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EFFECT OF SHAD RITU ON COLLECTION OF MANDUKPARNI ON PUSHYA NAKSHATRA

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ABSTRACT

This study explores the seasonal variations in the physico-chemical and phyto-chemical properties of *Mandukaparni* (*Centella asiatica*), a widely used medicinal plant in Ayurveda. Six different seasons (*Ritus*)—*Vasant*, *Grishma*, *Varsha*, *Sharad*, *Hemant*, and *Shishir*—were examined to understand how environmental changes affect the plant's chemical composition. Key physico-chemical parameters, including pH, total ash content, acid insoluble ash, loss on drying, water-soluble extractives, alcohol-soluble extractives, and triterpenoid content, were analyzed across the seasons. Additionally, phyto-chemical identification of alkaloids, glycosides, flavonoids, tannins, steroids, terpenoids, saponins, carbohydrates, and proteins was performed. HPTLC analysis at 254 nm, 366 nm, and 540 nm wavelengths was also conducted to detect variations in the plant's phytochemical profiles. The results indicate that while certain parameters, such as triterpenoid concentration and water-soluble extractive values, are higher in winter (*Shishir Ritu*), the overall chemical profile of *Mandukaparni* remains relatively consistent throughout the year. These findings suggest that optimal harvesting for specific bioactive compounds may vary by season, with winter providing the highest concentrations of key medicinal constituents. The research provides a comprehensive understanding of the influence of seasonal variation on *Mandukaparni*'s therapeutic efficacy, aiding in better harvesting and utilization practices.

KEYWORDS- *Mandukaparni*, *Centella asiatica*, physico-chemical analysis, phyto-chemical constituents, HPTLC analysis, Ritus

INTRODUCTION

Ayurveda, the ancient system of medicine, emphasizes the harmonious relationship between nature and human health. One of the core principles of Ayurveda is that the time and environmental conditions under which medicinal plants are collected can significantly influence their therapeutic efficacy. This understanding is deeply rooted in Ayurvedic scriptures, which advocate the collection of medicinal herbs at specific times—both seasonal and astrological—to maximize their potency. The concept of harvesting plants based on favorable conditions, such as *Shad Ritu* (six seasons) and *Nakshatras* (lunar constellations), reflects this ancient wisdom.[1]

Mandukaparni (Centella asiatica), commonly known as Indian pennywort or Gotu Kola, is one of the most revered herbs in Ayurveda. It is classified as a *medhya rasayana*, meaning it promotes intelligence, memory, and longevity. *Mandukaparni* is known for its broad spectrum of medicinal properties, such as enhancing cognitive function, supporting nervous system health, promoting wound healing, and acting as an anti-inflammatory and antioxidant agent. In Ayurveda, the optimal collection of this herb is believed to be linked to the lunar asterism *Pushya Nakshatra*, which is regarded as an extremely auspicious time for harvesting herbs due to its celestial influences. Collecting plants under this nakshatra is said to enhance their medicinal virtues and longevity.[2]

In addition to *Nakshatras*, Ayurveda also recognizes the significance of seasonal variations, known as *Shad Ritu*, in determining the potency of medicinal plants. The year in Ayurveda is divided into six distinct seasons:

1. *Vasanta* (spring),
2. *Grishma* (summer),
3. *Varsha* (monsoon),
4. *Sharad* (autumn),
5. *Hemanta* (pre-winter),
6. *Shishira* (winter).

Each of these seasons, governed by different climatic conditions, influences the biological processes in plants, including growth, phytochemical production, and overall therapeutic potency. Ancient texts like *Charaka Samhita* and *Sushruta Samhita* explain that herbs should be harvested at the peak of their seasonal potency, which varies based on the herb's individual characteristics and environmental preferences. The intricate interplay between these seasonal and astrological factors can optimize the bioavailability and concentration of active compounds in medicinal plants.[3]

The lunar asterism *Pushya Nakshatra* is believed to have a special effect on plants and natural substances. In Vedic astrology, *Pushya* is associated with nourishment, growth, and positive energy, making it an ideal time to perform agricultural and medicinal tasks. Collecting herbs during *Pushya Nakshatra*, as described in texts such as the *Brihat Samhita* and

Vrikshayurveda, is thought to fortify the plants with celestial energy, ensuring that they reach their peak therapeutic potential.[4]

Mandukaparni has a significant place in Ayurvedic medicine due to its adaptogenic properties, particularly in promoting mental clarity and brain function. As a *medhya rasayana*, it rejuvenates the nervous system, sharpens memory, and helps in managing stress-related disorders. The plant grows in diverse climatic conditions, but the environmental and seasonal factors heavily influence its growth pattern and the concentration of bioactive compounds like triterpenoids (asiaticoside, madecassoside), flavonoids, and other phytochemicals. These active compounds are responsible for the neuroprotective, antioxidant, anti-inflammatory, and wound healing properties of *Mandukaparni*.[5]

In this study, we aim to investigate the combined influence of *Shad Ritu* (six seasons) and *Pushya Nakshatra* on the collection of *Mandukaparni*. By examining the seasonal variations in the concentration of bioactive compounds, this research seeks to identify the optimal time for harvesting *Mandukaparni* to ensure maximum therapeutic efficacy. Traditional Ayurvedic practices suggest that harvesting during *Pushya Nakshatra* within specific seasons enhances the plant's potency, but modern scientific studies on this subject are limited. This research will bridge the gap by analyzing how each season's climatic conditions affect the bioavailability of *Mandukaparni*, and whether the combination with *Pushya Nakshatra* truly maximizes its medicinal potential.[6]

The specific objectives of this study are:

- To understand how the seasonal variations of *Shad Ritu* influence the bioactive content of *Mandukaparni*.
- To determine the impact of collecting *Mandukaparni* during *Pushya Nakshatra* in different seasons.
- To identify the season during which the bioactive compounds in *Mandukaparni* are at their peak, thereby optimizing the timing of its collection for medicinal use.

This investigation holds great importance not only in validating traditional Ayurvedic knowledge with scientific evidence but also in enhancing the standardization of medicinal plant collection practices, which can contribute to the improved efficacy of herbal medicines in both Ayurvedic and modern medical practices.[7]

Review of Literature

1. Importance of *Pushya Nakshatra* in Ayurveda

The *Pushya Nakshatra* holds a prominent place in Vedic astrology and Ayurvedic medicine due to its perceived cosmic influences, which are believed to enhance the efficacy and potency of medicinal plants. In Ayurveda, timing is an essential factor in determining the quality and effectiveness of medicinal herbs, and the *Pushya Nakshatra* is seen as a highly auspicious period for various activities, especially the collection of herbs. This Nakshatra is

ruled by Brihaspati (Jupiter), the planet associated with wisdom, growth, and nourishment, making it a favorable time for any activities related to health, healing, and growth.[8]

According to *Brihat Samhita* and other classical texts like *Vrikshayurveda*, plants and herbs collected during the *Pushya Nakshatra* are believed to be imbued with special qualities. The cosmic alignment during this Nakshatra is thought to infuse herbs with added vitality, energy, and healing properties, which are not present when they are collected during other times. This practice of timing herb collection based on lunar and planetary influences is deeply rooted in Ayurveda's holistic understanding of the interconnectedness of all life forms and their responsiveness to celestial cycles. The potency of herbs harvested during *Pushya Nakshatra* is believed to be enhanced by the cosmic energies, which are in perfect alignment during this time, resulting in a higher concentration of active phytochemicals and greater therapeutic benefits.[9]

This Nakshatra is particularly known for fostering nourishing and protective qualities, making it an ideal time for not only harvesting medicinal plants but also for performing various healing rituals and medical interventions. The influence of *Pushya Nakshatra* is often compared to the nourishing and life-sustaining energy of the mother's milk (*pushya* in Sanskrit means "to nourish"), which is why plants harvested during this Nakshatra are considered exceptionally potent and beneficial for health.[10]

In addition to harvesting, many Ayurvedic practitioners recommend initiating treatment plans, conducting *panchakarma* therapies, or even preparing specific Ayurvedic formulations during *Pushya Nakshatra* to harness these cosmic influences. This period is believed to amplify the therapeutic outcomes and enhance the effectiveness of Ayurvedic interventions.[11]

2. Concept of *Shad Ritu* in Ayurveda

Ayurveda categorizes the year into six distinct seasons, known as *Shad Ritu*, which are aligned with the environmental and climatic changes occurring throughout the year. These seasons each have unique characteristics that impact the health of living beings, the growth of plants, and the balance of the three doshas (*Vata*, *Pitta*, and *Kapha*). The six Ayurvedic seasons and their corresponding influences on medicinal plants are:

