Original Article

Pharmacognostic and physicochemical evaluation of homoeopathic drug: *Erigeron canadensis* L.

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Abstract

Background: *Erigeron canadensis* L. is an erect annual herb belonging to the family *Asteraceae*. Aerial parts are used in Homoeopathy for bruises, cough, dysuria, gonorrhea, haemorrhages, haemorrhoids, spermatorrhea, and wounds. **Objective:** The pharmacognostic and physicochemical studies have been carried out to facilitate the use of correct species and lay down standards of raw drug materials. **Materials and Methods:** Pharmacognostic studies of the leaf and stem of authentic samples of *E. canadensis* L. have been carried out. Physicochemical parameters of the raw drug include extractive values, ash value, and formulation; besides weight per mL, total solids, and alcohol content, high-performance thin layer chromatography (HPTLC) and ultraviolet (UV) studies are given. **Results:** Epidermal cells often possess crystals of calcium oxalate. Stomata are anomocytic, anisocytic, and tetracytic types. Trichomes are uniseriate and conical in structure. The mid vein in transection is flat on adaxial and is ribbed toward abaxial, with a secretory cavity beneath the central vascular bundle. Stem in transection is round. The vascular tissue is made of several vascular bundles in a ring. Crystals of calcium oxalate occur in the epidermis, cortex, and pith of stem. In mature stem, secondary xylem is well developed with a reduced phloem. The determined physicochemical data, namely, extractive values, ash values, and preparation of for raw drug and weight per mL, total solids, and alcohol content besides UV and HPTLC profile for finished product are provided. **Conclusions:** The presented morphoanatomical features along with powder microscopic and organoleptic characters and physicochemical data are diagnostic to establish the standards for ensuring quality and purity of the drug.

Keywords: *Erigeron canadensis*, High-performance thin layer chromatography, Homoeopathy, Pharmacognosy, Physicochemical, Secretory cavities

Introduction

Erigeron canadensis L. (Syn. *Conyza canadensis* (L.) Cronq.), popularly known as "Canada fleabane" in English, is a leafy annual herb belonging to the family *Asteraceae*.[1] It is distributed in the European part of the former USSR, the Caucasus Western and Eastern Siberia, Central Asia, Asia Minor, Iran, Japan, China, and North America.[2] In India, it is found growing in Western Himalayas, Punjab, Upper Gangetic plains, Valleys of Kashmir, Shillong, Western Ghats, and Nilgiris.[3] The tincture of fresh aerial parts is used as medicine in Homoeopathy. It is used in the treatment of black eye, bruises, cough, dysuria, gonorrhea, haematocele, haemorrhages, haemorrhoids, spermatorrhea, and wounds.[2] It has been proved in Homoeopathy by W.H. Burt as given in American Homoeopathic Observer, 1966. Wilmot Moore has given it with success in cases of placenta previa.[2] Development and utility of advanced analytical techniques are indispensable in drug research, for the identification of active compounds and characterization of chemical structure, quantification, and laying down standards for scientific quality control. To solve complex natural matrix, it is mandatory to use combination of advanced techniques. Further, it is also helpful to analyze multiple compounds or multiple classes of components. Advanced analytical techniques (high-performance thin layer chromatography [HPTLC], liquid chromatography-mass

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spectrometry, gas chromatography-mass spectrometry, and nuclear magnetic resonance) are needed for better understanding and to ensure the product credibility.

Chemically, the plant is reported to contain erigeron oil which acts like turpentine but is less irritant and stimulating. The oil consists of d-limonene and terpineol, besides solid dihydrochloride, camphene, and germacrene D [Figure 1].[4] Sphingolipids and its derivatives (β-D-glucopyranoside) were isolated from ethyl acetate fraction along with β-sitosterol, stigmasterol, β-sitosterol-3-O-β-D-glucoside, and harmine.[5] About fifty components were identified from the oil, the important being limonene and trans-alpha-bergamotene.[6] Five compounds were isolated from ethanol extract and identified as scutellarin, luteolin-7-O-beta-D-glucuronide, quercetin, quercetin-3-O-β-D-glucopyranoside, and luteolin.[7] Twelve flavonoids were isolated from ethanolic extract of the whole plant.[8]

A review of literature reveals no pharmacognostic standards laid down for this drug, except some review works on trichome diversity[9] and organoleptic distribution of calcium oxalate crystals in *Conyza* spp.[10] In view of the significance and importance of the drug, pharmacognostic and physicochemical standardization studies were carried out to lay down specific standards in homoeopathic perspective.

