, acid-insoluble ash, water-soluble extract, alcohol-soluble extract, pH, uniformity of weight, friability, hardness, disintegration time), as well as qualitative analysis and thin-layer chromatography (TLC).

**Aims and Objectives:-**

* Identification and authentication of raw drugs used for *Guduchyadi Rasayana*.
* Preparation of *Guduchyadi Rasayana* at GMP-certified pharmacy.
* Organoleptic characters, Physicochemical, and TLC analysis of *Guduchyadi Rasayana*.

**Materials and Methods: -**

*Guduchi (Tinospora Cardifolia), Apamarga (Achyanthes Aspera), Shankhpushpi (Convolvulus Pluricaulwas), Vacha (Acorus Calamus), Haritaki (Terminalia chebula), Shatavari(Asparagus racemosus), Vayvihdanga (Embelia ribes ),* and *Kushtha (Saussurea lappa)* were purchased from authenticated resources at Jodhpur.

**Identification and Authentication of Raw Drugs: -**

The drug identification and authentication were done by the Department of Dravya Guna, PGIA, Karwar Jodhpur.

**Method of preparation *Guduchyadi Rasayana*: -**

The classical formulation of *Guduchyadi Rasayana* includes a variety of *Medhya* herbs. This section provides a comprehensive discussion of these herbs, covering their synonyms, vernacular names, constituents, properties and actions, traditional classifications, therapeutic effects, pharmacological activities, recommended dosages, and the parts used, were thoroughly discussed in this section below. The drug was prepared at the Postgraduate Institute of *Ayurved* (formerly known as the University Postgraduate Institute of Ayurved Studies and Research and University College of Ayurveda) in Jodhpur. According to classical texts, all the ingredients of *Guduchyadi Rasayana—Guduchi, Apamarga, Shankhpushpi, Vacha, Haritaki, Shatavari, Vayvihdanga,* and *Kushtha*—were taken in equal proportions of 3 kg each. A total of 24 kg of raw material was processed through a pulverizer to obtain a fine powder of *Guduchyadi Rasayan*. A loss of 600 grams was observed during this process. After complete drying in sunlight, the *Guduchyadi Rasayan* was packed into 100 gm containers. All containers were labeled and distributed to patients for 21 days.

**Table 1: Showing Ingredients of *Guduchyadi Rasayan***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.No.** | **Name**  **of Drug** | **Latin Name** | **Family** | **Useful part** |
| 1. | *Guduchi* | *Tinospora Cardifolia* | *Menispermaceae* | Root, Stem, leaves |
| 2. | *Apamarg* | *Achyanthes Aspera* | *Amaranthaceae* | Mula,Tandul,patra  (Panchang) |
| 3. | *Shankhpushpi* | *Convolvulus Pluricaulis* | *Convolvulaceae* | Whole plant |
| 4. | *Vacha* | *Acorus Calamus* | *Araceae* | Mula & Bhoumik kand |
| 5. | *Haritaki* | *Terminalia chebula* | *Combretaceae* | Fruits (dry) |
| 6. | *Shatavri* | *Asparagus racemosus* | *Liliaceae* | Kandh |
| 7. | *Vayvihdanga* | *Embelia ribes* | *Myrsinaceae* | Phal |
| 8. | *Kushth* | *Saussurea lappa* | *Compositae* | Mula(Roots) |

**METHOD OF PREPARATION OF *GUDUCHYADI RASAYAN*: -**

The “Protocol for Testing Ayurvedic, Siddha, and Unani Medicines,”published by the National Institute of Science Communication and Information Resources (NISCAIR), CSIR, and released by the Government of India’s Department of Ayurveda, Yoga — Naturopathy, Unani, Siddha & Homeopathy (AYUSH), New Delhi, provided the foundation for the parameters used in various investigations.

**Place of work**

Cultivator Phyto Lab Pvt. Ltd. Sonamukhi Nagar, Sangaria Fanta, Jodhpur.