- **Vasanta (Spring)**: This season is considered the time of renewal and rejuvenation. Plants begin to grow vigorously due to the moderate temperatures and adequate moisture, making this season ideal for collecting herbs that promote health and wellness. In this season, the bitter and astringent tastes are dominant, making it suitable for collecting herbs that have cooling properties, such as *Mandukaparni*. The balance of moisture and temperature supports the synthesis of essential oils, alkaloids, and active compounds in plants.[12]
- **Grishma (Summer)**: Summer is characterized by intense heat and dryness, which can deplete moisture from plants, leading to a reduction in their bioactive compound

production. The dominance of the *Pitta* dosha makes this season less ideal for the collection of heat-sensitive herbs. The plants may exhibit lower potency during this time, as they struggle to thrive in the harsh conditions of high heat and low humidity. Herbs collected during *Grishma* may not possess their optimal medicinal qualities unless they are specifically suited to hot climates.[13]

- **Varsha (Monsoon)**: The rainy season brings an abundance of water, which can foster the growth of many plants. However, the excessive moisture and humidity during this time may also increase the risk of fungal contamination and degradation of medicinal compounds. The season is dominated by the *Vata* dosha, and although plants grow well in monsoon conditions, the waterlogged soil and reduced sunlight can sometimes compromise the quality of herbs collected during this period. It is crucial to ensure proper drying and storage techniques if herbs are harvested in this season.[14]
- **Sharad (Autumn)**: Autumn is known as the post-monsoon season, where the environment becomes dry and cool. The balance of moisture and temperature in *Sharad Ritu* creates an optimal environment for the accumulation of active compounds in medicinal plants. *Mandukaparni* collected during this season is considered to have its highest potency due to favorable conditions that promote the synthesis of phytochemicals like asiaticoside and madecassoside. This is often regarded as one of the best seasons for harvesting many medicinal herbs.[15]
- **Hemanta (Pre-winter)**: Pre-winter is characterized by cold and dryness, which can stimulate certain plants to store more nutrients and medicinal compounds to prepare for the upcoming winter. Herbs collected in this season, especially those with cooling properties, tend to have stable levels of bioactive compounds. The *Kapha* dosha becomes prominent, and this season is suitable for collecting herbs that promote nourishment and rejuvenation.[16]
- **Shishira (Winter)**: Winter is cold and dry, but it offers stability in the environment, which can be beneficial for certain plants. However, the intense cold may inhibit plant growth, leading to lower production of medicinal compounds. The potency of some herbs may diminish if they are collected in winter, as the physiological activity of the plants slows down during this time.[17]

In Ayurveda, each season brings about changes in the plants' internal chemistry, impacting their *rasa* (taste), *guna* (properties), *virya* (potency), and *vipaka* (post-digestive effect). Hence, the timing of herb collection is essential to ensure that the plants yield the highest possible therapeutic benefits. By aligning the collection time with the specific seasons, the potency and efficacy of Ayurvedic herbs can be optimized, leading to better health outcomes for patients.

3. Mandukaparni and Its Therapeutic Uses[18]

Mandukaparni (Centella asiatica) is a well-known herb in Ayurvedic medicine and is classified under the group of *medhya rasayana* (memory-boosting rejuvenatives). It has a long history of use in promoting cognitive function, treating neurological conditions, and enhancing mental clarity. Its key properties include:

- **Rasa (Taste)**: *Mandukaparni* is predominantly *tikta* (bitter) and *kashaya* (astringent) in taste. The bitter taste is associated with detoxifying and purifying properties, making it an excellent herb for removing metabolic waste and toxins from the body. The astringent taste, on the other hand, helps to tighten tissues and promote healing, especially in skin conditions.
- **Guna (Qualities)**: The herb is light (*laghu*) and drying (*ruksha*), which helps in pacifying *Kapha* and *Pitta* doshas, and is thus beneficial in treating conditions related to inflammation and sluggish metabolism.
- **Virya (Potency)**: *Mandukaparni* has a *sheeta* (cooling) virya, making it effective in calming *Pitta* dosha-related imbalances such as inflammation, heat-related disorders, and skin issues. Its cooling nature also contributes to its neuroprotective and anti-anxiety effects, soothing the nervous system and reducing stress.
- **Vipaka (Post-Digestive Effect)**: The herb has a *madhura* (sweet) post-digestive effect, which nourishes and supports the body's tissues, particularly the brain and nervous system.

Materials and Methods

1. **Study Design**- The study was designed to assess the effect of seasonal variations (*Shad Ritu*) on the collection of *Mandukaparni* during *Pushya Nakshatra*. Samples were collected during each of the six seasons over a one-year period, during the occurrence of *Pushya Nakshatra* in each season.
2. **Materials**
    - **Plant Material**: *Mandukaparni* (Centella asiatica) samples were collected from the same geographical area in each season to avoid geographic variability.
    - **Tools and Instruments**: Standard plant collection tools, drying equipment, and laboratory apparatus for phytochemical analysis.
3. **Methods**
    - **Collection of Samples**: *Mandukaparni* samples were collected during *Pushya Nakshatra* in all six seasons. The samples were dried, powdered, and stored in airtight containers.
    - **Phytochemical and HPLTC Analysis**: Each sample was subjected to phytochemical analysis to determine the concentration of bioactive compounds such as triterpenoids (asiaticoside, madecassoside), flavonoids, and polyphenols.

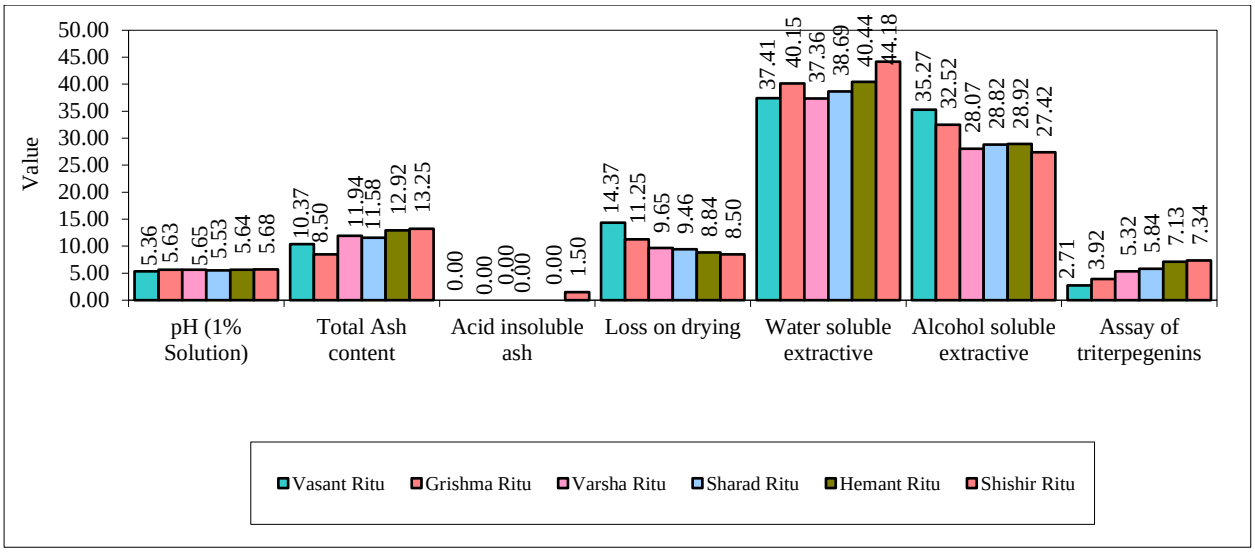
- **Comparative Evaluation**: The phytochemical content of the samples was compared across different seasons to assess how seasonal variations influenced the potency of the herb.

DRUG ANALYSIS

Table no. 1: Status of PHYSICO-CHEMICAL ANALYSIS in different Ritus

Parameters	Vasant Ritu	Grishma Ritu	Varsha Ritu	Sharad Ritu	Hemant Ritu	Shishir Ritu
pH (1% Solution)	5.36	5.63	5.65	5.53	5.64	5.68
Total Ash content	10.37	8.50	11.94	11.58	12.92	13.25
Acid insoluble ash	0.00	0.00	0.00	0.00	0.00	1.50
Loss on drying	14.37	11.25	9.65	9.46	8.84	8.50
Water soluble extractive	37.41	40.15	37.36	38.69	40.44	44.18
Alcohol soluble extractive	35.27	32.52	28.07	28.82	28.92	27.42
Assay of triterpegenins	2.71	3.92	5.32	5.84	7.13	7.34

Figure no.2: Status of PHYSICO-CHEMICAL ANALYSIS in different Ritus



1. pH (1% Solution)

The pH measures the acidity or alkalinity of a solution. In this case, the pH values of a 1% solution of *Mandukaparni* extract were recorded during different seasons. **Vasant Ritu (5.36)**: The pH is slightly acidic, indicating a stable solution. **Grishma Ritu (5.63)**: A slight increase in pH suggests the solution becomes less acidic, likely due to environmental conditions during summer, which can alter plant chemistry. **Varsha Ritu (5.65)**: The pH is similar to Grishma, slightly more alkaline. **Sharad Ritu (5.53)**: The pH is slightly lower than

in the previous seasons, possibly due to improved synthesis of acidic bioactive compounds during autumn. **Hemant Ritu (5.64)**: Similar to the summer and monsoon seasons. **Shishir Ritu (5.68)**: The highest pH value indicates the least acidity, which may reflect slower plant metabolism in the cold and dry winter season.

