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Correlating traditional harvest practices of roots of medicinal plant *Hemidesmus indicus* with therapeutic biomolecule levels: with reference to traditional inscriptions

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Traditional texts mention some specific guidelines for harvesting of medicinal plants based on the seasons. These ancient practices are grounded in centuries of empirical knowledge and observation, aiming to maximize the plant's medicinal efficacy and potency. In this light, the present study considered an important medicinal herb *Hemidesmus indicus* to quantify an active phytocompound lupeol present in the plant root collected in different seasons and variations in morphoanatomical and phytochemical assay. Flavonoid and phenolic contents were investigated as a phytochemical assay. To estimate the quantity of lupeol a reliable, precise, reproducible and validated HPLC method was conducted. The study revealed that the lupeol content is highest in winter season (0.4029%) while least in rainy season (0.2739%) and there were no significant differences in pharmacognostical characters, but phytochemical contents were noted with significant variations. The total phenolic contents were highest in spring days (8.34%) and total flavonoid contents are highest in summer season (4.16%). The significant outline of the present piece of work based on the lupeol content and concentration of other secondary metabolites assures the relevancy of ancient wisdom in contemporary times through prudent utilisation of natural resources and sustainable growth of mankind. This is because results obtained through the investigations ensure the seasonal preferences as stated in ancient texts. These findings help in bridging traditional knowledge with contemporary understanding, ensuring safe and effective utilization of medicinal plants.

Keywords: Ayurveda, Estimation, *Hemidesmus indicus*, HPLC-PAD quantification, Lupeol, Phytochemicals, Seasonal harvesting

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The harvesting of medicinal plants are described in ancient traditional texts. *Charak Samhita* (Verse. 1.10) advocates specific guidelines for harvesting based on the plant's growth stage, environmental conditions, and lunar cycles[1].

"There, the full-sized taste, semen, and smells of time, which are not affected by time, heat, fire, water, wind, animals, and the effects of smell, colour, taste, and touch, are directly situated in the northern direction; their branches and leaves are acceptable in the spring and summer, the roots in summer or winter, late winter, the leaves in winter, the skin and milk in autumn, the essences in winter, and the flowers and fruits in winter". Farmer should be of auspicious conduct, well-behaved, clean, dressed in white, worship the deity, the *Ashvins*, the cows and the *Brahmins*, and should fast, facing east or north, as described in *Charak Smahita Kalpsthana* Verse 1.10.

The above texts clearly state that, it relies on many natural factors like time, locations, hostilities, natural phenomena and directions for harvesting the medicinal plants for getting the highest therapeutic values of medicinal plants. Hence, the commentaries in old text guide us in maximum utilisation of best therapeutic potential from medicinal plants. At this point we have huge scope to validate these principles with the aid of modern instrumentations.

Modern investigation validates these practices by assessing the plant's chemical composition through scientific method like phytochemical analysis. Thus, right timing for collection of medicinal herbs ensure path towards successful drug development. Collection of roots for medicinal purpose is the one time utilisation of the natural resources. Unlike leaves,

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flowers, and fruits, the roots of medicinal herbs can only be harvested once. Therefore, untimely harvesting of roots of medicinal plant not only leads us to get a less efficacious and less potent drug but also causes underutilisation of natural resources. Possibly, because of this reason, ancient wisdom has advocacy for harvesting of roots as a medicinal resource. Such seasonal variations have been substantiated experimentally earlier by a few researchers[2].

With this reference, the present study considers the roots of an Ayurvedic medicinal herb *Hemidesmus indicus* (L.) R.Br. belongs to Apocynaceae family. *H. indicus* commonly known as *sveta sariva* or *anantamul*, is a prostrate or semi-erect shrub found throughout India from upper Gangetic plains east-wards to Assam, throughout Central, Western and Southern India up to 600 m elevation[3]. This medicinal herb is attributed with several health benefits with properties like, *Guna-Guru, Snigdha, Veerya-Sheeta, Vipaka-Madhura* and *Karma-Kapha, Pitta*, etc. in Ayurvedic texts[4,5]. It is frequently used in preparation of single and compound Ayurvedic formulations[6,7]. Root of *H. indicus* is of great interest to researchers across the globe because of its reported medicinal properties such as, anti-inflammatory, anti-leprotic, anti-oxidant, anti-arthitic, anti-microbial, anti-diarroheal, wound healing, anti-carcinogenic, hypoglycemic, hepatoprotective, antinociceptive activities[8-10]. It is used in traditional medicine in the treatment of a number of ailments such as snakebite, scorpion sting, diabetes, urinary diseases, menorrhagia, oligospermia, anorexia, fever, abdominal colic and pain, dysentery, diarrhoea, cough, rheumatism, pain, inflammation, pyrosis, headache, skin diseases, leprosy, sexually transmitted diseases and cancer[11,12]. It is also used as blood purifier, appetite stimulant, health promoter and body cooling agent.

