RESEARCH ARTICLE

Evaluation of the Procurement Time of Kalmegh [Andrographis paniculata (Burm.f.) Nees], through HPLC, Macro- and Microscopic Studies

Venkata N Cheemalapati1, Neelima Sharma2, Anupam K Mangal3, Prabhu Rekha4, Naraynam Srikanth5, Soma N Murthy6

ABSTRACT

Aim: This study was conducted to validate the most suitable collection time for Kalmegh [Andrographis paniculata (Burm.f.) Nees] by comparing the high-performance liquid chromatography (HPLC) estimation of andrographolide in different seasons and pharmacognostical study of aerial parts of the plant. In Ayurvedic texts, procurement time of A. paniculata has not been mentioned; hence, this study was performed to validate the most suitable time of collection of the plant.

Materials and methods: The aerial part of Kalmegh [Andrographis paniculata (Burm.f.) Nees] was collected from the Regional Ayurveda Research Institute (RARI) garden from the same locations in the different seasons described in Ayurveda, i.e., Shishir or winter season (Jan to Feb), Vasant or spring season (Mar to Apr), Grishm or summer season (May to June), Varsho or rainy season (Jul to Aug), Sharad or autumn season (Sept to Oct), and Hemant or early winter season (Nov to Dec). The source of the collected plant materials was authenticated at National Vrkshayurveda Research Institute (NVARI), Jhansi Herbarium acronym code “JHS” (Accession no. 23598). Identification, comparative macroscopic and microscopic along with powder microscopy, of the aerial parts of A. paniculata in each season was carried out, in addition to the extraction of different solvents such as methanol, alcohol, and hydroalcoholic through Soxhlet, and its comparative quantitative analysis of the extracted material in all six seasons through HPLC for the different seasons at Captain Srinivas Murthy Regional Ayurveda Drug Development Institute, Chennai.

Results: Extractive value of Andrographis paniculata (Burm.f.) Nees was found to be maximum, i.e., 1.3787 g (27.57%) of Varsha Ritu sample in methanol extract. The marker compound, andrographolide is high, i.e., 2.0312 to 2.2093 (% w/w) in Varsha ritu, through the HPLC analysis. The drug shows floral parts, i.e., pollen grains, epidermis of petals, fragments of trichome from the anthers in powder microscopy in all seasons through HPLC for the different seasons.

Conclusion: The study finds that the best procurement season for A. paniculata Nees. is Varsha Ritu for better therapeutic results in terms of assay of andrographolide.

Keywords: Andrographis paniculata, Andrographolide, Authentication and pharmacognosy, Kalmegh, Procurement time, Seasonal variation.

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INTRODUCTION

Kalmegh or Andrographis paniculata (Burm.f.) Nees is used in traditional medicines since ancient times to modern era for treating a variety of chronic and acute diseases such as liver diseases, diabetes mellitus, upper respiratory infections, fever, herpes, sore throat, etc. According to Indian Pharmacopoeia, it is used in at least about 26 Ayurvedic formulations as a predominant ingredient.1 Acharya PV Sharma in his Dravyaguna Vigyanam identifies Bhunimba as A. paniculata and assigns a Sanskrit name Kalmegh to it (Vaidya PK Warrier).2 The upper part of the plant is collected during October and November when it is full of flowers and wears maximum number of leaves.3 The plant is bitter, antipyretic, anti-inflammatory, expectorant, rhinothoracic, bitter, antipyretic, and digestive (Indian Medicinal Plants by PK Warrier 2010). Andrographis paniculata (Burm.f.) Nees is extensively used as a hepatostimulant and hepatoprotective agent in the Indian system of medicine.4 Andrographis paniculata (Burm.f.) Nees is also an ingredient in several polyherbal preparations used as hepatoprotectives in India.5

It is cultivated in India, Pakistan, and Indonesia, but it is extensively cultivated in China, Thailand, and Mauritius. In India, it is generally sown during September; and crops of upto 5 to 6 months are harvested in February to March. To achieve the above therapeutic indications, we need to understand the suitable time for drug procurement. Hence, it is necessary to validate the best suitable procurement time scientifically.