2. Total Ash Content

Ash content refers to the amount of inorganic minerals present in the plant material. It gives an indication of the plant's overall mineral content after combustion. **Vasant Ritu (10.37%)**: The total ash content is moderate, reflecting balanced nutrient absorption during spring. **Grishma Ritu (8.50%)**: A decrease in total ash content suggests that the plant's ability to absorb and store minerals is reduced during the hot and dry summer. **Varsha Ritu (11.94%)**: The highest ash content in monsoon reflects better mineral absorption due to the abundance of moisture. **Sharad Ritu (11.58%)**: Total ash content is still high, though slightly lower than in Varsha, indicating continued efficient nutrient absorption. **Hemant Ritu (12.92%)**: A further increase in total ash content is observed in the pre-winter season, possibly due to the plant's preparation for winter dormancy. **Shishir Ritu (13.25%)**: The highest ash content indicates maximum accumulation of inorganic minerals during winter, when the plant growth is minimal, and the nutrients are concentrated.

3. Acid Insoluble Ash

Acid insoluble ash refers to the portion of ash that is not soluble in acid, and it indicates the amount of silica and other insoluble material, which may come from soil contamination. **Vasant Ritu to Hemant Ritu (0.00%)**: There is no acid-insoluble ash detected in the plant during these seasons, suggesting minimal contamination. **Shishir Ritu (1.50%)**: During the winter season, a small amount of acid-insoluble ash is detected, possibly due to dust or soil contamination from the colder, drier conditions.

4. Loss on Drying

Loss on drying refers to the amount of water and volatile substances lost when the plant material is dried. It gives an indication of the moisture content of the plant. **Vasant Ritu (14.37%)**: The highest loss on drying, indicating that *Mandukaparni* collected in spring contains more moisture. **Grishma Ritu (11.25%)**: The loss on drying decreases in summer as the dry and hot conditions reduce the plant's moisture content. **Varsha Ritu (9.65%)**: In the monsoon, despite high rainfall, the loss on drying decreases further, likely due to internal water retention mechanisms in the plant. **Sharad Ritu (9.46%)**: Autumn exhibits one of the lowest moisture contents, indicating optimal drying and stable bioactive compound synthesis. **Hemant Ritu (8.84%)**: Pre-winter shows further reduction in moisture content, which could promote stability and better preservation. **Shishir Ritu (8.50%)**: The lowest moisture content is observed in the winter, as the cold and dry conditions lead to less water retention in the plant.

5. Water Soluble Extractive

Water-soluble extractive refers to the amount of plant material that dissolves in water, representing hydrophilic compounds like sugars, tannins, and some phytochemicals. **Vasant Ritu (37.41%)**: The water-soluble extractive content is moderate in spring, reflecting the presence of hydrophilic bioactive compounds. **Grishma Ritu (40.15%)**: A slight increase is seen in summer, possibly due to stress-related metabolic changes in the plant that lead to an increase in water-soluble compounds. **Varsha Ritu (37.36%)**: The content decreases slightly in the rainy season, indicating that water-soluble compounds are less concentrated due to high moisture levels. **Sharad Ritu (38.69%)**: In autumn, the water-soluble extractive increases, reflecting enhanced synthesis of compounds like tannins and phenolics due to optimal growing conditions. **Hemant Ritu (40.44%)**: The highest water-soluble extractive content in the pre-winter season reflects good retention of hydrophilic compounds. **Shishir Ritu (44.18%)**: The winter season shows the highest extractive content, likely due to concentration effects as the plant loses moisture and internalizes more compounds.

6. Alcohol Soluble Extractive

Alcohol-soluble extractive represents lipophilic compounds like alkaloids, resins, and essential oils that dissolve in alcohol. **Vasant Ritu (35.27%)**: The highest alcohol-soluble extractive content is observed in spring, indicating active synthesis of lipophilic compounds. **Grishma Ritu (32.52%)**: A decrease is observed in summer, possibly due to the stress from high temperatures reducing the plant's metabolic output of certain compounds. **Varsha Ritu (28.07%)**: The lowest alcohol-soluble extractive content is observed in the monsoon season, likely due to the dilution of compounds caused by excess moisture. **Sharad Ritu (28.82%)**: The extractive content increases slightly in autumn as the plant recovers from monsoon conditions and focuses on generating more secondary metabolites. **Hemant Ritu (28.92%)**: The levels are stable in pre-winter, reflecting good storage and minimal degradation of alcohol-soluble compounds. **Shishir Ritu (27.42%)**: A further decrease in winter, as the plant enters dormancy and produces fewer lipophilic secondary metabolites.

7. Assay of Triterpenoids (Triterpegenins)

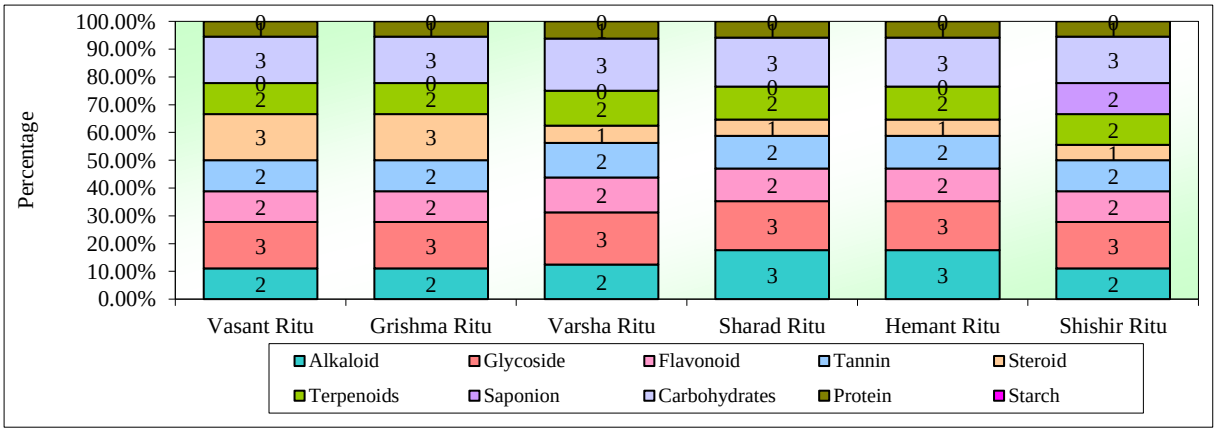
Triterpenoids, such as asiaticoside and madecassoside, are key bioactive compounds in *Mandukaparni*, known for their neuroprotective, wound-healing, and anti-inflammatory effects. **Vasant Ritu (2.71%)**: The lowest concentration of triterpenoids is observed in spring, suggesting that while the plant grows actively, it does not yet accumulate large quantities of these bioactive compounds. **Grishma Ritu (3.92%)**: The concentration increases in summer, despite the challenging conditions, as the plant produces more secondary metabolites to cope with environmental stress. **Varsha Ritu (5.32%)**: The highest concentration of triterpenoids in the rainy season may reflect the plant's need to produce protective compounds to guard against excess moisture and fungal attack. **Sharad Ritu (5.84%)**: In autumn, the concentration peaks, as the favorable climate promotes the synthesis of triterpenoids, making this season the optimal time for harvesting *Mandukaparni*.

Hemant Ritu (7.13%): The concentration of triterpenoids is still high in pre-winter, suggesting that the cold and dry conditions support the retention of these compounds. **Shishir Ritu (7.34%)**: The highest concentration is observed in winter, as the plant concentrates its bioactive compounds in preparation for dormancy. However, growth is minimal, so collection is not ideal.

Table no.2: Status of PHYTO-CHEMICAL IDENTIFICATION in different Ritus

Phyto-Chemical Identification	Vasant Ritu	Grishma Ritu	Varsha Ritu	Sharad Ritu	Hemant Ritu	Shishir Ritu
Alkaloid	2	2	2	3	3	2
Glycoside	3	3	3	3	3	3
Flavonoid	2	2	2	2	2	2
Tannin	2	2	2	2	2	2
Steroid	3	3	1	1	1	1
Terpenoids	2	2	2	2	2	2
Saponion	0	0	0	0	0	2
Carbohydrates	3	3	3	3	3	3
Protein	1	1	1	1	1	1
Starch	0	0	0	0	0	0

Figure no.2 : Status of PHYTO-CHEMICAL IDENTIFICATION in different Rutus



1. Alkaloids

Alkaloids are nitrogen-containing compounds that have a significant role in plant defense and are often responsible for a plant's medicinal properties, such as anti-inflammatory and neuroprotective actions. **Vasant Ritu (2)**: Moderate levels of alkaloids are present during spring, indicating that the plant is synthesizing these compounds in response to new growth. **Grishma Ritu (2)**: Similar moderate levels of alkaloids are seen in summer, likely due to the plant's adaptation to hot and dry conditions. **Varsha Ritu (2)**: Alkaloid concentration remains stable in the monsoon, with no significant increase despite abundant moisture.