The plant root is a rich source of several secondary metabolites belonging to the flavonoids, glycosides and polyphenols, terpenoids classes[13,14]. Flavonoids and phenolics are considered to have high therapeutic potentials[15-17]. Their presence as active ingredient in herbal preparations and phyto-pharmaceutical preparations are very well known[18-20]. Among several active phytocompounds in root of this plant, hemidesmin, lupeol, lupeol acetate, hindicusine, β-amyrin are major[21,22]. Among these major phytoconstituents lupeol is probably the most studied compound which bears several biological activities[23-25]. Quantity of lupeol in roots and in different extracts has been determined earlier, but the seasonal variations of lupeol and other phytochemicals in roots remain undone[26,27].

With the above said background, the present piece of work tried to determine the variation in quantity of lupeol by an affordable reliable HPLC method and other secondary metabolites by spectrophotometric methods for validating the ancient claim of seasonal preferences. In addition to that, the present study also tried to investigate the dynamicity in morphoanatomical characters. The objective of the present investigation may help to bridge traditional knowledge with contemporary understanding, ensuring safe and effective utilization of medicinal plants. Investigation through the modern techniques may ensure the relevancies of ancient texts. Such investigations not only validate the scripture but also open a path of sustainable utilisation of the natural resources for medicinal purposes. The most significant point of this work is that the findings not only give us a close insight of the old texts but also emphasise the prudential use of natural resources and sustainable development of mankind in healthcare.

Methodology

Chemicals and solvents

Ethanol (99%) of GR grade used in the experiment was procured from Fischer Pvt. Ltd. All other chemicals, reagents and solvents of HPLC grade were purchased from E. Merck Ltd., Mumbai, India and standard lupeol was procured from Sigma-Aldrich bearing Batch No. 658921.

Collections and authentication of plant materials

The emphasis on the importance of the collection period and time underscores the significance of these factors hence, a special attention was given to detail in collecting and documenting the plant samples is commendable. Ensuring the accuracy of collection time and location is crucial for drawing reliable conclusions from the study. Preserving voucher specimens for future reference is also a good practice in botanical studies, ensuring that the identity of the specimens can be verified later if needed. Matured plant roots were collected naturally from medicinal plant garden, CARI Jhansi, Uttar Pradesh (Latitude: 25028’06.965” N, Longitude: 78033’38.533” E); during May 2021 to April 2022 in all six seasons. Collected plant samples were authenticated by Central Ayurveda Research Institute, Survey of Medicinal Plant Unit (SMPU), Jhansi, Uttar Pradesh and deposited as museum sample at CARI, Kolkata with voucher specimen number CARI/KOL/RT-06 for

Table 1 — Details of plant root collections

Season (Abbreviated)	Summer (SS)	Rainy (RS)	Autmn (AS)	Late Autumn (LA)	Winter (WS)	Spring (SP)
Seasons name in Sanskrit	*Grishma*	*Varsha*	*Sharad*	*Hemant*	*Shishir*	*Vasant*
Seasonal duration	May-Jun	Jul-Aug	Sep-Oct	Nov-Dec	Jan-Feb	Mar-Apr
Month of collection	May	August	October	December	February	April
Plant description	Flowering stage	Vegetative growth	Vegetative growth	Vegetative growth	Vegetativestage	Vegetativestage with flowering
Reference number	CARI/KOL/RT-06A	CARI/KOL/RT-06B	CARI/KOL/RT-06C	CARI/KOL/RT-06D	CARI/KOL/RT-06E	CARI/KOL/RT-06F

future reference. Seasons, period of collections and the reference sample numbers are mentioned in Table 1.

Processing of plant materials

Using proper techniques for cleaning, drying, and processing the plant materials is essential to maintain the integrity of the samples for analytical evaluation. Grinding the dried materials to obtain both coarse and fine powders allows for a comprehensive analysis of the plant constituents. Plant roots were washed properly under running tap water and then washed with ethanol and finally dried up in tray drier at of 37-39°C temperature for 3 h[28]. Dried materials were randomly taken and observed very thoroughly to remove foreign matter if any adhered with the plant materials. For the purpose of analytical assessment, a certain amount of the dry material was processed using a mixer grinder to obtain both coarse and fine powder, which was then passed through for additional evaluation.

Morpho anatomical evaluation of plant samples

The macroscopic and organoleptic characteristics of the root including its size, shape, surface, texture, colour, odour etc. of dried root were observed under stereo zoom microscope (Radical RSMr-3) and measuring the characters with centimetre scale. The characters were compared with the same as mentioned in standard books. The transverse section of dried root sample was done with sharp razor blade. The chosen sections were subjected to ethanol treatment, commencing with a concentration of 30% ethanol and gradually increasing to absolute grade, accompanied by the application of safranin stain. The processed sections were then placed on slides and fixed in Canada balsam. They were viewed using a digital trinocular compound microscope (Olympus CX21i) equipped with a Magcam DC14 camera, using 10X and 40X lenses. Ultimately, at a magnification of 10 times, photomicrographs were captured. The dried powdered root specimen, weighing approximately 2 g, was individually treated with various solutions. These solutions included aqueous saturated chloral hydrate for maceration, 50% glycerine, phloroglucinol in concentrated hydrochloric acid for staining lignified tissues, and 0.02 N iodine reagent for starch grains. The treated samples were subsequently mounted on slides with 50% glycerine, following a standardized protocol. Finally, the samples were examined under a digital microscope (Olympus CX21i trinocular compound microscope) at magnifications of 10X and 40X. The microscope images of various cellular components and inclusions were captured at a magnification of 10X using a Magcam DC14 camera that was connected to the microscope[29-31].