Sample Registration No. – CPL/O/24/09/01473/1

Sample Code – CPL/ 2024/07728-N

Date of Sample sent to Lab & Sample Registration -12/09/2023

Date of start of analysis: 13/09/2024

Date of completion of Analysis-18/09/2024

Duration- 6 days

**An analytical study was conducted under the following headings:**

1. Organoleptic Characters
2. Physiochemical Parameters
3. Chromatographic Fingerprint - TLC

**Organoleptic Characters:**

Organoleptic characteristics refer to the sensory properties of a substance that can be evaluated using the five senses: sight, smell, taste, touch, and, occasionally, hearing. In the context of herbal products and natural remedies, organoleptic analysis is crucial for assessing quality, authenticity, and overall acceptability. The color, aroma, and flavor of an herb can provide important information about its freshness, potency, and potential adulteration. This type of analysis is especially important in traditional systems of medicine, like Ayurveda, where the sensory traits of herbs are often linked to their therapeutic properties.

**Table -2: Organoleptic Properties of Guduchyadi Rasayana**

|  |  |  |
| --- | --- | --- |
| **S. No.** | **Macroscopic Study** | ***Guduchyadi Rasayan*** |
| **1.** | Appearance | Powder |
| **2.** | Color | Greenish Yellow |
| **3.** | Odor | pleasant |

**Physiochemical Parameters:**

To ensure the safety, potency, and efficacy of the prepared formulation, several critical parameters were assessed. These included the Alcohol Soluble Extractive, which measures the amount of active constituents that can be extracted with alcohol, providing insights into the bioactive components in the formulation. The Loss on Drying at 105°C(moisture content) was evaluated to determine the level of water present, as excessive moisture can lead to microbial growth or degradation of the formulation. The pH Value was assessed to ensure the formulation maintains a stable acidic or alkaline environment suitable for its intended use. The Total Ash content was measured to determine the amount of inorganic matter left after combustion, which can indicate the presence of contaminants or adulterants. Finally, the Water-Soluble Extractive was analyzed to identify the compounds that can be extracted by water, providing additional information aboutthe chemical profile and potential therapeutic properties of the formulation.[[7]](#endnote-7)

* **Alcohol-Soluble Extractive: -**

To determine the alcohol-soluble extractive value from an Ayurvedic powder, a specific procedure is followed to accurately assess the extractive components. First, approximately 5 grams of the powdered sample are weighed with precision and placed in a glass-stoppered flask. Next, 100 ml of 90% ethanol is added to the flask, and the mixture is shaken for the first 6 hours, after which it is allowed to stand for 18 hours to ensure proper extraction. The mixture is then rapidly filtered to minimize solvent loss, and 25 ml of the filtrate is transferred into a pre-weighed evaporating dish. The solvent is carefully evaporated in a water bath, and the residue is dried at 105°C until it reaches a constant weight[[8]](#endnote-8). Finally, the residue is weighed, and the percentage of alcohol-soluble extractive is calculated using the appropriate formula, which allows for an accurate evaluation of the extractable compounds from the Ayurvedic powder.

**Percentage of alcohol-soluble extractive= (Weight of residue / Weight of sample)×100**

This method aids in identifying the quantity of active ingredients that dissolve in alcohol, which can be vital for the effectiveness of Ayurvedic formulations[[9]](#endnote-9).

* **Moisture Content (Loss on Drying at 105°C):**

The process for determining the loss on drying (LOD) begins with sample preparation, where a clean, dry, and pre-weighed glass-stoppered shallow weighing bottle is used. Approximately 1-2 grams of the sample are carefully weighed into the bottle. Next, the bottle containing the sample is placed in a drying oven set at 105°C. The stopper is removed and placed in the oven alongside the bottle to ensure proper drying. The sample is dried to a constant weight, typically for about 3 hours. Once drying is complete, the bottle is removed from the oven and immediately sealed with the stopper. The bottle is then allowed to cool to room temperature in a desiccator to prevent moisture absorption. After cooling, the bottle with the dried sample is reweighed. The loss on drying (LOD) is then calculated using the formula

**LOD (%) = (Initial Weight−Final weight / Initial weight) ×100**

This method helps assess the moisture content and volatile substances in the sample, which is essential for quality control.[[10]](#endnote-10)