Sharad Ritu (3): In autumn, the alkaloid levels increase, reflecting optimal environmental conditions for the production of these bioactive compounds. **Hemant Ritu (3)**: Alkaloids remain high in pre-winter, possibly due to the plant preparing for the colder months. **Shishir Ritu (2)**: The alkaloid levels decrease slightly in winter as the plant slows down in growth and metabolic activity.

2. Glycosides

Glycosides are compounds that play a key role in plant metabolism and medicinal activity, often used for their cardiac, anti-inflammatory, and antimicrobial properties. **All Ritus (3)**: Glycoside levels remain consistently high across all seasons, indicating that *Mandukaparni* maintains stable production of these compounds year-round, regardless of the environmental conditions.

3. Flavonoids

Flavonoids are antioxidants and anti-inflammatory agents that protect plants from oxidative stress and also have a range of health benefits for humans, including supporting cardiovascular health and cognitive function. **All Ritus (2)**: The levels of flavonoids remain consistent and moderate across all seasons, suggesting that *Mandukaparni* synthesizes these compounds steadily, without significant seasonal fluctuation.

4. Tannins

Tannins are astringent compounds known for their antimicrobial, antioxidant, and wound-healing properties. **All Ritus (2)**: Tannins are present at moderate levels throughout all seasons, indicating that *Mandukaparni* consistently produces tannins, irrespective of the seasonal changes.

5. Steroids

Steroids in plants contribute to the formation of important structural components like membranes and are also involved in the plant's response to environmental stress. **Vasant and Grishma Ritu (3)**: High levels of steroids are observed in the spring and summer, possibly due to the plant's need to protect itself from environmental stress like heat. **Varsha, Sharad, Hemant, and Shishir Ritu (1)**: Steroid levels drop significantly during the rainy, autumn, pre-winter, and winter seasons. The reduced stress from heat and moisture likely causes the plant to produce fewer steroids during these periods.

6. Terpenoids

Terpenoids are essential for plant survival and play a role in defense against herbivores and pathogens. They are also key medicinal compounds in *Mandukaparni* for their anti-inflammatory and neuroprotective properties. **All Ritus (2)**: Terpenoid levels remain moderate across all seasons, indicating consistent synthesis of these important bioactive compounds throughout the year.

7. Saponins

Saponins have soap-like properties and are known for their role in reducing cholesterol, enhancing immune response, and acting as antimicrobial agents. **Vasant to Hemant Ritu (0)**: No saponins are detected during these seasons, suggesting that *Mandukaparni* does not focus on producing these compounds during most of the year. **Shishir Ritu (2)**: Saponins are present only in winter, which might indicate a defensive mechanism activated by the plant in response to the harsh, cold conditions of the season.

8. Carbohydrates

Carbohydrates are essential energy stores in plants and are critical for supporting plant growth and metabolism. **All Ritus (3)**: High levels of carbohydrates are consistently detected in all seasons, reflecting the plant's steady energy production to sustain growth and metabolism year-round.

9. Proteins

Proteins are essential for plant growth and the formation of enzymes, which are necessary for biochemical reactions. **All Ritus (1)**: The protein content remains low throughout all seasons, which indicates that *Mandukaparni* produces minimal amounts of protein, as it is not a major protein-synthesizing plant.

10. Starch

Starch is a storage form of carbohydrates in plants, providing energy during periods of dormancy or growth spurts. **All Ritus (0)**: No starch is detected in *Mandukaparni* across any season, indicating that the plant does not store energy in this form, and may instead rely on soluble sugars or other forms of carbohydrates for energy storage.

Table no.3 : Comparison of six groups with HPTLC Chromatogram @ 254 nm scores by Kruskal Wallis ANOVA

Groups	Mean	SD	Median	IQR	Mean Rank	H-value	p-value
Vasant Ritu	0.6083	0.3090	0.7000	0.1500	19.75	0.1040	1.0000
Grishma Ritu	0.5967	0.3052	0.6650	0.1500	18.25		
Varsha Ritu	0.5967	0.3052	0.6650	0.1500	18.25		
Sharad Ritu	0.5967	0.3052	0.6650	0.1500	18.25		
Hemant Ritu	0.5967	0.3052	0.6650	0.1500	18.25		
Shishir Ritu	0.5967	0.3052	0.6650	0.1500	18.25		

The data provided compares the results of HPTLC (High-Performance Thin Layer Chromatography) chromatogram scores at 254 nm for *Mandukaparni* collected during six different *Ritus* (seasons), using the **Kruskal-Wallis ANOVA** test.

Results:

Mean Values: The mean HPTLC scores are very close across all seasons. *Vasant Ritu* has the highest mean (0.6083), while the other seasons (*Grishma*, *Varsha*, *Sharad*, *Hemant*, *Shishir*) all have the same mean value of 0.5967. **Standard Deviation (SD)**: The standard deviation,

which measures how much the individual scores deviate from the mean, is slightly higher in *Vasant Ritu* (0.3090) compared to the other seasons, which all have an SD of 0.3052. This indicates that there is slightly more variation in the scores during *Vasant Ritu*. **Median**: The median, which is the middle value in the data set, is higher in *Vasant Ritu* (0.7000) compared to the other seasons (0.6650). This suggests that, in *Vasant Ritu*, the scores tend to be a bit higher overall. **Interquartile Range (IQR)**: The IQR, which measures the spread of the middle 50% of the data, is consistent across all seasons at 0.1500. This indicates that the range of central values is similar regardless of the season. **Mean Rank**: The mean rank, used in the Kruskal-Wallis ANOVA test, shows a slight difference between *Vasant Ritu* (19.75) and the other seasons (18.25). The higher mean rank for *Vasant Ritu* suggests that its HPTLC scores are generally higher than those in other seasons.

Interpretation of data:

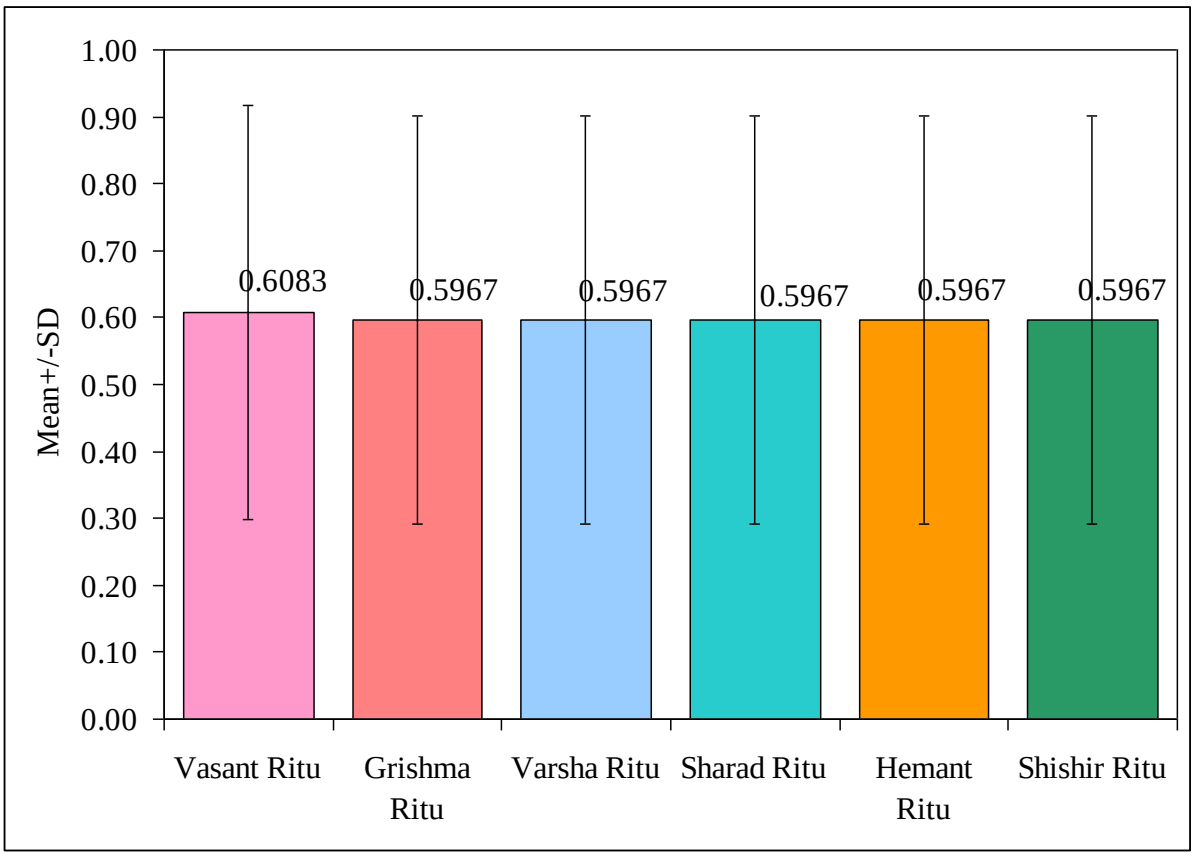
Mean and Median: The mean and median values are almost identical across the seasons, indicating that there is little variation in the HPTLC scores of *Mandukaparni* collected during different *Ritus*. **IQR**: The interquartile range (0.1500) remains consistent across all groups, suggesting a similar spread in the middle 50% of the data for all seasons. **Mean Rank**: The *Vasant Ritu* has a slightly higher mean rank (19.75) compared to the other seasons (18.25), indicating that the scores during *Vasant* were marginally higher, but this difference is not substantial. **p-value (1.0000)**: The p-value is greater than the commonly used significance level of 0.05, meaning there is **no statistically significant difference** between the groups. In simpler terms, the HPTLC chromatogram scores at 254 nm do not vary significantly across the six different *Ritus* for *Mandukaparni*.