Evaluation of physicochemical properties

Dried roots samples from each of the six seasons were tested to determine the physicochemical properties, like loss on drying at 105°C, total ash values, acid insoluble ash[32].

Determination of gross extractive values

The importance of solvent selection in medicinal plant extraction is a crucial aspect of herbal medicine and pharmaceutical research. Solvents vary in polarity, which affects their ability to dissolve different compounds present in medicinal plants[33,34]. To evaluate the efficiency of solvents for extraction, we tested a variety of non-polar, medium polar, and polar solvents to determine the total extract yield. We choose this wide range of solvents because, plant secondary metabolites have different extractability which depends on the polarity of the solvent employed. Many of the secondary metabolites are efficiently extractable in non-polar media some in medium polar media or in polar media. Seeing this variability, we considered solvents of different polarities. In this study, 10 g of roots from each season underwent Soxhlet extraction using 100 mL of hexane, ethyl acetate, chloroform, methanol, and water[35]. The resulting extracts were filtered using Whatman filter paper (No. 40), and the volume of each

extract was adjusted to 100 mL in a volumetric flask. These extracts were then evaporated under vacuum to obtain dried extracts, and the weight of the extracts was recorded.

Proximate analysis of aqueous extracts of roots

Having highest extractive yield in water, the aqueous extract of the roots were analysed to get the total phenolic content as gallic acid equivalent and total flavonoid content as quercetin equivalent by spectrophotometric analysis. Total phenolic contents were spectroscopically determined by colour reaction using Folin-Ciocalteu reagent[36,37]. The quantity of total flavonoid content was estimated by aluminium chloride reagent[38].

Preparation of standard solution of lupeol

To prepare the stock standard solution, 100 mg of lupeol was dissolved in 100 mL methanol in a volumetric flask to obtain 1000 ppm solutions of the standard. This stock solution was diluted to get variable concentration of 10, 20, 40, 60, 80 ppm of lupeol as working standards. These working standard solutions were used for the estimation of lupeol in roots by HPLC.

Preparation of plant extracts for HPLC quantifications

The measured quantity of 1 g of coarsely powdered root samples of six different seasons was separately extracted with methanol in Soxhlet apparatus for three hours[35]. Final volume of each extract was made up in 100 mL volumetric flask. Extracts were preserved at approximately 4°C temperature for further use in HPLC analysis.

High Performance Liquid Chromatography estimation of lupeol

Chromatographic instrumentation

For estimation of lupeol in the methanolic extracts of roots of *H. indicus*, chromatographic experiments were carried out in Agilent make HPLC 1260 Infinity II series equipped with autosampler (DEAEQ41150), quaternary pump (DEAEX03076) and diode array detector (DEAC614541). Chromatographic separation was performed by using ODS-RP column with dimensions of 150 mm x4.6 mm and 4 µm, with poroshell stationary phase materials.

Chromatographic condition and method development

While doing column chromatographic separation the mobile phase and the flow rate play the most vital role in exact measurement of the analyte of interest. The combination of solvents which would give well resolute peak with appropriate and significantly separated in R_t value is highly desirable. With this view, a number of mobile phases have been tried and optimised as an isocratic elution with water, acetonitrile and methanol (40: 50: 5, v/v) having a flow rate of 1 mL min^{-1} at a system operated pressure of 430-432 psi. The operation was allowed for a total runtime of 10 min and injection volume was kept constant for 10 µL throughout the experiment. Standard solution of lupeol of variable concentrations of 20, 40, 60, 80 and 100 ppm were injected and responses were recorded to get calibration curve.

Validation of chromatographic methods

The validation of newly developed chromatographic method ensures that the method is reliable and suitable for analytical applications. The described method was validated with respect to several key parameters like, determination of absorption maximum, sensitivity, linearity and range, precision, accuracy, specificity, ruggedness were performed according to the established guidelines[39]. Validation parameters ensure the method's reliability and establish the method as a trusted one.

An absorption maximum (λmax) to determine the specific wavelength where the analyte exhibits maximum absorbance is crucial for accurate quantification. For determining the λ_{max}, repeated (*n=6*) responses of the standard lupeol for a specific concentration was scanned across the entire UV-Vis range were recorded. Specificity ensures that the method can accurately measure the analyte in the presence of potentially interfering substances like other components in the matrix. Specificity was assessed by measuring the response of the system while determining the absorption maxima using various concentrations of standard solutions, a blank, and a placebo sample matrix. An overlapping Gaussian curve was employed to confirm the specificity in determining the absorption maxima. Sensitivity is the determination of the limits of detection (LOD) and quantification (LOQ) which provides insight into the method's ability to detect and quantify low concentrations of the analyte with acceptable precision and accuracy. LOD and LOQ were determined by recording the response at different dilution of the standard, where the signal to ratio is 3 and 5, respectively. Establishing a linear relationship between concentration and response over a specified range ensures accurate quantification of analyte concentrations within that range. This is performed from responses at absorption maximum of variable concentration of the standard. Precision is evaluating the repeatability