Materials and Methods

Pharmacognosy

The plant material *E. canadensis* was supplied by the Survey of Medicinal Plants and Collection Unit, Nilgiris, Tamil Nadu. The leaves and stems were fixed in formaldehyde-acetic acid-alcohol, dehydrated through xylene-alcohol series, and embedded in paraffin wax. The sections cut between 8 and 10 μm were stained with crystal violet and basic fuchsin combination as per the Johansen method.[11] Epidermal peels were obtained by gently scraping and peeling with a razor blade. Then, the peels were stained in safranin and mounted in glycerin. The photomicrography was done on BX-53 trinocular microscope attached with a digital Camera.

Physicochemical

The air-dried whole plant was coarsely powdered to 10/44 (sieve size) and subjected to determination of loss on drying at 105°C, total ash, water soluble ash, acid insoluble ash, extractability in different solvents, physicochemical constants, and ultraviolet (UV) aspects of mother tincture, following official methods.[12] Mother tincture was prepared as per the Homœopathic Pharmacopœia of the United States Convention[13] by percolation method.[12] One hundred grams of coarse powder of the drug was suspended in 677 mL of 95% alcohol and 350 mL of purified water for 24 h at room temperature. It was filtered and made up to 1000 mL using the same solvent ratio.[12,13]

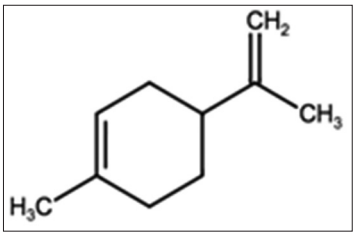

Figure 1: Chemical structure of limonene

High-performance thin layer chromatography analysis

25 mL mother tincture was evaporated on water bath to remove alcohol. The residue was extracted thrice with 25 mL chloroform. Concentrated chloroform extract was used for the HPTLC study. The concentrated chloroform extract was spotted in the form of bands of length 6 mm with 100 μL syringe on precoated silica gel aluminum plate 60 F_{254}, (5 cm × 10 cm with 0.25 mm thickness; Merck, Darmstadt, Germany) using a Linomat-V sample applicator (Camag, Muttenz, Switzerland). A constant application rate of 6 μL/s was employed. The space between the bands was 10 mm. The slit dimension was kept at 5 mm × 0.45 mm and scanning speed at 20 mm/s was employed. The mobile phase of chloroform: methanol (9:1 v/v) and 10 mL was used for chromatography. Linear ascending development was carried out in a 10 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase at room temperature for 20 min. The length of the chromatogram run was 8.5 cm, and subsequent to the development, the thin layer chromatography (TLC) plates were dried in a current of air with the help of hot air dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed (Camag TLC scanner III) at 254 nm and 366 nm by reflectance scanning and operated by *winCATS* software (v 1.4.3 Camag Muttenz, Switzerland).[14-16]

Observations and Results

Pharmacognosy

Macroscopy

The leaves are 2.5–4 cm long, sessile, linear-obovate with rough surface. Stems are 2–5 mm thick, ridged with coarse surface and densely hairy.

Leaf microscopy

In surface, epidermal cells of adaxial are polygonal anisodiametric to linear, sides thin, curved to wavy and sinuate, surface smooth and those of abaxial are similar with deeply sinuate sides, contents scanty with sandy crystals of calcium oxalate occurring frequently. Epidermal cells are more frequent on adaxial, 2430/mm^2 and lesser toward abaxial, 1440/mm^2; stomata present on either sides of anomocytic, anisocytic, and tetracytic types. Stomatal number was 150/mm^2 for adaxial and 162/mm^2 for abaxial; stomatal index was 5.82 for adaxial and 6.2 for abaxial. Trichomes are uniseriate macroform conical hair, occurring common, more on veins [Figure 2a and b]. In transection, leaf midvein is flat on adaxial and ridged prominently on abaxial, 302–432 μm (376) in thickness, covered by few conical hairs. Lamina is undulated