The secondary metabolites present in the plant enhanced the medicinal value of the herbal medicines. A number of glycosides of similar carbon structure are isolated from the plant, mainly

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the most bitter compounds; among them are andrographolide, neoandrographolide, and deoxyandrographolide.⁶

Ayurveda suggested the collection of particular part of the plant in a particular season, i.e., leaves and branches should be procured in rainy and spring (early summer) seasons.⁷ Economically important part of Kalmegh is the upper part of the plant possessing leaves and branches.⁸ Selected marker compound in the present study is andrographolide. It was quantified by HPLC in six seasons in the samples collected from the same location. The variations in the morphological and microscopical characters of A. paniculata were recorded in all six seasons. Hence, an attempt is made to select an appropriate season to collect the useful parts of the plant.

Materials and Methods
Collection of Plant Material and Authentication
Plant materials/aerial parts of the plant were procured from the RARI Jhansi Garden and authenticated at the Herbarium of RARI, Jhansi, with accession no. 23598 (acronym code “JHS”). Triplicate plant samples were washed and shade dried. Useful plant parts, i.e., whole plant, for histological studies were preserved in 70% alcohol. This process was repeated for each season.

Drying of Plant Materials
The material was shade dried for about 4 weeks. The dried drug was powdered and the powder was strained by the mesh 22 and stored in airtight container for further analysis. The process was repeated for each season.

Macroscopic and Microscopic Analyses
Macroscopic Study
Macroscopic features were observed and documented and photographs were taken with Nikon DSLR Camera in different seasons (Figs 1 to 6). Morphological characters were studied by floras.⁹ Documentation and comparison of the morphological characters such as vegetative stage, flowering, and fruiting time in different seasons were documented (Table 1).

Morphology
Procured drug material was studied and documented for its morphological features. Morphological and organoleptic characters such as color, shape, and size were documented (Fig. 7).

Microscopic Analysis
Microscopic screening was carried out according to the standard method.¹⁰–¹³ Transverse sections of the root, stem, petiole, and leaf were prepared and stained with safranin and fast green per the procedure. The same procedure was followed for powder microscopy.¹⁴ The microphotographs were taken by bright field microscope with digital camera (Fig. 8) and microscopic characters were recorded (Table 2). Powered drug was studied for its organoleptic characters (Table 2) also. Salient features of powered drug were observed microscopically.

Estimation of andrographolide using HPLC¹⁵–²⁰
Extraction
The dried powdered whole-plant material (5 g) was extracted with 200 mL of methanol by using Soxhlet for 24 hours. The extracts were evaporated to dryness under reduced pressure. The same procedure was followed for ethanol and hydroalcoholic extraction. The obtained residue weights for the above extractions given in Table 3.

Test Solution
The residues obtained from methanol, ethanol, and hydroalcoholic extracts of each six seasons were weighed in triplicate and dissolved in methanol using 10 mL volumetric flask, filtered through 0.22 μ membrane filter, and used for HPLC analysis (Figs 10 to 12).

Standard Solution
Andrographolide of 1.6 mg with 99% purity, which is procured from Natural Remedies Pvt. Ltd., Bengaluru, India, was dissolved in HPLC grade methanol in a 10 mL volumetric flask to make up the volume.

Chromatographic Conditions
- Instruments—Agilent 1200 series with manual sampler
- Column—C₁₈ eclipse, XBD, 4.6 mm × 150 mm
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Detection—VWD Detector at 223 nm
Mobile phase—buffer and acetonitrile (20:80)
Flow rate—1.4 mL/minute
Injection volume—10 μL

Calibration Curve
Andrographolide of 1.6 mg of accurately weighed and added to a 10-mL volumetric flask, dissolved in HPLC grade methanol, and the volume was made up to 10 mL to obtain a concentration of 0.16 mg/mL. This solution was appropriately diluted further to get a concentration of 0.16, 0.08, and 0.04 mg/mL of andrographolide. Each of the standard solution was run through the HPLC and recorded the respective peak areas. Calibration curve was established for the peak area vs the concentration of andrographolide applied (Fig. 13).

Estimation of Andrographolide in the Drug
10 μL of the test solution was injected to the HPLC system. The chromatogram was recorded and determine the peak area of the test solution corresponding to that of andrographolide from the calibration curve, as described above. The amount of andrographolide present in the residues extracted in various solvents such as hydroalcohol, ethanol, and methanol was calculated for each of the test samples obtained from various seasons of A.paniculata, as given in the Table 3.