(intraday), intermediate precision (interday), and method precision provides an understanding of the method's reliability and reproducibility. This was evaluated by measuring the repeatability of sample applications and peak areas of standard solution from six replicate analyses of the same concentration (60 ppm). Method precision, including intraday and interday variations, was assessed using sample solutions at three different concentrations of 20, 40, and 80 µg mL^{-1}. Intermediate precision was also tested by changing the analyst performing the measurements and recovery values were recorded for this purpose. Assessing the method's accuracy through standard addition experiments verifies its ability to provide results close to the true values, even in the presence of potential errors. Accuracy was determined using an external standard addition method at three levels, 80%, 100%, and 120% of the standard solution. This experiment was repeated six times, and the ranges of quantified values were noted for each level to detect both negative and positive errors, thus providing the method's accuracy. Monitoring the stability of the analyte over time ensures that measurements remain consistent and reliable over the desired duration of analysis. The stability of standard scopoletin in methanol was monitored by measuring the area under the curve of a 60 µg mL^{-1} solution at regular 24 h intervals over six consecutive days. Stability was confirmed by calculating the % RSD of peak areas at the absorption maxima. Ruggedness is testing the method's sturdiness against small variations in parameters such as temperature, flow rate, pH, and mobile phase ratio confirms its reliability under different conditions. Robustness, or ruggedness, was tested by calculating the % RSD of peak areas and noting changes in retention time (Rt) with small variations in method parameters. These included column temperature (±5°C), flow rate (±0.15 mL min^{-1}), pH of the buffer solution (±1), and mobile phase ratio adjustments (±1.0%). This thorough validation ensures that the chromatographic method is reliable, specific, sensitive, precise, accurate, stable, and robust, making it suitable for the intended analytical applications.

Results and Discussion

Macroscopic and morpho-anatomical characters

Roots are fragmented, pieces cylindrical, thick, hard, 10-20 cm. long and 2-5 mm in diameter, tortuous, with few rootlets or secondary roots, outer surface dark brown, corky with longitudinal fissures and transverse cracks, easily detachable from the hard central core, centre woody, creamish to yellowish, surrounded by a mealy white cortical layer. Organoleptic study revealed, roots have distinct aromatic odour and bitter and sweetish, to slightly acrid in taste.

Under microscopy the transverse section of root from periphery towards inside shows rectangular, radially flattened cork cells filled with dark brown contents, compressed cork cambium followed by 3-4 cell layers of transparent secondary cortex, similar to cork cells; secondary phloem consists of phloem parenchyma, sieve elements, along with starch grains in parenchyma, scattered lactiferous ducts and occasional prismatic crystals of calcium oxalate; xylem region composed of metaxylem and protoxylem alternating with narrow medullary rays, fibres and tracheids. Central region made up of woody parenchyma with starch grains and other cell content, pith mostly absent. These morpho-anatomical features of root are represented in (Fig. 1).

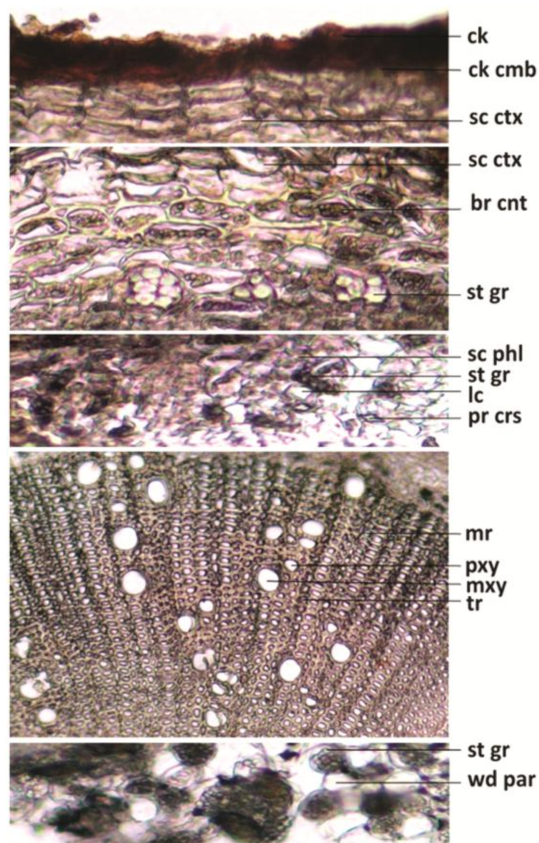

Fig. 1 — Transverse section of root of *Hemidesmus indicus* (L.) R. Br. (100x) [ck: cork; ckcmb: cork cambium; scctx: secondary cortex; brcnt: brown cell content; st gr: starch grains; scphl: secondary phloem; lc: laticiferous canal; prcrs: prismatic crystals of Cal oxalate; mr: medullary rays; pxy: protoxylem; mxy: metaxylem; tr: tracheid]