* **pH Value Determination:**

To determine the pH value of an Ayurvedic powder, a specific procedure is followed to assess its acidity or alkalinity. First, 1 gram of the Ayurvedic powder is weighed accurately and dissolved in 100 ml of distilled water to prepare a 1% w/v solution. The solution is thoroughly stirred to ensure the powder is completely dissolved. The pH meter is then calibrated using standard buffer solutions, typically at pH 4.0, 7.0, and 9.0, to ensure accurate readings. After calibration, the electrode is rinsed with distilled water and blotted dry before being immersed into the prepared solution. The pH value is recorded once the reading stabilizes. After measurement, the electrode is cleaned and stored according to the manufacturer’s instructions to maintain its longevity and accuracy. This method is essential for determining the pH value of the Ayurvedic powder, which provides insights into its potential effectiveness and stability, as the pH can influence the solubility and bioavailability of its active components[[11]](#endnote-11)**.**

* **Total Ash Value Determination:**

To determine the total ash value of a churn, a specific procedure is followed. First, 2 grams of the churn are accurately weighed and transferred to a previously ignited and tared silica crucible. The crucible is then gradually heated to a temperature range of 500-600°C, ensuring the material turns white, which indicates complete combustion of the sample. Once the ignition is complete, the crucible is allowed to cool in a desiccator to room temperature to prevent moisture absorption. After cooling, the crucible with the ash is weighed. The total ash value is then calculated using the appropriate formula, providing important information about the inorganic content of the churn.

**Total Ash (%) =(Weight of ash​ / Weight of sample)×100**

This method helps assess the quantity of inorganic material in the churna after complete incineration.[[12]](#endnote-12)

* **Water Soluble Extractive Determination:**

To determine the water-soluble extractive value of a churn, the following procedure is carried out. First, 5 grams of the churn are accurately weighed and placed into a glass-stoppered flask. Then, 100 ml of chloroform water (a mixture of chloroform and water) is added to the flask. The mixture is shaken frequently for the first 6 hours to ensure thorough mixing, then allowed to stand for 18 hours to facilitate proper extraction. After this maceration period, the mixture is rapidly filtered to prevent any loss of solvent. Next, 25 ml of the filtrate is transferred into a pre-weighed evaporating dish. The solvent is then evaporated using a water bath, and the residue is dried at 105°C until it reaches a constant weight. Finally, the residue is weighed, and the percentage of water-soluble extractive is calculated using the appropriate formula, which provides insight into the extractable components of the churn.

**Percentage of water-soluble extractive =(Weight of residue​ / Weight of sample)×100**

This method aids in measuring the quantity of active ingredients that dissolve in water, which is essential for the formulation's therapeutic effects.[[13]](#endnote-13)

**Phytochemical Parameters:**

**FINGERPRINTING OF *GUDUCHYADI RASAYAN* BY THIN LAYER CHROMATOGRAPHY (TLC):-**

Thin-layer chromatography (TLC) was used to develop a chromatographic fingerprint for Guduchyadi Rasayana, which serves as a unique profile of its chemical constituents. TLC allows for identifying key compounds present in the formulation, aiding in quality control and standardization. This technique is particularly useful in confirming the presence of active ingredients and ensuring consistency in the formulation’s composition.

To perform Thin Layer Chromatography (TLC) on an Ayurvedic powder (Churna), a systematic procedure is followed. First, approximately 1 gram of the Churna is weighed, and the sample is extracted with a suitable solvent, such as methanol or ethanol, by shaking it for 30 minutes. The extract is then filtered and concentrated to a smaller volume. A pre-coated silica gel TLC plate is used for the analysis, and a baseline is drawn about 1 cm from the bottom of the plate using a pencil. Small spots of the concentrated extract are applied along the baseline using a capillary tube or micropipette, and the spots are allowed to dry completely. The TLC plate is then placed in a TLC chamber containing a prepared mobile phase, such as a mixture of chloroform and methanol, ensuring that the baseline remains above the solvent level. The chamber is covered, and the solvent rises up the plate through capillary action until it reaches about 1 cm from the top. After the development process, the plate is removed, dried, and the spots are visualized either under UV light or by spraying with a suitable detecting reagent, such as iodine vapor or anisaldehyde-sulfuric acid. Finally, the distance traveled by each spot and the solvent front are measured to calculate the Rf (retention factor) values, which provide information about the individual components in the Ayurvedic powder. Calculate the Rf value for each spot using the formula:

**Rf=Distance travelled by the compound​Distance travelled by the solvent front**

This procedure helps in identifying and analyzing the compounds present in the Ayurvedic formulation, enabling quality control and standardization.[[14]](#endnote-14)

**TLC Profile:**

Sample Name: *Guduchyadi Rasayan*

sample Id: CPL\_2024\_07728

Sample Preparation: 1ml in 10ml methanol.