Results and Discussion
Common Macroscopic and Microscopic Features of the Whole-plant Part using Powder Microscopy (Figs 1 to 6, 8 and 9)
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Macroscopic

The aerial parts consisting of the following are highly bitter in taste:

Stem—greenish with short fracture semiwoody or woody, bearing branches in decussate pattern, upper branches are clearly quadrangular, with swollen nodes and four winged projections, lower basal somewhat spherical and bearing thin, slender, and hard adventitious roots at the nodes.

Leaf—lanceolate, simple, opposite, exstipulate, thin, 3–8 cm in length and 1–2 cm in width, entire or slightly undulated, acute or acuminate, upper surface dark green and shiny and glabrous lower granular, 4–6 pairs of lateral veins; petiole winged and short, 5–8 mm in length.

Flower—small about 1 cm long, bracteate, pedicellate, zygomorphic, two-lipped, white, pink to purple with purple dots, lower lip darker; bracts, calyx, and corolla are pubescent; pedicel very short up to 4 mm long, gland dotted, arranged on about 10 cm long cross armed, terminal to axillary semipaniculate racemes producing flowers on one side of their ultimate branchlets.

Table 1: Differentiating macro- and microscopic features of Kalmegh (Andrographis paniculata) in different seasons (Figs 1 to 6, 8 and 9)

| Ritu       | Macroscopic features                                                                 | Microscopic features                                                                 |
|------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| Shishir    | Fruiting and scanty flowers are present (Fig. 1)                                      | Powder: shows abundant simple, uni- and 2- to 3-celled trichomes; glandular trichomes |
| Vasant     | Only flowering present (Fig. 2)                                                      | Powder: pollen, pollen grains, epidermis of petals, epidermis of the corolla, pollen |
| Grishma    | Both flowering and fruiting present (Fig. 3), vegetative stage, stem, immature tissue | Powder: Same as Vasant ritu                                                        |
| Varsha     | Both flowering and fruiting present (Fig. 5), immature, protoderm, metaxytem are    | Powder: Same as Shishir ritu                                                       |
| Sharad     |                                                                         | Powder: Same as Shishir ritu                                                       |
| Hemant     |                                                                         | Powder: Same as Shishir ritu                                                       |

Table 2: Organoleptic study: Organoleptic characters of powder of the samples of Andrographis paniculata (Burm.f.) Nees

| S no. | Test  | Observation | Inference |
|-------|-------|-------------|-----------|
| 1     | Color | Green       | Herbal    |
| 2     | Odor  | None        | –         |
| 3     | Taste | Extremely    | bitter    |

Table 3: Estimation of andrographolide in the drug

| S no. | Season name | *Andrographolide (w/w) |
|-------|-------------|------------------------|
| 1     | Shishir ritu| 0.8474–1.2985          |
| 2     | Vasant ritu | 1.6738–2.0088          |
| 3     | Grishma ritu| 0.9084–1.3616          |
| 4     | Varsha ritu | 2.0312–2.2093          |
| 5     | Sharad ritu | 1.4241–2.0747          |
| 6     | Hemant ritu | 1.5021–1.6764          |

*Range of results was given from the means of triplicates of optimized three solvents of hydroalcohol, ethanol, and methanol for all seasons

From the table, it was observed that Andrographolide is more abundant in Varsha ritu samples received from National Vrkhayurveda Research Institute, Jhansi

Table 4: Extractive value of Andrographis in various solvents in different seasons

| S. no. | Seasonal name | Residue weight (g) |
|--------|---------------|--------------------|
| 1      | Shishir ritu  | Methanol extract   |
|        |               | Ethanol extract    |
|        |               | Hydroalcoholic extract |
| 1      | S1 (5 g)      | 0.6275             |
| 2      | S2 (5 g)      | 0.4011             |
| 3      | S3 (5 g)      | 0.4335             |
| 4      | S4 (5 g)      | 1.2080             |
| 5      | S5 (5 g)      | 0.7694             |
| 6      | S6 (5 g)      | 1.0757             |
| 1      | Shishir ritu  | 0.9111             |
| 2      | Vasant ritu   | 0.5090             |
| 3      | Grishma ritu  | 1.3787             |
| 4      | Varsha ritu   | 0.9580             |
| 5      | Sharad ritu   | 1.1592             |
| 6      | Hemant ritu   | 0.8807             |

Macroscopic

The aerial parts consisting of the following are highly bitter in taste:

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Figs 8A to J: Transverse section (TS) of matured stem of Andrographis paniculata (Burm.f) Nees: (A) 4×; (B) 10×. glt, glandular trichome; chl, chlorenchyma; col, collenchyma; cys, cystolith; e, epidermis; end, endodermis; ph, phloem; pi, pith; xy, xylem. TS of Andrographis paniculata leaf: (C) 4×; (D) Lamina. ue, upper epidermis; col, collenchyma; chl, chlorenchyma; pal, palisade; me, mesophyll; xy, xylem vessel; ph, phloem; cys, cystolith; pa, parenchyma; le, lower epidermis. TS of Andrographis paniculata (Burm.f) Nees petiole: (E) 4×; (F) 10×. ue, upper epidermis; le, lower epidermis; t, trichome; cys, cystolith; chl, chlorenchyma; vb, vascular bundle; xy, xylem; ph, phloem; pa, parenchyma; col, collenchyma; sg, stach grains. (G and H) Andrographis paniculata (Burm.f) Nees powder microscopy in sharad riti: fragment of spiral and pitted vessels, starch grains, and sclereidal layer of the anther at 400×. (I and J) Simple and glandular trichomes from leaf, stem and flowers. Andrographis paniculata (Burm.f) Nees powder microscopy in sharad riti: simple and glandular trichomes from leaf, stem, and flowers and fragments of tracheids at 400×.
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Varsha Ritu

Fruit—a capsule, narrowing at both ends, slightly flattened to cylindric with a septum at the center, elliptic to linear-oblong, gland dotted when young, glabrous when fully mature, 2 cm long, 3 mm wide, encloses 6–12 seeds attached to the flattened retinacula.

Microscopic

Stem—diagrammatic transverse section (TS) of the stem is quadrangular in outline with winged corners. The sections from young and mature stems differ slightly from each other; in young, the central pith is wider and encircled by discontinuous ring of vascular bundles, unlike the older one with a continuous ring of xylem.

Detailed TS of the mature stem shows a layer of epidermis covered with thin cuticle and few sessile glandular trichomes interrupted at places with bigger sized cells embedded with cystolith, cortex is narrow, the cells lying under the wing and at places in between the wings are collenchymatous, the remaining major cortical cells being chlorenchymatous; endodermis is distinct; phloem is narrow, and it is traversed with few isolated thin-walled fibers and small acicular crystals of calcium oxalate; few layers of cambium lies underneath it; xylem is very wide and consists of few small-sized, isolated, scattered vessels, tracheids, fibers, and parenchyma, the major elements being of fibers. Pith is parenchymatous, occasionally embedded with cystolith and acicular crystals of calcium oxalate and few simple starch grains.

Lamina—diagrammatic TS of the leaf passing through the middle of the midrib region of lamina is broadly irregularly planconvex with lateral elevated corners at the lower side and squarishly elevated on the upper side; collenchymatous tissue being located underneath of its both sides and palisade tissue under the upper epidermis only. Detailed TS shows upper and lower epidermis covered with thin cuticle, cells at places embedded with cystolith and stomata, the latter on lower sides only and bearing simple and glandular trichomes; simple trichomes are 1–3-celled long and majority of them being located toward the margin of leaf; glandular trichomes are with unicellular stalk and multicellular head; a layer of palisade runs under the upper epidermis, the remaining mesophyll tissue consists of 4–5 rows of spongy parenchyma, underneath both the epidermis of midrib lie few layers of collenchymatous tissue; they being more celled in the lateral elevated region; the ground tissue of the midrib is parenchymatous and is embedded with an arc of meristele.

Petiole—diagrammatic TS of the petiole is almost rectangular in outline with four winged projections at the corner, the two upper ones being longer and embedded with vascular strands; under both the epidermis of the midrib lie the collenchymatous tissue and an arc of centrally located meristele in the ground tissue, and one to two very small extra meristele being located at its terminal end.

The detailed section shows epidermis bearing simple and glandular trichomes as mentioned above, followed by 3–5–celled thick collenchymatous tissue, occupying almost the whole area of the lower and terminals of the upper wings; the remaining cells being chlorenchymatous, containing small acicular crystals of calcium oxalate; an arc of well-developed centrally located conjoint collateral meristele of the midrib shows isolated phloem fibers, exhibiting cells of endodermis at places and 1–2 small rudimentary meristele near its terminals.