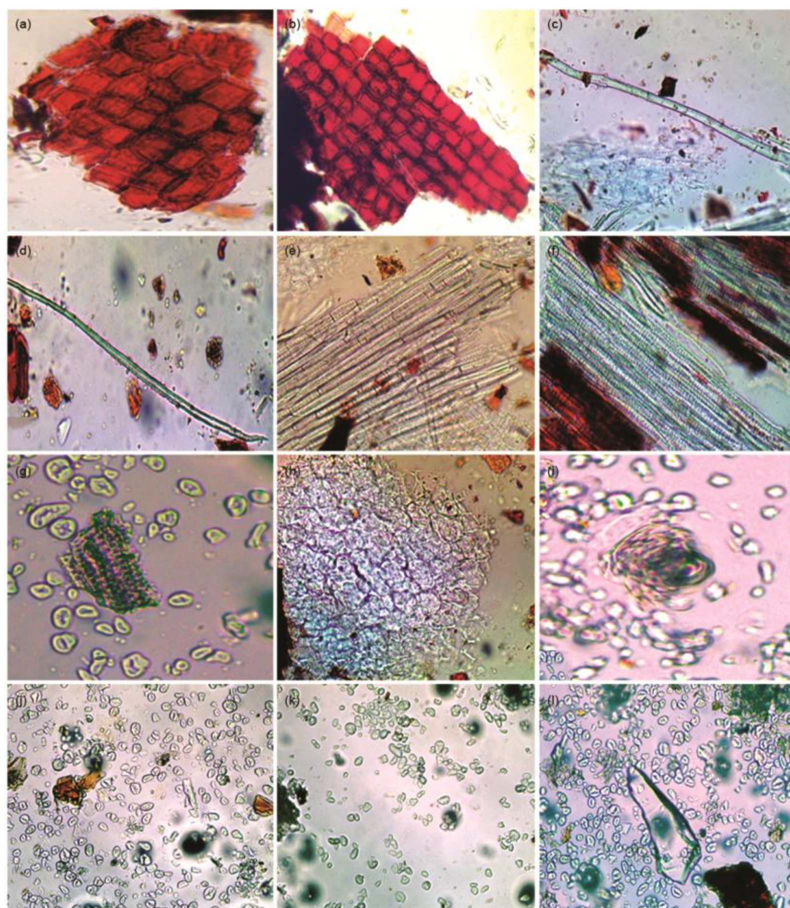

Fig. 2 — Powder microscopy of root of *Hemidesmus indicus* (L.) R. Br. (100x) [a,b: Groups of polygonal cork cells with reddish brown contents; c,d: Aseptatefibres; e, f: Groups of pitted tracheids; g: Fragmented pitted vessels; h: Groups of parenchymatous cells with cell content and few laticiferous canals (lc); i: laticiferous canal; j, k: Profuse starch grains each with prominent hilum; l: Prismatic crystal of Ca-oxalate]

Powder microscopy of root shows the presence of polygonal cork cells in groups with reddish brown contents, groups of parenchymatous cells with starch grain and other cell content, groups of medullary ray cells, profuse starch grains (8 to 10 micrometer) with prominent hilum.; prismatic crystals of Ca-oxalate; groups of xylem parenchyma with medullary rays, few aseptate fibres, few groups of pitted tracheids and fragmented pitted vessels. These cellular characters of root powder are represented in (Fig. 2).

The current study demonstrated that the morphological and internal microscopic characteristics derived from the analysis of transverse sections and powder microscopy was consistent throughout the year, regardless of the season.

Physicochemical analysis, gross extractive yield, proximate analysis and HPLC analysis

Physicochemical data clearly indicate that root samples can meet the pharmacopoeial quality irrespective of collection time, as the values obtained are within the permissible limit laid down by the Pharmacopoeia[40]. The physicochemical parameters and gross extractive yields are represented in Table 2. From the loss on drying information it was noted that the roots of rainy seasons were containing highest moisture trapped within it, which indicates that the preserving of rainy seasons samples should have extra care to avoid any fungal growth during storage due to trapped moisture.

Based on the observations and analysis of the extractive values of phytocompounds using different solvents, we can derive several key points. Water is the most efficient solvent for extracting phytocompounds whereas hexane and ethyl acetate were proven to be weakest. Generally, polarity of solvent has a major role in extracting phytocompounds. Here polar solvents like methanol, ethanol and water are showing such trend. Another, significant observation is that, roots collected

Table 2 — Physicochemical evaluation, gross extractive yield and proximate analysis of roots of different seasons

Sample	LOD (%)	Ash content (%)		Extractive values (%)						Total Phenolic Content (%)	Total Flavonoid Content (%)
		Total	Acid Insoluble	Hexane	Chloroform	Ethyl acetate	Ethanol	Methanol	Water		
SS	2.10	3.25	0.65	2.74	3.40	3.40	16.30	15.70	18.20	4.31	4.16
RS	6.50	1.90	0.35	2.10	3.30	2.25	14.20	15.95	15.40	5.12	3.12
AS	2.80	2.20	0.45	2.16	4.10	2.45	14.90	19.10	18.90	6.16	3.56
LA	2.60	2.45	0.45	2.37	3.50	2.90	15.60	19.65	19.20	5.49	3.98
WS	2.03	2.20	0.50	2.95	2.10	4.35	16.90	21.25	21.30	5.75	3.69
SP	2.50	1.95	0.40	2.54	4.15	3.80	14.05	19.50	18.70	8.34	3.86