often with elevated trichome bases, 108–194 μm (150) thick. Epidermis is single-layered, cells tabular and barrel-shaped, few oval to spherical, larger as trichome bases, cuticle slightly thick, contents scanty, with acicular crystals of calcium oxalate in few. Epidermal cells lie over the midvein oval to spherical, tabular and barrel-shaped, larger on abaxial. Stomata occur on both sides with guard cells raised above the surface. Mesophyll is dorsiventral, palisade two-layered on adaxial, cells cylindrical, columnar, 22–38 μm long and 8–19 μm (14) wide, filled with chloroplasts. Spongy parenchyma cells are polygonal, spherical, and cylindrical with few intercellular spaces, contents dense with chloroplasts [Figure 2a and b]. Ground tissues at midvein are with two-layered collenchyma on adaxial side and single-layered on abaxial, cells angularly thickened. Parenchyma is 2–3 layered on adaxial and 4–6 layered on abaxial contents in few with chloroplasts. A secretory canal enclosed by an epithelial layer is conspicuous beneath the central vascular bundle, 16–50 μm (29) in diameter [Figure 3a]. Secretory canals also occur attached to veinular bundles of lamina [Figure 3b]. Midvein vascular bundle is arcuate, endarch, collateral, conjoint, 112–134 μm (125) in diameter. The tracheids/vessels arranged in radial rows, and in longitudinal section, they show helical or scalariform thickenings and rarely annular. The phloem is toward abaxial with phloem parenchyma, blast fibers, and sieve elements associated with companion cells. The margin is rounded [Figure 3a].

Stem microscopy

In transection, it is rounded with ridges and furrows covered by uniseriate hairs. Epidermis is single-layered, cells tabular and barrel-shaped, polygonal to spherical, and larger over ridges, walls thick, contents in few with sandy crystals and prisms of calcium oxalate [Figures 3c, 4a and b]. Collenchymatous hypodermis is 3–5 layered in ridges with cells angularly thickened, while in furrows, it is 3–4 layered and chlorenchymatous. Cortex is scanty, cells polygonal to spherical, large, often with small sandy crystals of calcium oxalate [Figures 3c, 4a and b]. Vascular tissue is made of several vascular bundles arranged in a ring, the smallest 130–238 μm (172) in diameter and largest 184–302 μm (226) in diameter. The vascular bundles are capped by 3–8 layered sclerenchymatous sheath. The vascular ring is enclosed by endodermis of larger polygonal to elongated cells. The vascular bundles are endarch, collateral, and separated by cambium [Figures 3c, 4a and b]. The xylem consists of vessels/tracheids in radial rows, few isolated and interspersed with fibers and few xylem parenchyma cells. The secondary walls of vessels/tracheids are made of scalariform and helical thickenings and a few bordered pitted and annular. The central pith parenchyma consists of large polygonal to spherical cells often with acicular and raphidal needle-like crystals. The pith parenchyma disintegrates and appears hollow at center [Figures 3c and 4a].

Mature stem microscopy

In transection, the structure is almost similar, except the outermost epidermis is replaced by 3–4 layered cork at some places. The cork is often with chloroplasts and is followed by a narrow cortex [Figure 4c]. The secondary xylem is well developed and the phloem is reduced. The vascular cambium is present between xylem and phloem. The interfascicular

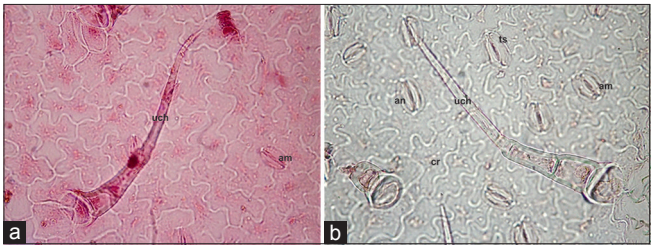

Figure 2: (a) Epidermal cells of leaf adaxial surface X 416, (b) epidermal cells of leaf abaxial surface X 625. Uch: Uniseriate conical hair; am: Anomocytic stomata; ts: Tetracytic stomata; an: Anisocytic stomata; cr: crystals