Table no.3 : Pair wise comparison of six groups with HPTLC Chromatogram @ 254 nm scores by Mann-Whitney U test

Groups	U-value	Z-value	P-value
Vasant Ritu vs Grishma Ritu	16.50	0.1601	0.8728
Vasant Ritu vs Varsha Ritu	16.50	0.1601	0.8728
Vasant Ritu vs Sharad Ritu	16.50	0.1601	0.8728
Vasant Ritu vs Hemant Ritu	16.50	0.1601	0.8728
Vasant Ritu vs Shishir Ritu	16.50	0.1601	0.8728
Grishma Ritu vs Varsha Ritu	18.00	-	1.0000
Grishma Ritu vs Sharad Ritu	18.00	-	1.0000
Grishma Ritu vs Hemant Ritu	18.00	-	1.0000
Grishma Ritu vs Shishir Ritu	18.00	-	1.0000
Varsha Ritu vs Sharad Ritu	18.00	-	1.0000
Varsha Ritu vs Hemant Ritu	18.00	-	1.0000
Varsha Ritu vs Shishir Ritu	18.00	-	1.0000

Sharad Ritu vs Hemant Ritu	18.00	-	1.0000
Sharad Ritu vs Shishir Ritu	18.00	-	1.0000
Hemant Ritu vs Shishir Ritu	18.00	-	1.0000

Figure no.3 : Comparison of six groups with HPTLC Chromatogram @ 254 nm scores



Pairwise Comparison using Mann-Whitney U Test (HPTLC Chromatogram @ 254 nm)

The Mann-Whitney U test was used to compare the HPTLC chromatogram scores at 254 nm for *Mandukaparni* collected during six different *Ritus* (seasons). Below is an interpretation of the table and results, including the U-value, Z-value, and p-value for each pairwise comparison.

Pairwise Comparisons:

1. **Vasant Ritu vs Grishma Ritu**: **U-value**: 16.50, **Z-value**: 0.1601, **p-value**: 0.8728- There is no statistically significant difference between *Vasant Ritu* and *Grishma Ritu* ($p > 0.05$), suggesting that the HPTLC scores for *Mandukaparni* collected during these two seasons are similar.
2. **Vasant Ritu vs Varsha Ritu**: **U-value**: 16.50, **Z-value**: 0.1601, **p-value**: 0.8728- Similarly, no significant difference is observed between *Vasant Ritu* and *Varsha Ritu* ($p > 0.05$), indicating consistent HPTLC scores across these two seasons.

3. **Vasant Ritu vs Sharad Ritu**: **U-value**: 16.50, **Z-value**: 0.1601, **p-value**: 0.8728-There is no significant difference between *Vasant* and *Sharad Ritu*, meaning the chemical composition at 254 nm is similar in these two seasons.
4. **Vasant Ritu vs Hemant Ritu**: **U-value**: 16.50, **Z-value**: 0.1601, **p-value**: 0.8728-No significant difference is found between *Vasant* and *Hemant Ritu*, suggesting consistent phytochemical profiles between these seasons.
5. **Vasant Ritu vs Shishir Ritu**: **U-value**: 16.50, **Z-value**: 0.1601, **p-value**: 0.8728-Again, no significant difference exists between *Vasant* and *Shishir Ritu*, indicating that the seasonal variation does not have a notable impact on the HPTLC scores between these two seasons.
6. **Grishma Ritu vs Varsha Ritu**: **U-value**: 18.00, **p-value**: 1.0000-The U-value of 18.00 and a p-value of 1.0000 suggest that there is **no difference** between *Grishma Ritu* and *Varsha Ritu*, showing that the phytochemical composition is identical.
7. **Grishma Ritu vs Sharad Ritu**: **U-value**: 18.00, **p-value**: 1.0000-No significant difference between *Grishma* and *Sharad Ritu*, indicating similar phytochemical content during these seasons.
8. **Grishma Ritu vs Hemant Ritu**: **U-value**: 18.00, **p-value**: 1.0000-No difference is observed between *Grishma* and *Hemant Ritu* as indicated by the identical U-value and p-value.
9. **Grishma Ritu vs Shishir Ritu**: **U-value**: 18.00, **p-value**: 1.0000-No significant difference between *Grishma* and *Shishir Ritu*.
10. **Varsha Ritu vs Sharad Ritu**: **U-value**: 18.00, **p-value**: 1.0000-No significant difference is observed between *Varsha* and *Sharad Ritu*.
11. **Varsha Ritu vs Hemant Ritu**: **U-value**: 18.00, **p-value**: 1.0000-The U-value and p-value indicate no difference between *Varsha* and *Hemant Ritu*.
12. **Varsha Ritu vs Shishir Ritu**: **U-value**: 18.00, **p-value**: 1.0000-No significant difference is found between *Varsha* and *Shishir Ritu*.
13. **Sharad Ritu vs Hemant Ritu**: **U-value**: 18.00, **p-value**: 1.0000-No significant difference between *Sharad* and *Hemant Ritu*.
14. **Sharad Ritu vs Shishir Ritu**: **U-value**: 18.00, **p-value**: 1.0000-No difference is observed between *Sharad* and *Shishir Ritu*.
15. **Hemant Ritu vs Shishir Ritu**: **U-value**: 18.00, **p-value**: 1.0000-No significant difference between *Hemant* and *Shishir Ritu*.

Table no.5: Comparison of six groups with HPTLC Chromatogram @ 366 nm scores by Kruskal Wallis ANOVA

Groups	Mean	SD	Median	IQR	Mean Rank	H-value	p-value
Vasant Ritu	0.5329	0.3687	0.7000	0.7700	23.07	0.7550	0.9800
Grishma Ritu	0.6229	0.2834	0.7000	0.1200	24.07		

Varsha Ritu	0.5057	0.3567	0.6500	0.7700	19.71		
Sharad Ritu	0.5057	0.3567	0.6500	0.7700	19.71		
Hemant Ritu	0.5157	0.3624	0.6800	0.7700	21.43		
Shishir Ritu	0.4357	0.4107	0.7000	0.7700	21.00		

Mean Values: The **highest mean score** is observed in **Grishma Ritu** (0.6229), indicating a slightly higher HPTLC score in summer. The **lowest mean score** is in **Shishir Ritu** (0.4357), reflecting lower HPTLC scores in the winter season. The other seasons (*Vasant*, *Varsha*, *Sharad*, and *Hemant*) show similar mean scores, all within the 0.50–0.53 range.

Standard Deviation (SD): **Shishir Ritu** has the **highest standard deviation** (0.4107), indicating more variability in the HPTLC scores during winter. **Grishma Ritu** shows the **lowest SD** (0.2834), suggesting more consistent HPTLC scores during summer.

Median: The median value is **0.7000** for most seasons, except for **Varsha** and **Sharad Ritu**, where it is **0.6500**. This shows a slight shift in the distribution of HPTLC scores in these two seasons.

IQR (Interquartile Range): The IQR remains consistent at **0.7700** for all seasons except for **Grishma Ritu**, where it is much smaller (**0.1200**). This suggests that the HPTLC scores during *Grishma Ritu* are tightly clustered around the median, with less variability.

Mean Rank: **Grishma Ritu** has the highest mean rank (24.07), indicating that the HPTLC scores for *Mandukaparni* collected during this season tend to be higher than those in other seasons. **Varsha Ritu** and **Sharad Ritu** have the lowest mean rank (19.71), suggesting lower HPTLC scores compared to other seasons.

H-value and p-value: **H-value**: 0.7550. This value is the Kruskal-Wallis H statistic. **p-value**: 0.9800, which is **greater than 0.05**, indicating that there is **no statistically significant difference** between the groups. The similarity in p-values suggests that the variations in HPTLC chromatogram scores across the different *Ritus* are likely due to random variation rather than meaningful differences.