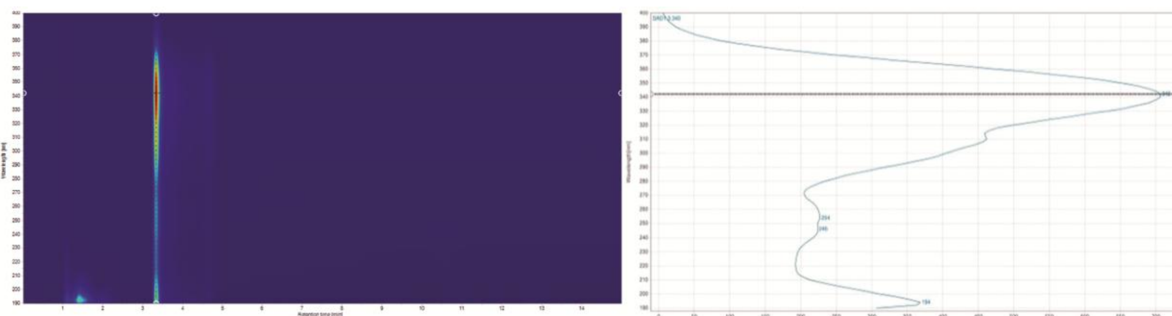

Fig. 3 — Iso-absorbance plot for lupeol at λ_{max} of 342 nm at R_t 3.346

in rainy season yielded least extractive values and that is also true for any of the solvents. This is may be due to the fact that rainy season could affect the concentration and composition of phytocompounds in the roots due to factors like water saturation, nutrient leaching, and microbial activity.

The total phenolic content and total flavonoid content in aqueous extracts of all seasons were determined as gallic acid equivalent quercetin equivalent respectively and results are represented here as percentage in Table 2.

The results of proximate analysis show that the total phenolic content was highest in roots of spring season and the total flavonoids content was found to be highest in summer season sample. Presence of lupeol in roots of different seasons identified by reversed phase column at Rt of 3.346 min with well resolved peak at absorption maxima of 342 nm, represented in (Fig. 3). Lupeol was estimated in roots by plotting the absorbance in calibration curve having linearity range of 20-100 ppm. From the quantification curve it was noted that the roots collected in winter season contained highest quantity of lupeol (0.4049%) and lowest in rainy (0.2739%) among the roots of all six seasons. An image for overlaying peaks of extracts of six season's samples for quantification by HPLC–DAD is represented in (Fig. 4). The quantitative assay of lupeol in roots of different seasons is mentioned in Table 3. On comparison the above facts an interesting finding is that the roots having greater extractive values are not necessarily to have higher content of a particular class of phytocompounds. The trend of extractive yield, phenolic contents, flavonoids contents and quantity of lupeol considering the all seasons are represented in (Fig. 5).

From the above data it has been observed that, roots collected in summer are best when we consider flavonoids, whereas, roots of spring days that is in late winter are best in terms of phenolic contents. Similarly, roots of winter contain highest amount of lupeol among all the seasons. These observations are somehow in alignment with the ancient literature where collections of roots are preferred in summer, winter or in late winter. The guidance for practicing the seasonal collections are validated by the present dataset obtained and substantiated the fact of seasonal preferences. Hence, the conceptualisation of ancient wisdom for seasonal harvesting is still relevant in present days. Therefore, need of the days is validation of the ancient claim by modern instrumentation and adapting those practices in achieving potent drug development.

A notable observation is that the plants of different seasons did not show any morphological variations but there were notable variations in phytochemicals array that may be due to genetic differences leading to variations in phytochemical profiles. These differences

Table 3 — Concentration of lupeol in extracts of all seasons

Seasons	Regression via area	r	sdv (%)	Concentration[a] (%)
SS				0.3194±0.009
RS				0.2739±0.006
AS	1.5566+60.7398X	1.0000±0.0004	2.9166	0.3162±0.006
LA				0.3724±0.010
WS				0.4049±0.011
SP				0.2946±0.009