Powder

It shows abundant simple, uni- and 2–3-celled multicellular short and long trichomes; glandular trichomes with short and long multicellular stalk and multicellular head from leaf, stem, and all parts of the flower; long acicular fibers from the xylem region of stem, epidermal cells of the leaf and petiole in surface view embedded with diacytic stomata and cystolith; parenchymatous cells of the pith of the stem embedded with acicular crystals of calcium oxalate and simple starch grains; fragments of spiral, pitted, and scalariform vessels; polygonal thick-walled cells of the corolla tube, pollen grains and sclereidal layers from the stamen; and fragments of cotyledon and endosperm, fibers, and sclereids from the fruit wall.

This study was carried out to observe any histological change in the samples of whole plant of Kalmegh collected in different seasons from the same habitat. It has been observed that it is a herb that completes its life cycle in 1 year approximately. At the end of summer season, the life cycle is completed, and vegetative parts start to grow in the beginning of rainy season (Varsha Ritu). Therefore, it shows immature part.

Salient Microscopic Features of Identification in Powder Drug Analysis (Figs 8G to J and 9)

Powder Microscopy

The dried leaves, root, and stem of Andrographis paniculata (Burm.f.) Nees were analyzed for powder characteristics. Microscopic examination showed following structures:

- Stomata: Fragments of leaf epidermis with diacytic, stomata
- Cystoliths: Fairly large in upper and lower epidermis. Parenchymatous cells of the pith of stem embedded with acicular crystals of calcium oxalate.
- Crystals: Abundant pyramidal calcium oxalate crystals in stem and root. Parenchymatous cells of the pith of stem embedded with acicular crystals of calcium oxalate.
- Vessels: Fragments of spiral, pitted, and scalariform vessels. Elements with bordered pits and intervessel pitting in alternate position and pointed lignified fibers were observed.
- Fragment of parenchymatous tissues: Here the cortex was also observed.
- Trichomes: It shows abundant simple, uniseriate and 2–3-celled multicellular short and long trichomes; glandular trichomes with short and long multicellular stalk and multicellular shows head from leaf, stem, and all parts of the flower.
- Starch grains: Simple starch grains found embedded in the parenchymatous cells of the pith of the stem.
- Floral parts: Pollen grains and epidermis of the petals, and fragments of trichome from the anthers, fibers, and sclereids from the fruit wall are seen.

Selected chemical constituent:

Extraction

Discussion

Mostly leaves and roots have been traditionally used over centuries for different medicinal purposes in Ayurvedic formulations.\(^{20}\) To increase the efficacy of these formulations, it is very important to procure the useful plant part of this plant in suitable season. This study was carried out to observe any histological change in the samples of whole plant of Kalmegh procured in different seasons from same habitat RARI garden. It has been observed that it is a herb that completes its life cycle in approximately 1 year. In the ending of summer season, the life cycle is completed; and vegetative parts start to grow in the beginning of rainy season (Varsha Ritu). Plant is procured from the same habitat (RARI garden).
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in all six seasons. In Shishir, Sharad, and Hemant Ritu, the plant is found in flowering and fruiting stages, while in Vasant, Grishm, and Varsha Ritu are devoid of floral parts. Therefore, the drug shows floral parts, i.e., pollen grains, epidermis of petals, fragments of trichome from the anthers in powder microscopy in Shishir, Sharad, and Hemant Ritu, while in Vasant, Grishm, and Varsha Ritu are devoid of floral parts.

Selected marker compound andrographolide was estimated in each season by HPLC. Extractive value was found to be maximum, i.e., 1.3787 g (27.57%) of Varsha Ritu sample of methanol extract (Table 4). The HPLC estimation showed that abundant andrographolide can be observed in Varsha Ritu sample, e.g., 2.0312–2.2093 (Table 3).

**Conclusion**

In Varsha Ritu, A. paniculata (Burm.f.) Nees., Kalmegh possesses its bioactive secondary metabolite in the highest concentration compared to other seasons. Hence, it may be concluded that the best suitable procurement time is Varsha Ritu (July–Aug). It validates the principle of collection of leaves and branches in Varsha Ritu as quoted in Charakasamhita, Kalpasthana—1/10. In conclusion, the study finds that the best procurement time for Andrographis
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Andrographis paniculata (Burm.f.) Nees is in the season of Varsha Ritu for the better therapeutic results in terms of andrographolide assay.