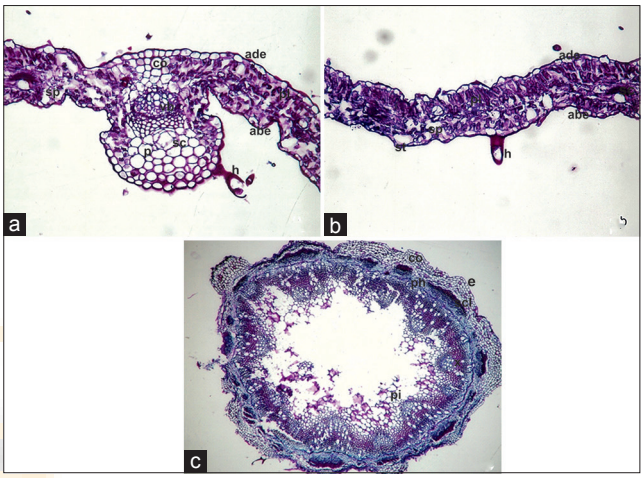

Figure 3: (a) Transection of leaf at midvein X 162, (b) Transection of leaf lamina X 111, (c) Transection of young stem X 45. Ade: adaxial epidermis; co: Collenchyma; pl: Palisade; sp: Spongy tissue; p: Parenchyma; sc: Secretory cavity; vb: Vascular bundle; abe: Abaxial epidermis; h: Hair; st: Stomata; e: Epidermis; scl: Sclerenchyma; ph: Phloem; x: Xylem; pi: Pith

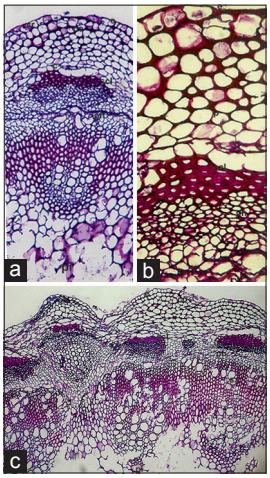

Figure 4: (a) Transection of young stem at the ridge (enlarged) X 146, (b) Transection of stem at furrow region (enlarged) X 432, (c) Transection of mature stem (a portion) X 92. E: Epidermis; ck: Cork; co: Collenchyma; ch: Chorenchyma; p: Parenchyma; c: Cambium; scl: Sclerenchyma; ph: Phloem; x: Xylem; sx: Secondary xylem; pi: Pith

tissue is replaced by continuous growth of xylem. Pith is hollow [Figure 4c].

Powder microscopy

1. Pieces of upper epidermis with anomocytic stomata and epidermal cells with straight to curved sides
2. Uniseriate macroform conical hair long, either whole or fragments, numerous
3. Pieces of lower surface with wavy to sinuate sides and stomata anomocytic and tetracytic types
4. Pieces of vessels with scalariform, bordered pits and helical thickenings
5. Secretory canals with brownish contents
6. Pieces of cortical tissue with attached sclerenchyma.

Organoleptic characters

- Color – Light green
- Taste – Not characteristic
- Odor – Pungent
- Touch – Slightly coarse.

Physicochemical

Qualitative phytochemical tests

Loss on drying reveals the presence of water in the plant powder and also some volatile organic matter. Results of physicochemical studies[17-18] are summarized in Tables 1 and 2.

High-performance thin layer chromatography fingerprinting

The profile of chromatographic separation scanned at 254 nm reveals six spots [Figures 5 and 6], out of which one possess maximum composition with R_f at 0.89, while the densitogram scanned at 366 nm revealed nine spots [Figures 7 and 8], with spot no. 4 and 5 showing maximum composition at R_f 0.33 and 0.43, respectively. It is evident from the data that these are characteristic for the studied drug, which will help in identification and authentication of the mother tincture of the drug. These are considered as valuable standards in pharmacopeia. At 254 nm, six spots appear at R_f 0.17, 0.33, 0.45, 0.61, 0.76, and 0.89 (all are brown) with various concentrations, while at 366 nm, nine spots appears at R_f 0.12 (blue),0.17 (blue), 0.26 (blue), 0.33 (red), 0.43 (blue), 0.56 (red), 0.70 (blue), 0.76 (blue), and 082 (blue). These are again vital fingerprint parameters for the prepared mother tincture and ensure the reliability and reproducibility of the drug.