[a]Each value represents the mean of triplicate analyses ± SD

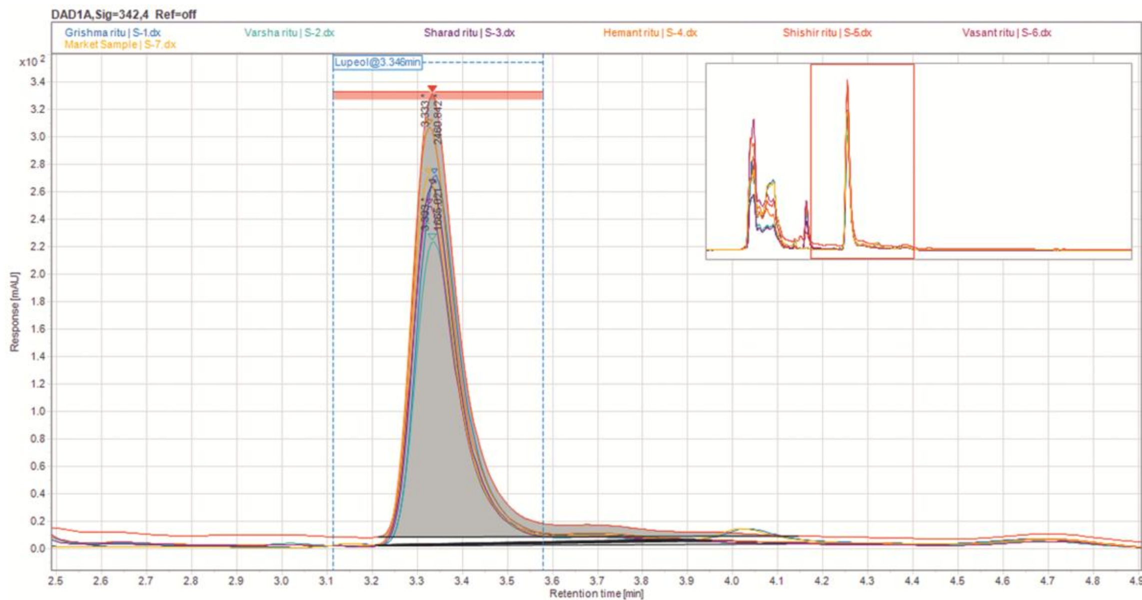

Fig. 4 — Image for overlaying peaks of extracts of six seasons samples for quantification by HPLC

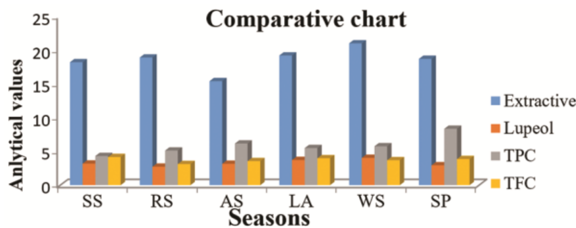

Fig. 5 — Comparative diagram representing extractive yields, lupeol content, TPC and TFC of different seasons

may not affect morphology but influence metabolic pathways. There may be several other factors which govern the variations in chemical profiles, like environmental factors, soil type, humidity, temperature, light intensity etc. Other possible factors working behind the phytochemicals variability may be, ecotypic variation, chemotype, epigenetics, developmental stages etc.

Validation of chromatographic method

The validation of the method confirms its suitability and efficiency in determining the assay of lupeol in roots. The method was found to be repeatable, precise, and accurate. The wavelength for maximum absorption for lupeol was determined to be 204 nm with a variation of ±1 nm. The sensitivity of the method for detection and quantification were 10 ppm and 20 ppm, respectively. The method showed a linear relation of concentration-dependent absorption between 20 and 100 µg mL^{-1} (Fig. 6a & Fig. 6b). The regression analysis data for the sensitivity are represented in Table 4. The precision, recovery rate, and stability of lupeol in solution were also found to be satisfactory. The method's accuracy in terms of recovery or external addition method was in the range of 97 to 103% considering both the positive and negative errors. The method was found to be very stable and robust because the fluctuations in results, in terms of R_t and area under the curve were noted within 1.81% from the actual values. Method precision in terms of relative standard deviation and accuracy data in terms of recovery percentage and data for robustness study are presented in Table 4.