Mobile Phase: Toluene: Chloroform: methanol

Derivatization: Anisaldehyde +Sulphuric Acid +Acetic Acid

Sample Injection volume: 10 µl

Solvent distance travelled: 8 cm.

Visualization after derivatization Rf Value: 0.48,0.51,0.73,0.83,0.90

**Test Method:** API Part II Vol IV: 2017[[15]](#endnote-15)

**Table 3. Showing Physio-chemical Parameters & TLC of *Guduchyadi Yog***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S. No.** | **Test Parameters** | **Unit** | **Result** | **Test Method** |
| 1. | Alcohol soluble extractive | % | 29.94 | API Part I Vol IX: 2016 |
| 2. | pH Value | - | 4.81 | API Part I Vol IX: 2016 |
| 3. | Total Ash | % | 9.54 | API Part I Vol IX: 2016 |
| 4. | Water Soluble extractive | % | 22.07 | API Part I Vol IX: 2016 |
| 5. | Moisture | % | 8.51 | API Part I Vol IX: 2016 |
| 7. | Thin-Layer Chromatography | - | 0.48,0.51,0.73,0.83,0.90 | API Part II Vol IV: 2017 |

**RESULT**

The formulation of *Guduchyadi Rasayana* was prepared following standard operating procedures at the GMP-certified Nagarjuna Pharmacy of the Post Graduate Institute of Ayurveda and subjected to quantitative analysis. An analytical study of *Guduchyadi Rasayana* was conducted at Cultivator Phyto Lab Pvt. Ltd. in Sonamukhi Nagar, Sangaria Fanta, Jodhpur, to evaluate its organoleptic characteristics, physicochemical properties, and chromatographic analysis. The results of the pharmaceutical analysis are discussed below.

* **Organoleptic Characteristics -** The organoleptic characteristics of *Guduchyadi Rasayan* in powder form include a greenish-yellow color and a pleasant odor. In contrast, the powder form of *Guduchyadi Rasayan* is characterized by a reddish-brown color and also has a pleasant odor. The typical color and odor of *Guduchyadi Rasayan* is greenish-yellow with a pleasant aroma. The justification for the powder's color and odor is that the contrasting colors and forms (powder) suggest different processing methods and possibly varying herbal constituents. The pleasant odor in form indicates a favorable aromatic profile, which is essential for patient acceptance and therapeutic efficacy.
* **Physicochemical Parameters-** The physicochemical parameters of *Guduchyadi Rasayan* were evaluated based on criteria from API I, Vol. IX, 2016, which were shown as follows:–

1. **Alcohol-Soluble Extractive**:-The alcohol-soluble extractive was reported at 29.94%, indicating that *Guduchyadi Rasayan* effectively extracted active principles, enhancing its therapeutic efficacy. This value fell within the range of 20-30%, suggesting consistency in preparation methods.
2. **Loss on Drying (Moisture):-** A moisture content of 8.51% was deemed reasonable, ensuring that the product maintained stability without becoming overly dry, which could degrade active ingredients.
3. **pH:-** With a pH of 4.81, *Guduchyadi Rasayan* was mildly acidic, which could benefit the solubility of certain compounds and enhance absorption in the gastrointestinal tract.
4. **Total Ash Content:-** The total ash content of 9.54% reflected the presence of inorganic minerals. This value was acceptable and suggested a quality product rich in beneficial minerals
5. **Water-Soluble Extractive:-** At 22.07%, this value indicated that a significant proportion of the compounds were soluble in water, which was crucial for the bioavailability of herbal medicines. This fell within the typical range of 15-25%, further affirming the product's quality.