Table no.6: Pair wise comparison of six groups with HPTLC Chromatogram @ 366 nm scores by Mann-Whitney U test

Groups	U-value	Z-value	P-value
Vasant Ritu vs Grishma Ritu	24.50	-0.0639	0.9491
Vasant Ritu vs Varsha Ritu	20.50	0.4472	0.6547
Vasant Ritu vs Sharad Ritu	20.50	0.4472	0.6547
Vasant Ritu vs Hemant Ritu	23.00	0.1278	0.8983
Vasant Ritu vs Shishir Ritu	23.00	0.1278	0.8983
Grishma Ritu vs Varsha Ritu	18.00	0.7667	0.4433
Grishma Ritu vs Sharad Ritu	18.00	0.7667	0.4433
Grishma Ritu vs Hemant Ritu	21.50	0.3194	0.7494
Grishma Ritu vs Shishir Ritu	22.50	0.1917	0.8480

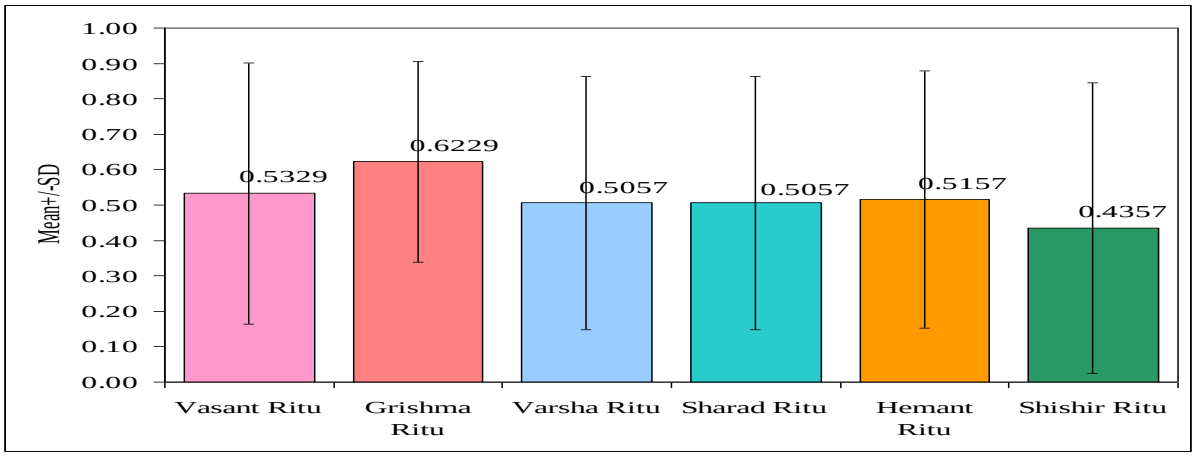
Varsha Ritu vs Sharad Ritu	24.50	0.0000	1.0000
Varsha Ritu vs Hemant Ritu	23.00	-0.1278	0.8983
Varsha Ritu vs Shishir Ritu	24.00	0.0000	1.0000
Sharad Ritu vs Hemant Ritu	23.00	-0.1278	0.8983
Sharad Ritu vs Shishir Ritu	24.00	0.0000	1.0000
Hemant Ritu vs Shishir Ritu	23.50	0.0639	0.9491

Pairwise Comparisons:

1. **Vasant Ritu vs Grishma Ritu**: **U-value**: 24.50, **Z-value**: -0.0639, **p-value**: 0.9491- There is **no statistically significant difference** between *Vasant* and *Grishma Ritu* (p > 0.05). This suggests that the HPTLC chromatogram scores at 366 nm are similar between these two seasons.
2. **Vasant Ritu vs Varsha Ritu**: **U-value**: 20.50, **Z-value**: 0.4472, **p-value**: 0.6547- The p-value indicates **no significant difference** between *Vasant* and *Varsha Ritu*. The HPTLC scores are similar in both seasons.
3. **Vasant Ritu vs Sharad Ritu**: **U-value**: 20.50, **Z-value**: 0.4472, **p-value**: 0.6547-Again, **no significant difference** is observed between *Vasant* and *Sharad Ritu*, indicating comparable chromatogram scores.
4. **Vasant Ritu vs Hemant Ritu**: **U-value**: 23.00, **Z-value**: 0.1278, **p-value**: 0.8983- There is **no statistically significant difference** between *Vasant* and *Hemant Ritu*.
5. **Vasant Ritu vs Shishir Ritu**: **U-value**: 23.00, **Z-value**: 0.1278, **p-value**: 0.8983-No significant difference between *Vasant* and *Shishir Ritu*, showing similar HPTLC scores in these seasons.
6. **Grishma Ritu vs Varsha Ritu**: **U-value**: 18.00, **Z-value**: 0.7667, **p-value**: 0.4433- There is **no significant difference** between *Grishma* and *Varsha Ritu*.
7. **Grishma Ritu vs Sharad Ritu**: **U-value**: 18.00, **Z-value**: 0.7667, **p-value**: 0.4433-No significant difference is observed between *Grishma* and *Sharad Ritu*.
8. **Grishma Ritu vs Hemant Ritu**: **U-value**: 21.50, **Z-value**: 0.3194, **p-value**: 0.7494- The p-value suggests **no significant difference** between *Grishma* and *Hemant Ritu*.
9. **Grishma Ritu vs Shishir Ritu**: **U-value**: 22.50, **Z-value**: 0.1917, **p-value**: 0.8480- There is **no statistically significant difference** between *Grishma* and *Shishir Ritu*.
10. **Varsha Ritu vs Sharad Ritu**: **U-value**: 24.50, **Z-value**: 0.0000, **p-value**: 1.0000, The p-value of 1.0000 indicates **no difference** between *Varsha* and *Sharad Ritu*. The HPTLC scores are exactly the same in these seasons.
11. **Varsha Ritu vs Hemant Ritu**: **U-value**: 23.00, **Z-value**: -0.1278,**p-value**: 0.8983-No significant difference is observed between *Varsha* and *Hemant Ritu*.
12. **Varsha Ritu vs Shishir Ritu**: **U-value**: 24.00, **Z-value**: 0.0000, **p-value**: 1.000-The p-value suggests **no difference** between *Varsha* and *Shishir Ritu*.

13. **Sharad Ritu vs Hemant Ritu**: **U-value**: 23.00, **Z-value**: -0.1278, **p-value**: 0.8983-There is **no significant difference** between *Sharad* and *Hemant Ritu*.
14. **Sharad Ritu vs Shishir Ritu**: **U-value**: 24.00, **Z-value**: 0.0000, **p-value**: 1.0000-No significant difference is found between *Sharad* and *Shishir Ritu*.
15. **Hemant Ritu vs Shishir Ritu**: **U-value**: 23.50, **Z-value**: 0.0639, **p-value**: 0.9491-No statistically significant difference between *Hemant* and *Shishir Ritu*.

Figure no.6: Comparison of six groups with HPTLC Chromatogram @ 366 nm scores



e

Table no.7: eComparison of six groups with HPTLC Chromatogram @ 540 nm scores by Kruskal Wallis ANOVA

Groups	Mean	SD	Median	IQR	Mean Rank	H-value	p-value
Vasant Ritu	0.4790	0.2634	0.5300	0.3600	33.00	2.0380	0.8440
Grishma Ritu	0.4850	0.2721	0.5300	0.3600	33.25		
Varsha Ritu	0.4850	0.2721	0.5300	0.3600	33.25		
Sharad Ritu	0.4230	0.3064	0.4300	0.5200	29.60		
Hemant Ritu	0.4230	0.3064	0.4300	0.5200	29.60		
Shishir Ritu	0.3370	0.2904	0.3500	0.5800	24.30		

Observations:

1. **Mean Values**: The **highest mean score** is observed in **Grishma Ritu** and **Varsha Ritu** (0.4850), indicating slightly higher HPTLC scores in these two seasons. The **lowest mean score** is in **Shishir Ritu** (0.3370), reflecting lower HPTLC scores in the winter season. *Vasant* and *Hemant* Ritu have mean scores similar to *Sharad Ritu*, but all are lower than *Grishma* and *Varsha*.
2. **Standard Deviation (SD)**: **Sharad Ritu** and **Hemant Ritu** have the **highest standard deviation** (0.3064), indicating more variability in the HPTLC scores during these seasons. **Grishma Ritu** and **Varsha Ritu** have the **lowest SD** (0.2721), suggesting more consistent HPTLC scores during these seasons.

3. **Median**: The median value is **0.5300** for *Vasant Ritu*, *Grishma Ritu*, and *Varsha Ritu*, indicating higher HPTLC scores in these seasons. The median value is **0.4300** for *Sharad* and *Hemant Ritu*, and **0.3500** for *Shishir Ritu*, showing a decline in scores during these seasons.
4. **IQR (Interquartile Range)**: **Shishir Ritu** has the **highest IQR** (0.5800), suggesting a wider spread of scores in winter. **Grishma Ritu**, **Varsha Ritu**, and **Vasant Ritu** have a smaller IQR of **0.3600**, indicating more concentrated HPTLC scores during these seasons.
5. **Mean Rank**: **Grishma Ritu** and **Varsha Ritu** have the highest mean rank (**33.25**), indicating that their HPTLC scores tend to be higher compared to the other seasons. **Shishir Ritu** has the lowest mean rank (**24.30**), reflecting lower HPTLC scores in winter.
6. **H-value and p-value**: **H-value**: 2.0380, which is the Kruskal-Wallis H statistic. **p-value**: 0.8440, which is **greater than 0.05**, indicating that there is **no statistically significant difference** between the groups. This suggests that the variation in HPTLC scores across the different seasons is likely due to random variation rather than meaningful differences.