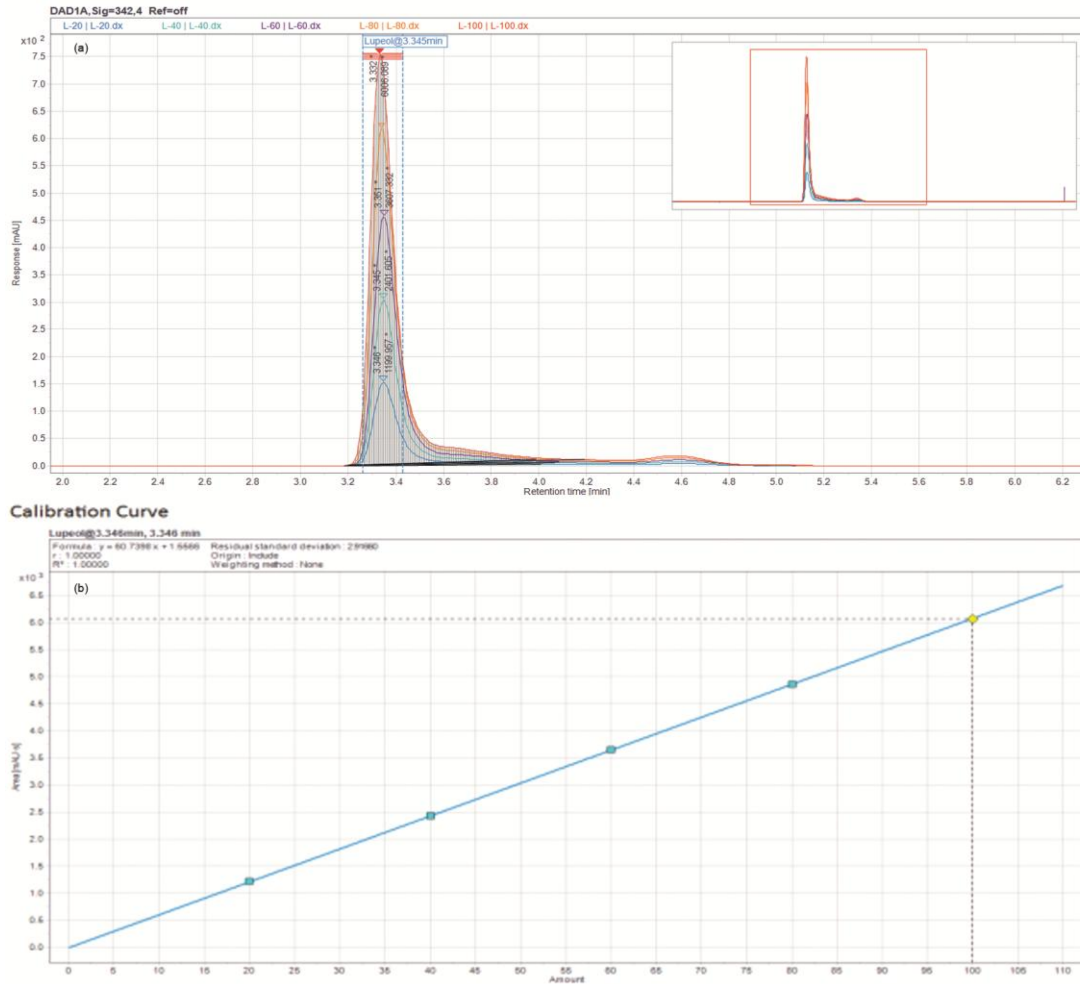

Fig. 6 — (a) Image for overlaying peaks of standards for specificity study in HPLC method and (b) Absorbance-based linearity curve for scopoletin in HPLC–DAD

Table 4 — Results of regression analysis and method validation parameters of HPLC-DAD analysis

Validation parameters	**HPLC**
Absorption maxima (λ_{max})	342 nm
Calibration range	20–100 ppm
R_t	3.346 min
Slope±SD	60.73±0.63
Intercept±SD	1.55±0.87
Regression coefficient (r^2) ± SD	1.0000±0.0004
LOD	10 ppm
LOQ	20 ppm
Residual standard deviation	2.91660

Precision	**Recorded as (% RSD)**
Intra-day precision	0.9998
Inter-day precision	0.9997
Repeatability (n=3)	0.9997
Robustness	**Changes in R_t value (RSD for AUC)**
Column temperature (C)	0.13 (1.81%)
pH level (0.5)	0.11 (1.13%)
Change of mobile phase composition (5%)	0.11 (0.47%)
Flow rate (0.15 ml min^{-1})	0.13 (1.63%)
Change of analyst (Intermediate precision)	0.09 (1.81)
Accuracy (by external addition method)	**Percentage of recovery from (AUC)**
Level 1 (80%)	97.42%
Level 2 (100%)	103.27%
Level 3 (120%)	102.36%

The newly developed method for quantifying lupeol in water extracts of roots of *H. indicus* have shown promising results based on the validation data table. The method appears to be suitable for its intended purpose, providing trustworthy and dependable results in terms of quantifying lupeol content.

Conclusions

Traditional knowledge often emphasizes the importance of harvesting medicinal plants at specific times of the year to ensure their potency and efficacy. This practice is rooted in observations of natural cycles and the understanding that environmental conditions can influence the biochemical composition of plants, including their medicinal properties. By adhering to these seasonal and lunar guidelines, traditional healers aim to harvest plants when they are believed to be at their peak potency, thereby maximizing their therapeutic benefits for medicinal use. This approach reflects a deep understanding of the interconnections between plants, seasons, and human health, honed over generations of empirical observation and experience. The main focus of this work is to connect traditional knowledge with modern understanding. This approach not only enhances our appreciation of the natural world but also offers potential solutions to contemporary healthcare challenges in a sustainable, culturally sensitive, and holistic way. The present study delves into the seasonal variations only, whereas, the ancient text also has mentioned the different timelines of collection like full moon, new moon, twilight, dawn etc. In that sense the present study limited in validating the seasonal dynamicity. The concluding statement is that the relevance of ancient wisdom in modern times cannot be overstated, especially in fields like herbal medicine and natural health which offers valuable insights into sustainable healthcare practices and validating the ancient scriptures through modern investigations definitely enhance the enhance the quality of herbal products.

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Conflict of Interest

The authors of the present work declare that there is no conflict of interest associated with this manuscript. All the co-authors have gone through the manuscript and they have given their consent to the corresponding author to handle the manuscript and to publish.

Author Contributions

SD: Formal analysis, Investigation, Methodology, AM: Conceptualisation, Visualisation, KH: Formal analysis, Validation, Methodology, Data Curation, Writing, Reviewing, and editing, SS: Formal analysis, Validation, Methodology, GP: Resources, Literary review, SM: Data Curation, Reviewing and editing, RB, JD & SM: Validation, Data Curation, SS: Literary review, Translation, GB: Project administration, Supervision.

Ethics Approval

Not applicable.

Data Availability

The supporting data associated with these work will be made available on reasonable request to the corresponding author.

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