* **Thin layer Chromatography (TLC) -** The chromatographic fingerprint (TLC) analysis of *Guduchyadi Rasayan* was conducted using a 1 ml solution in 10 ml of methanol, with a mobile phase of chloroform and methanol. The solvent traveled a distance of approximately 8 cm, and after derivatization, the final Rf values obtained were 0.48, 0.51, 0.73, 0.83, and 0.90. The TLC analysis of Guduchyadi Rasayan yielded Rf values of 0.48, 0.51, 0.73, 0.83, and 0.90. When compared to typical Rf ranges for herbal extracts, it showed interpretation as
* **Rf 0.48**:- The normal range was 0.4 - 0.6, suggesting polar compounds, often critical for therapeutic effects.
* **Rf 0.51**:- Also within the 0.4 - 0.6 range, indicating similar polar constituents that could enhance the efficacy.
* **Rf 0.73**:- Fits within the 0.7 - 0.9 range, reflecting moderately polar compounds that contributed to the formulation's complexity.
* **Rf 0.83**:- This value was consistent with the 0.7 - 0.9 range, indicating non-polar to moderately polar substances that might enhance therapeutic action.
* **Rf 0.90**:- Within the 0.8 - 1.0 range, suggested less polar compounds, valuable for broadening the spectrum of activity. The diversity of Rf values confirms effective extraction methods, highlighting the presence of a rich array of phytochemicals. This complexity was essential for the synergistic effects often seen in herbal formulations, supporting the overall therapeutic potential and quality of *Guduchyadi Rasayan.* Thus, the results were not only aligned with expected norms but also underscored the formulation's efficacy.

**DISCUSSION**

The analytical study of *Guduchyadi Rasayana* was carried out to assess its organoleptic, physicochemical, and chromatographic properties, which are essential for its quality control and therapeutic efficacy. The formulation, prepared at a GMP-certified pharmacy, was subjected to rigorous testing at Cultivator Phyto Lab Pvt. Ltd. in Jodhpur. The organoleptic characteristics revealed that the powder form of *Guduchyadi Rasayana* had a greenish-yellow color and a pleasant odor. These sensory properties are important, as they reflect the quality and acceptability of the formulation, which is crucial for its therapeutic effectiveness. Physicochemical analysis provided critical insights into the formulation’s composition and stability. The alcohol-soluble extractive content of 29.94% indicates a high concentration of active compounds that are likely to contribute to the therapeutic actions of *Guduchyadi Rasayana*. This result is consistent with the expected range, affirming the efficacy of the extraction process used in preparation. The moisture content of 8.51% and a pH value of 4.81 suggest that the formulation is stable and mildly acidic, which may enhance the solubility of certain compounds, thus improving their bioavailability. The total ash content of 9.54% is a reflection of the inorganic material in the formulation, while the water-soluble extractive content of 22.07% indicates a significant proportion of bioavailable compounds that dissolve in water, facilitating their absorption in the body. Thin Layer Chromatography (TLC) fingerprinting revealed multiple Rf values (0.48, 0.51, 0.73, 0.83, and 0.90), demonstrating the presence of a diverse array of chemical constituents. These values are typical for herbal formulations, indicating a mix of polar and moderately polar compounds, each contributing to the overall therapeutic efficacy. The variety of Rf values confirms the presence of a broad spectrum of active ingredients, ensuring the formulation’s therapeutic potential. Overall, the findings underscore the high quality and therapeutic potential of *Guduchyadi Rasayana,* supporting its use in traditional and modern medicinal practices. The comprehensive analysis provides a strong basis for the standardization and validation of this formulation in clinical applications.

**CONCLUSION: -**The Ayurvedic system of medicine is increasingly being relied upon for stress management, various health issues, and particularly lifestyle diseases. The ingredients used in these formulations have been pharmacognostically identified, authenticated, and utilized for preparation. Any plant or formulation used medicinally requires a detailed study before its use, as the therapeutic efficacy depends on the quality of the ingredients involved in the preparation of the medicinal product[[16]](#endnote-16). The prepared drug, *Guduchi Rasayana*, was pharmacologically subjected to physicochemical analysis, qualitative testing, and Thin Layer Chromatography (TLC). The ingredients of *Guduchi Rasayana* include *Guduchi, Apamarga, Shankhpushpi, Vacha, Haritaki, Shatavari, Vayvihdanga,* and *Kusth*, making it an herbal formulation. In this study, *Guduchi Rasayana* was prepared per classical references, following standard operating procedures at a GMP-certified pharmacy[[17]](#endnote-17). The raw drugs were identified and authenticated before use. The drug underwent physicochemical analysis, qualitative testing, and TLC analysis. Present analytical study proved that the This study aims to establish the groundwork for the standardization of *Guduchi Rasayana* and to prepare a monograph for the Ayurvedic Formulary of India (AFI)[[18]](#endnote-18).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