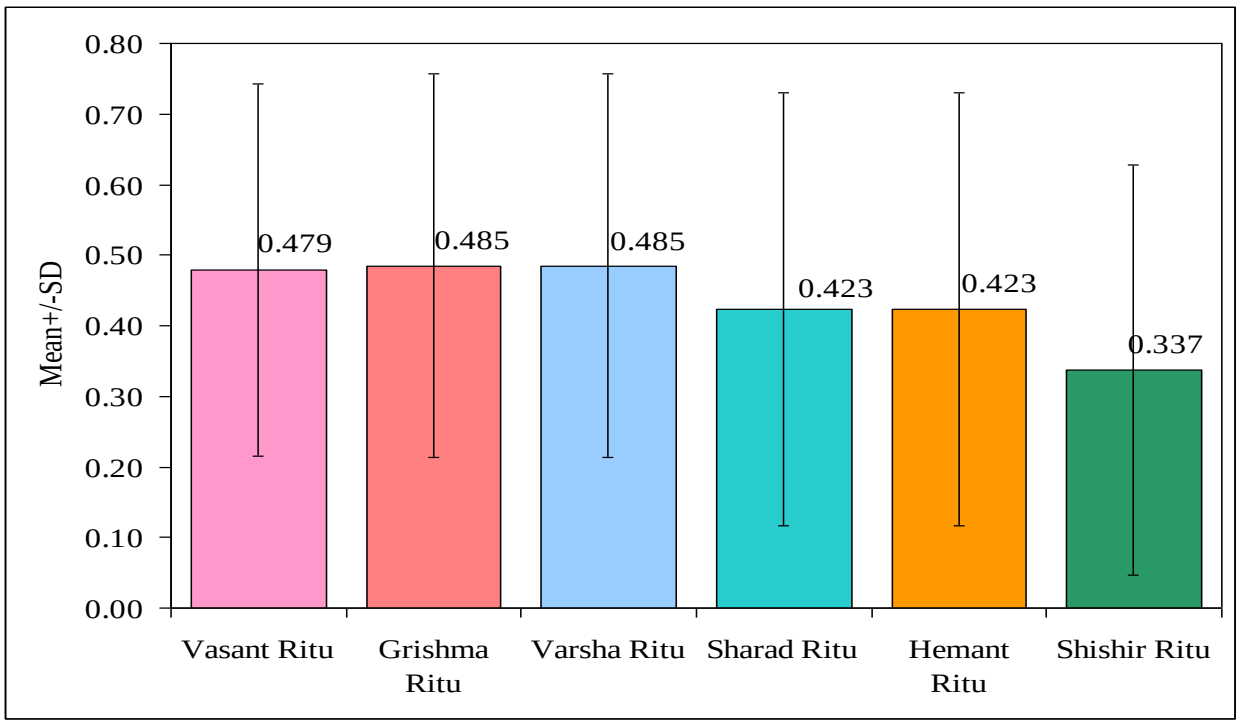
Table no. 7: Pair wise comparison of six groups with HPTLC Chromatogram @ 540 nm scores by Mann-Whitney U test

Groups	U-value	Z-value	P-value
Vasant Ritu vs Grishma Ritu	49.50	0.0000	1.0000
Vasant Ritu vs Varsha Ritu	49.50	0.0000	1.0000
Vasant Ritu vs Sharad Ritu	44.50	0.3780	0.7055
Vasant Ritu vs Hemant Ritu	44.50	0.3780	0.7055
Vasant Ritu vs Shishir Ritu	35.00	1.0961	0.2730
Grishma Ritu vs Varsha Ritu	50.00	-0.0378	0.9699
Grishma Ritu vs Sharad Ritu	44.00	0.4158	0.6776
Grishma Ritu vs Hemant Ritu	44.00	0.4158	0.6776
Grishma Ritu vs Shishir Ritu	35.00	1.0961	0.2730
Varsha Ritu vs Sharad Ritu	44.00	0.4158	0.6776
Varsha Ritu vs Hemant Ritu	44.00	0.4158	0.6776
Varsha Ritu vs Shishir Ritu	35.00	1.0961	0.2730
Sharad Ritu vs Hemant Ritu	50.00	0.0378	0.9699
Sharad Ritu vs Shishir Ritu	41.50	0.6047	0.5454
Hemant Ritu vs Shishir Ritu	41.50	0.6047	0.5454

Pairwise Comparisons:

1. **Vasant Ritu vs Grishma Ritu**: **U-value**: 49.50, **Z-value**: 0.0000,**p-value**: 1.0000 There is **no statistically significant difference** between *Vasant* and *Grishma Ritu*, indicating that their HPTLC chromatogram scores at 540 nm are essentially the same.
2. **Vasant Ritu vs Varsha Ritu**: **U-value**: 49.50, **Z-value**: 0.0000, **p-value**: 1.0000 No significant difference between *Vasant* and *Varsha Ritu*, showing identical HPTLC profiles.
3. **Vasant Ritu vs Sharad Ritu**: **U-value**: 44.50, **Z-value**: 0.3780,**p-value**: 0.7055 There is **no significant difference** between *Vasant* and *Sharad Ritu*, suggesting that their phytochemical profiles are similar.
4. **Vasant Ritu vs Hemant Ritu**: **U-value**: 44.50, **Z-value**: 0.3780 **p-value**: 0.7055 Similar to the previous comparison, **no significant difference** is found between *Vasant* and *Hemant Ritu*.
5. **Vasant Ritu vs Shishir Ritu**: **U-value**: 35.00,**Z-value**: 1.0961 **p-value**: 0.2730 There is **no statistically significant difference** between *Vasant* and *Shishir Ritu*, although the p-value is slightly lower than in other comparisons, indicating some variability but not statistically significant.
6. **Grishma Ritu vs Varsha Ritu**: **U-value**: 50.00, **Z-value**: -0.0378, **p-value**: 0.9699 No significant difference exists between *Grishma* and *Varsha Ritu*.
7. **Grishma Ritu vs Sharad Ritu**: **U-value**: 44.00, **Z-value**: 0.4158, **p-value**: 0.6776 The comparison between *Grishma* and *Sharad Ritu* shows **no significant difference**.
8. **Grishma Ritu vs Hemant Ritu**: **U-value**: 44.00,**Z-value**: 0.4158, **p-value**: 0.6776 No significant difference is observed between *Grishma* and *Hemant Ritu*.
9. **Grishma Ritu vs Shishir Ritu**: **U-value**: 35.00, **Z-value**: 1.0961,**p-value**: 0.2730 There is **no statistically significant difference** between *Grishma* and *Shishir Ritu*.
10. **Varsha Ritu vs Sharad Ritu**: **U-value**: 44.00, **Z-value**: 0.4158, **p-value**: 0.6776 **No significant difference** is found between *Varsha* and *Sharad Ritu*.
11. **Varsha Ritu vs Hemant Ritu**: **U-value**: 44.00, **Z-value**: 0.4158, **p-value**: 0.6776 The comparison between *Varsha* and *Hemant Ritu* shows **no significant difference**.
12. **Varsha Ritu vs Shishir Ritu**: **U-value**: 35.00, **Z-value**: 1.0961, **p-value**: 0.2730 There is **no statistically significant difference** between *Varsha* and *Shishir Ritu*.
13. **Sharad Ritu vs Hemant Ritu**: **U-value**: 50.00, **Z-value**: 0.0378 **p-value**: 0.9699 **No significant difference** is found between *Sharad* and *Hemant Ritu*.
14. **Sharad Ritu vs Shishir Ritu**: **U-value**: 41.50, **Z-value**: 0.6047, **p-value**: 0.5454 The comparison between *Sharad* and *Shishir Ritu* shows **no significant difference**.
15. **Hemant Ritu vs Shishir Ritu**: **U-value**: 41.50, **Z-value**: 0.6047, **p-value**: 0.5454 There is **no statistically significant difference** between *Hemant* and *Shishir Ritu*.

Figure: Comparison of six groups with HPTLC Chromatogram @ 540 nm scores



Discussion

Drug Analysis for *Mandukaparni* Across Ritus

The comprehensive drug analysis of *Mandukaparni* (*Centella asiatica*) across different *Ritus* (seasons) provides significant insights into the seasonal variations of its physico-chemical and phyto-chemical properties, as well as its chromatographic profiles. The data reveals how seasonal factors influence the concentration of various bioactive compounds, providing valuable information for determining optimal harvesting periods to maximize the therapeutic potential of *Mandukaparni*.