Discussion

The fresh leaves and stems of *E. canadensis* L. are used as medicine in Homoeopathy for various therapeutic conditions (*Loc. cit*). Epidermal cells in the leaf surface show curved to wavy and sinuate sides often with sandy crystals of calcium oxalate as also reported earlier. Stomata occur on either surface with anomocytic, anisocytic, and tetracytic types. Trichomes have been reported as uniseriate and multicellular in the species,[9] which is currently confirmed as uniseriate macroform conical hair. Epidermal cells in transection are found to possess acicular crystals of calcium oxalate in few cells as also reported earlier.[10] Secretory canal is conspicuous and found beneath the vascular bundle of midvein besides close to secondary and tertiary vein bundles of lamina, which confirms the earlier studies.[19]

Stem in transection is rounded with ridges and furrows. Epidermis is single-layered, often containing prismatic crystals of calcium oxalate as reported.[10] The hypodermis is collenchymatous in the ridges and conspicuously possess angular thickening, while furrows have chlorenchymatous cells. The cortical parenchyma possesses some small sandy and acicular crystals and confirms earlier studies.[10] Vascular bundles are several, arranged in a ring covered by a sclerenchymatous cap. The pith parenchyma has large polygonal to spherical cells, often containing acicular or raphidal crystals of calcium oxalate as also earlier reported.[10] The mature stem also shows nearly similar structure, except the epidermis being replaced by 3–4 layered cork at some places. Secondary xylem is well developed; the interfascicular tissue is replaced by secondary xylem. The presence of acicular and raphidal needles of calcium oxalate in the cortex and pith as reported earlier is presently confirmed.[10]

Physical parameters include color, appearance, odor, pH, sedimentation, moisture content, and ash values. Chemical

Table 1: Standardization of raw drug

Parameters	Quantitative values (%w/w)
Loss on drying at 105°C	8.80-9.20
Total ash	9.20-9.87
Acid insoluble ash	1.62-1.76
Water soluble ash	2.50-3.00
Alcohol soluble extractive	8.50-9.50
Water soluble extractive	18.62-19.80
Extractive values in	
Toluene	2.20-2.50
Chloroform	3.01-3.95
Methanol	10.40-11.20

Table 2: Standardization of *mother tincture*

Parameters	Observations
Organoleptic profile	
Appearance	Clear, nonviscous, transparent and foamy on shaking
Color	Yellowish green
Odor	Fruity and aromatic
Sediments	Absent
Weight/mL	0.87-0.89 g
Total solids	1.31-1.37 %w/v
Alcohol content	63-64 %v/v
pH	5.0-6.0
Ultraviolet absorbance (λ max)	233.4 and 278.6 nm

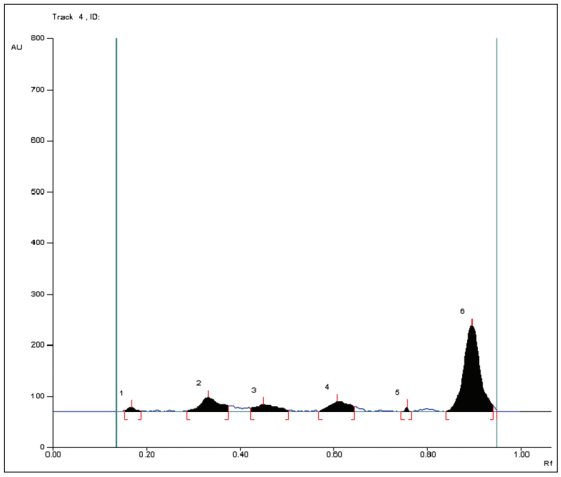

Figure 5: High-performance thin layer chromatography densitogram of *Erigeron canadensis* scanned at 254 nm using chloroform: methanol (9:1 v/v)

Figure 6: High-performance thin layer chromatography video image of *Erigeron canadensis* at 254 nm in chloroform: methanol (9:1 v/v)