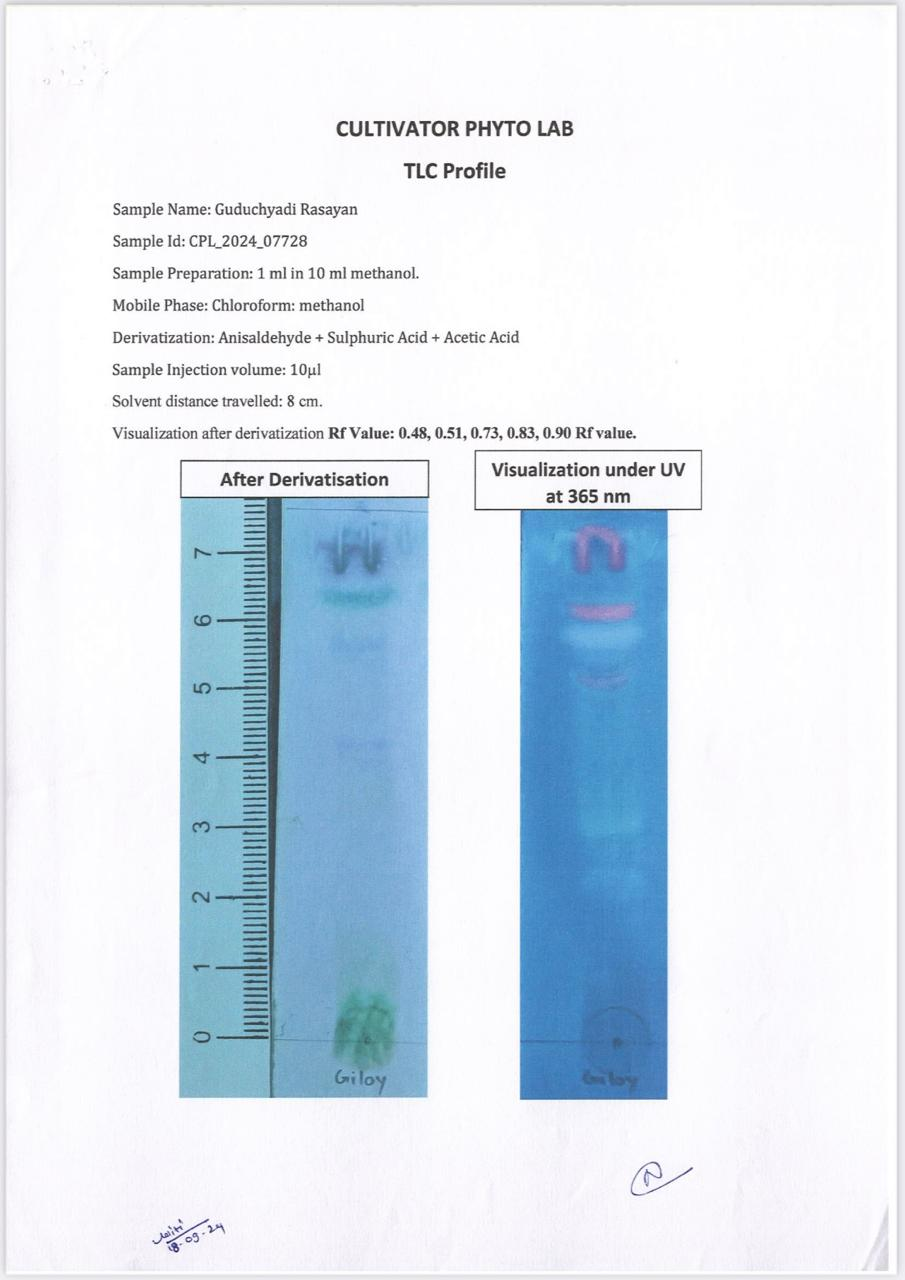


Fig 1- TLC Profile of the sample

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