Seasonal Variation and Its Impact on Physico-Chemical Properties:

pH (1% Solution): The pH values remained within a slightly acidic range (5.36 to 5.68) across all seasons, indicating that while *Mandukaparni* maintains a generally stable solution, the slight variations in acidity suggest environmental factors such as temperature and moisture influence the plant's chemical balance. The most alkaline pH was observed during *Shishir Ritu* (5.68), reflecting decreased plant metabolic activity during the colder months. **Ash Content**: Total ash content, which reflects the plant's mineral composition, was highest in *Shishir Ritu* (13.25%) and lowest in *Grishma Ritu* (8.50%), indicating a higher accumulation of inorganic minerals during winter. This could be attributed to decreased growth and water retention, allowing for nutrient concentration in the plant. The seasonal variations suggest that harvesting *Mandukaparni* during pre-winter or winter may provide the highest mineral content for therapeutic use. **Loss on Drying**: The highest moisture content was observed in *Vasant Ritu* (14.37%), while the lowest was in *Shishir Ritu* (8.50%).

This indicates that *Mandukaparni* retains more moisture during its active growth phase in spring and loses it during the colder winter months. Lower moisture levels in winter could favor the preservation of bioactive compounds, making it an ideal season for harvesting with reduced risk of microbial contamination. **Water and Alcohol Soluble Extractive**: The water-soluble extractive content was highest in *Shishir Ritu* (44.18%), indicating that hydrophilic compounds are concentrated in winter, likely due to reduced water content in the plant. On the other hand, the alcohol-soluble extractive, representing lipophilic compounds like alkaloids and essential oils, was highest in *Vasant* (35.27%) and lowest in *Shishir* (27.42%). This suggests that spring may be the optimal time for harvesting *Mandukaparni* to maximize lipophilic compounds, while winter may be best for water-soluble compounds. **Triterpenoids (Triterpegenins)**: The concentration of triterpenoids, key bioactive compounds in *Mandukaparni* with neuroprotective and wound-healing properties, showed a clear seasonal trend, with the highest levels observed in *Shishir Ritu* (7.34%) and the lowest in *Vasant* (2.71%). This indicates that winter provides the most optimal conditions for triterpenoid accumulation, likely as part of the plant's defense mechanism in preparation for dormancy. Thus, harvesting during winter maximizes the therapeutic potential of triterpenoids.

Phyto-Chemical Analysis:

Alkaloids: The concentration of alkaloids, which have neuroprotective and anti-inflammatory properties, increased during *Sharad* and *Hemant* (score of 3), suggesting optimal environmental conditions during autumn and pre-winter for alkaloid synthesis. However, these bioactive compounds remained stable across other seasons, highlighting *Mandukaparni*'s resilience and consistent medicinal value year-round. **Glycosides, Carbohydrates, and Terpenoids**: These compounds showed little seasonal variation, with stable levels observed across all seasons. Glycosides and carbohydrates, essential for plant energy and medicinal properties, were consistently high (score of 3). Terpenoids, known for their anti-inflammatory and neuroprotective effects, were stable across seasons (score of 2), ensuring that *Mandukaparni* retains its medicinal value regardless of the time of harvest. **Steroids and Saponins**: Steroid levels were highest in *Vasant* and *Grishma* (score of 3) but decreased significantly in the later seasons, reflecting the plant's response to environmental stress. Saponins were detected only in *Shishir* (score of 2), suggesting that their production may be triggered by the cold, dry conditions of winter, possibly as a defense mechanism. **Proteins and Starch**: Protein content remained low (score of 1) across all seasons, indicating that *Mandukaparni* is not a significant source of protein. Starch was absent in all seasons, suggesting that the plant does not rely on this form of carbohydrate for energy storage, instead using soluble sugars.

HPTLC Chromatographic Analysis:

The HPTLC analysis at 254 nm, 366 nm, and 540 nm wavelengths provided an in-depth look at the seasonal variations in the chemical profile of *Mandukaparni*. **254 nm (UV Analysis)**: The HPTLC scores across all seasons were consistent, with no statistically significant differences (p-value: 1.0000). This suggests that the chemical composition of *Mandukaparni* is stable across *Ritus*, making it suitable for harvesting at any time of the year without significantly affecting the overall phytochemical profile. **366 nm (Fluorescence Analysis)**: Although *Grishma Ritu* exhibited slightly higher mean scores, no significant differences were observed across seasons (p-value: 0.9800). The minimal variation in fluorescence suggests that the production of fluorescent compounds is not strongly influenced by seasonal factors. **540 nm (Visible Spectrum Analysis)**: The chromatogram scores at 540 nm revealed slight variations, with *Grishma* and *Varsha* showing the highest scores, while *Shishir* had the lowest. However, the Kruskal-Wallis ANOVA indicated no statistically significant differences (p-value: 0.8440), reinforcing that seasonal variation does not drastically impact the phytochemical profile of *Mandukaparni*.

CONCLUSION:

In conclusion, the seasonal analysis of *Mandukaparni* reveals that while certain physico-chemical parameters, such as ash content, moisture retention, and triterpenoid concentration, vary significantly across seasons, the overall chemical profile remains relatively stable. The consistent HPTLC scores across different wavelengths further support the finding that the plant's medicinal value is preserved year-round. However, based on the specific bioactive compounds of interest, different seasons may offer optimal harvesting windows. For example, winter (*Shishir Ritu*) appears to be the best season for harvesting *Mandukaparni* to maximize triterpenoid and water-soluble compound content, while spring (*Vasant Ritu*) may be preferable for maximizing lipophilic compounds like alcohol-soluble extractives.

CONFLICT OF INTEREST -NIL

SOURCE OF SUPPORT -NONE

REFERENCES

1. Sharma PV. *Dravyaguna Vijnana* Vol 2. 2nd ed. Varanasi: Chaukhambha Bharati Academy; 2008. p. 275-85.
2. Nadkarni KM. *Indian Materia Medica*. Vol 1. 3rd ed. Bombay: Popular Prakashan; 2002. p. 316-20.
3. Chatterjee A, Pakrashi SC. *The Treatise on Indian Medicinal Plants*. Vol 4. New Delhi: Publications and Information Directorate; 1995. p. 189-93.
4. Gohil KJ, Patel JA, Gajjar AK. Pharmacological review on *Centella asiatica*: A potential herbal cure-all. *Indian J Pharm Sci.* 2010;72(5):546-56.
5. Brinkhaus B, Lindner M, Schuppan D, Hahn EG. Chemical, pharmacological and clinical profile of the East Asian medicinal plant *Centella asiatica*. *Phytomedicine*. 2000;7(5):427-48.

6. Maquart FX, Bellon G, Gillery P, Wegrowski Y, Borel JP. Stimulation of collagen synthesis in fibroblast cultures by a triterpene extracted from *Centella asiatica*. *Eur J Dermatol*. 1999;9(4):289-96.
7. Suguna L, Sivakumar P, Chandrakasan G. Effects of *Centella asiatica* extract on dermal wound healing in rats. *Indian J Exp Biol*. 1996;34(12):1208-11.
8. Singh S, Singh M, Srivastava A, Ahmad N, Misra N, Khare P. A comparative study of the seasonal variation in the bioactive compound content of *Centella asiatica*. *Indian J Tradit Knowl*. 2014;13(4):714-9.
9. Jayathirtha MG, Mishra SH. Immunomodulatory effect of *Centella asiatica* extract and asiaticoside in experimental animals. *Indian J Pharmacol*. 2004;36(6):347-50.
10. Thomas J, Kumar NS, Kumar N. Seasonal variation of total phenolics, flavonoids, and antioxidant activity in *Centella asiatica* grown in two different geographical locations. *J Pharm Phytochem*. 2017;6(4):195-200.
11. Jamil SS, Nizami Q, Salam M. *Centella asiatica* (Linn.) Urban: A review of its ethnomedicinal, chemical, and pharmacological properties. *J Pharm Phytochem*. 2007;45(3):93-103.
12. Chopra RN, Nayar SL, Chopra IC. *Glossary of Indian Medicinal Plants* (Including the Supplement). 2nd ed. New Delhi: Council of Scientific and Industrial Research; 1992. p. 46.
13. Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*. 49th ed. Pune: Nirali Prakashan; 2014. p. 36-9.
14. Wang Z, Luo X, Zhao W, Song J. Seasonal variation in bioactive compounds and antioxidant activity in *Centella asiatica*. *Nat Prod Res*. 2020;34(7):1045-51.
15. Shankar M, Tandon S, Srivastava S. A study on the seasonal variation in the yield of secondary metabolites in *Centella asiatica* collected from different regions of India. *J Med Plants Res*. 2012;6(3):493-8.
16. Li H, Wang Z, Wang Y, Zhao W, Song J. Comparative analysis of antioxidant activities and bioactive compounds in *Centella asiatica* during different growth stages. *J Chromatogr Sci*. 2019;57(6):463-71.
17. Madhavi DL, Deshpande SS, Salunkhe DK. *Food Antioxidants: Technological, Toxicological, and Health Perspectives*. 1st ed. New York: CRC Press; 1996. p. 107-22.
18. Paliwal P, Vyas N, Paliwal S. A review on phytochemistry and pharmacological activities of *Centella asiatica*. *Acta Pol Pharm Drug Res*. 2011;68(3):413-21.