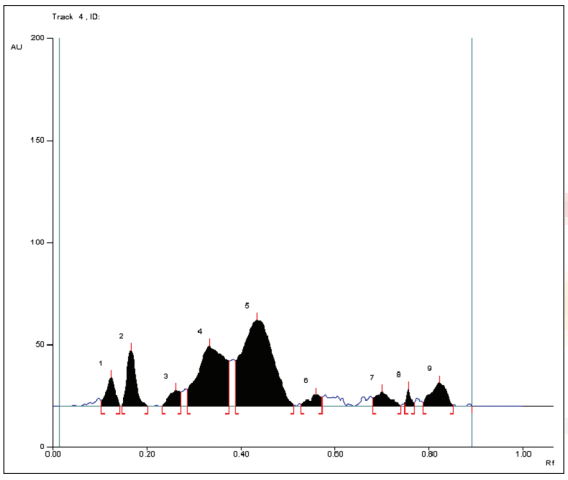

Figure 7: High-performance thin layer chromatography densitogram of *Erigeron canadensis* scanned at 366 nm using chloroform: methanol (9:1 v/v)

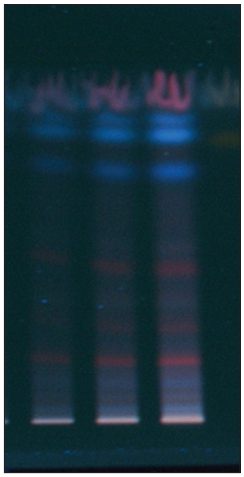

Figure 8: High-performance thin layer chromatography video image of *Erigeron canadensis* at 366 nm in chloroform: methanol (9:1 v/v)

parameters include limit tests, extractive values, and chemical assay; these standards and R_f values are worked out for the first time, form an invaluable data, will serve as a good standardization tool for *E. canadensis* L.

Conclusion

The macroscopical, microscopical, and organoleptic characters along with the anatomical and methodology used for the studies are diagnostic and establish the standards.

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Conflict of interest

None declared.

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Pharmakognostische und physikalisch-chemische Evaluation des homöopathischen Arzneimittels: Erigeron canadensis L

Auszug

Hintergrund: *Erigeron canadensis* Lin. ist ein aufrechtes einjähriges Kraut aus der Familie der Asteraceae. Oberirdische Teile werden in der Homöopathie zur Behandlung von Prellungen, Husten, Dysurie, Gonorrhoe, Hämorrhagien, Hämorrhoiden, Spermatorrhoe und Wunden eingesetzt.

Ziel: Pharmakognostische und physikalisch-chemische Untersuchungen wurden durchgeführt, um die Verwendung der richtigen Spezies zu erleichtern und Standards für Rohmaterialien festzulegen.

Materialien und Methoden: Pharmakognostische Untersuchungen von Blatt und Stengel von authentischen Proben von Erigeron canadensis wurden durchgeführt. Für die Urtinktur werden physikalisch-chemische Parameter des Rohstoffs, Extraktionswerte, Aschegehalt und des weiteren Gewicht pro ml, Gesamtfeststoffe, Alkoholgehalt, HPTLC und UV-Untersuchungen angegeben.

Ergebnisse: In epidermalen Zellen finden sich oft Kalciumoxalatkristalle. Die Stomata sind anomozytische, anisozytische und tetracytische Typen. Die Trichome sind unregelmäßig konisch. Die Mittelrippe ist im Transektionsbereich flach, adaxial und gerippt in abaxialer Richtung mit einem sekretorischen Hohlraum unter dem zentralen Gefäßbündel versehen. Der Stengel ist bei der Transektion abgerundet. Das Gefäßgewebe besteht aus mehreren Gefäßbündeln in einem Ring. Kalziumoxalatkristalle treten in der Epidermis, Kortex und Stamm des Stammes auf. Im reifen Stengel ist das sekundäre Xylem mit einem reduzierten Phloem gut entwickelt.

Fazit: Die gezeigten morpho-anatomischen Merkmale dienen zusammen mit pulvermikroskopischen und organoleptischen Charakteristika und physiko-chemischen Angaben als Qualitäts- und Sicherheitsstandards der Arznei.

Evaluación farmacognósica y físico-química del medicamento homeopático: *Erigeron Canadensis* L.

RESUMEN

Fundamento: *Erigeron canadensis* Linn., es una hierba erecta anual perteneciente a la familia de las Asteraceas. Las partes aéreas se utilizan en homeopatía para contusiones , tos, disuria, gonorrea, hemorragias, hemorroides, espermatorrea y heridas.

Objetivos: Los estudios farmacognósicos y físico-químicos se realizan para facilitar el uso de las especies correctas y establecer los estándares de la materia prima para los medicamentos.