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हिंदी सारांश

हाई परफोर्मेंस लिक्विड क्रोमेटोग्राफी, मैक्रो और माइक्रोक्सिकोपिक अध्ययनों के माध्यम से कालमेघ (एंड्रोग्राफिस पेनीक्युलेटा नीस.) के संग्रहण काल का मूल्यांकन।

उद्देश्य: यह अध्ययन पदार्थ के एरियल भागों के फार्मॉकोम्यूडिकल अध्ययन और विभिन्न अड्डाओं में हाई परफोर्मेंस लिक्विड क्रोमेटोग्राफी (एचपीएलसी) के एप्सोड्रोग्राफोलाइड के आकलन के लिए का उपयोग करके, कालमेघ (एंड्रोग्राफिस पेनीक्युलेटा नीस.) के लिए सबसे उपयुक्त बनावट समय को मान्य करने के लिए आयोजित किया गया था। आयुर्वैदिक ग्रंथों में के. पेनीक्युलेटा के संग्रहण समय का उल्लेख नहीं किया गया है; इसलिए यह अध्ययन इस पदार्थ के सबसे उपयुक्त मूल्यांकन समय को मान्य करने के लिए किया गया था।

समारोह और विधियाँ: कालमेघ (ए. पेनीक्युलेटा नीस.) का एरियल भाग क्षेत्रीय आयुर्वेद अनुसंधान संस्थान (आयोजित) उद्योग में वर्षित विभिन्न अड्डाओं में अर्थात शिशिर अड्डा (जनवरी से फरवरी), वसंत अड्डा (मार्च से अप्रैल), शीत अड्डा (मई से जून), वर्षा अड्डा (जुलाई से अगस्त), शरद अड्डा (सितंबर से अक्टूबर) और हेमंत अड्डा (नव. दिसंबर) में समान स्थानों से एकत्र किया गया था। एकत्रित पदार्थ सामग्रियों का सोत राष्ट्रीय वृक्षारोपण अनुसंधान संस्थान (एनवीआरआई), द्वारा हैवियरिम के संचिप्त कोड "जेएचसी" (एक्सेस नंबर 23598) में रखा किया गया था। प्रत्येक अड्डा में के. पेनीक्युलेटा के एरियल भागों की पाउडर माइक्रोक्सिकोपी के साथ तुलनात्मक मैक्रोक्सिकोपी और माइक्रोस्कीमिक पहचान की गई। इसके अतिरिक्त सोक्सलेट और इसके तुलनात्मक मात्रात्मक विश्लेषण के माध्यम से विभिन्न सॉल्वेंट्स जैसे मेथनॉल, अल्कोहोल और हाइड्रोक्लोरोइड को विभिन्न अड्डाओं की एचपीएलसी परीक्षण हेतु सभी छह अड्डाओं में निकाली गई सामग्री को कैंपस श्रीनिवास भूरि क्षेत्रीय आयुर्वेद औषध विकास संस्थान, चेन्नई में भेजा गया।

परिणाम: के. पेनीक्युलेटा का मेथनॉल एक्स्ट्रैक्ट में एक्स्ट्रैक्टिव वैश्विक वर्षा अड्डा के नमूना में अवधिक से 1.3787 ग्राम (27.57%) पाया गया । एचपीएलसी विश्लेषण के माध्यम से वर्षा अड्डा में मार्कर कंपनी, एप्सोड्रोग्राफोलाइड उच्च अर्थात 2.0312 से 2.2093 (w/w) है। ऊष्ठी गुण भागों अर्थात पराग कण, पंखुड़ीयां के एप्सोड्रोग्राफोलाइड, मार्कर, शरद और हेमंत अड्डा नमूनों में पाउडर माइक्रोस्कीमिक दो पंखों के टाइम को दर्शाती है, जबकि कस्त, शीत और वर्षा अड्डा पुष्प भागों में रहित रहती है।

विषय: अध्ययन में पाया गया है कि एप्सोड्रोग्राफोलाइड की परीक्षण के संदर्भ में भेदतर विचित्त्सा परिणामों के लिए के. पेनीक्युलेटा को प्राप्त करने की सबसे अच्छी अड्डा वर्षा अड्डा है।

मेधय शब्द: एंड्रोग्राफिस पेनीक्युलेटा, एप्सोड्रोग्राफोलाइड, प्रमाणीकरण और फार्मॉकोम्यूडिकल, हाई परफोर्मेंस लिक्विड क्रोमेटोग्राफी, कालमेघ, संग्रहण काल, अड्डा परिवर्तन।