Materiales y métodos: Se realizaron estudios de farmacognosia de las hojas y el tallo de muestras auténticas de E. canadensis. Para las tinturas madre, se presentan los parámetros físico-químicos de la materia prima, a saber, los valores de extracción, el valor de cenizas, la formulación, peso por ml, sólidos totales, contenido en alcohol, y los estudios por HPTLC y UV.

Resultados: A menudo, las células epidérmicas poseen cristales de oxalato de calcio. Los estomas son de tipo anomocítico, anisocítico y tetracítico. Los tricomas tienen una estructura uniseriada cónica. La sección de la vena central es plana en adaxial y estriada hacia abaxial con una cavidad secretora debajo del haz vascular central. El tallo es tiene una sección redonda. El tejido vascular está formado por varios haces vasculares formando un anillo. Los cristales de oxalato de calcio se presentan en la epidermis, la corteza y el mesocarpio del tallo. En el tallo maduro, el xilema secundarios está bien desarrollado con un floema reducido.

Conclusiones: Las características morfoanatómicas presentadas junto con los caracteres microscópicos del polvo y organolépticos y los datos físico-químicos sirven de diagnóstico para establecer los estándares y asegurar la calidad y pureza del medicamento.

होम्योपैथिक औषधि इरिजरॉन कैनाडैनसिस एल. का फार्मकॉग्नोस्टिक और भौतिक–रासायनिक मूल्यांकन

सार

पृष्ठभूमि: इरिजरॉन कैनाडैनसिस एल., एक सीधी, वर्ष भर पाये जाने वाली जड़ी बूटी है जिसका संबंध एस्टरेसी परिवार से है। इसकी शाखा व पत्तियों द्वारा होम्योपैथी औषधि का निर्माण किया जाता है। यह दवा चोट के निशान, खांसी, पेशाब में जलन, सूजाक, रक्तस्त्राव, बवासीर, अज्ञात में शुक्रपात और घाव आदि के उपचार के लिए इस्तेमाल की जाती है।

उद्देश्य: औषधि हेतु कच्चे माल के मानकों के निर्धारण और सही प्रकार की औषधि के उपयोग को सुविधाजनक बनाने के लिए फार्मकॉग्नोस्टिक और भौतिक–रासायनिक अध्ययन किए गये।

सामग्री और विधि: इरिजरॉन कैनाडैनसिस के पत्ते तथा तने के प्रामाणिक नमूनों का फार्मकॉग्नोस्टिक अध्ययन किया गया। मदर टिंक्चर के लिए अनिर्मित औषधि के भौतिक–रासायनिक पैरामीटर जैसे उदरण मूल्य, राख मूल्य, एमएल प्रति वजन के अलावा सूत्रीकरण, कुल गैस, एल्कोहल सामग्री, एचपीटीएलसी तथा यूवी अध्ययन किये गए।

परिणाम: एपिडर्मल कोशिकाओं में अक्सर कैल्शियम आक्ज़लेट के क्रिस्टल पाए जाते है। रंध्र तीन प्रकार के होते है– एनोमोसाइटिक, एनिसोसाइटिक और टेट्रासाइटिक। ट्राइकोम यूनीसिरिएट शंक्वाकार संरचना के होते हैं। ट्रानसेक्सन मे मिड वेन अक्ष के विपरी और सपाट तथा केन्द्रीय नाड़ी बंडल के नीचे स्रावी गुहा के साथ अध्र के विपरीत की ओर धारीदार। ट्रानसेक्सन में छाल गोल आकृति की है। संवहनी ऊतक गोल आकृति में कई संवहनी बंडलों से निर्मित है। कैल्शियम ओक्ज़लेट के क्रिस्टल (कण) एपिडर्मिस, छाल तथा स्टेम की मज्जा में पाए जाते है। परिपक्व स्टेम में माध्यमिक जाइलम अल्प फलोएम के साथ पूर्ण विकसित हैं।

निष्कर्ष: औषधि की गुणवत्ता और शुद्धता सुनिश्चित करने के लिए आवश्यक मानकों की स्थापना के लिए प्रस्तुत आकृति–संरचनात्मक विशेषताओं के साथ–साथ भौतिक–रासायनिक मानक निदानकारी हैं।