

ORIGINAL ARTICLE

Pharmacognostic and high performance thin layer chromatography finger printing of *Pyrus malus* Linn

P. Padma Rao, P. Subramanian¹, P. Sudhakar, T. Sheshashena Reddy¹,
P. Ramachandra Reddy², D. Suresh Baburaj³

ABSTRACT

Background: *Pyrus malus* L. Syn. *Malus sylvestris* Mill., commonly known as 'Apple' is a well known tree belonging to the family Rosaceae. Leaves and young aerial parts are used as medicine in homoeopathy. **Objective:** The Pharmacognostic and physico-chemical studies are carried out to facilitate use of correct species and standard raw materials. **Material and Methods:** Pharmacognostic examinations of aerial parts (leaf, petiole and stem) of authentic sample of *Pyrus malus* L., have been carried out. Physico-chemical parameters of raw drug viz., extractive value, ash values, formulation besides weight per mL, total solids, alcohol content along with high performance thin layer chromatography (HPTLC) and UV-visible studies have been worked out for mother tincture. **Results:** The leaves are ovate to oblong, margin serrate, acute, glabrous above and tomentose beneath with a short petiole. The leaf is hypostomatic with anomocytic stomata. Uniseriate macroform flagellate conical and unicellular filiform cylindrical hairs occur on the leaf. In transverse section (TS) the midvein is conspicuously ridged on abaxial side. Epidermis is 1-layered with larger cells on abaxial. Cells over midvein are papillated. Palisade is 2-layered. The vascular bundle at midvein is single, large and arc shaped. The petiole in T.S. is shield like with an adaxial groove. The cortex is aerenchymatous towards adaxial. The vascular tissue consists of a single, large, arcuate bundle and two small adaxial bundles. The young stem is oval to spherical. The vascular tissue is in the form of a continuous cylinder. 1-3 seriate rays are present in the xylem. The xylem towards the centre possess secretory canals arranged in a ring. **Conclusion:** The powder microscopic features and organoleptic characters along with the anatomical and physico-chemical studies are diagnostic to establish the standards for the drug.

Keywords: High performance thin layer Chromatography, Homoeopathy, Pharmacognosy, Physico-chemical, *Pyrus malus* L., Standardization

INTRODUCTION

Pyrus malus L., Syn. *Malus sylvestris* Mill, commonly called as 'apple' or 'crab apple' in English and 'seb' in Hindi locally, is a well known tree belonging to the

family Rosaceae. It is indigenous to Europe and West Asia and grow wild in N.W. Himalayas and is also cultivated.^[1] The leaves and young aerial parts are medicinally useful.^[1] Leaves contain phloretin which is a natural antibiotic.^[2] Crushed leaves are useful for

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Pharmacognosy and ¹Chemistry Sections, Drug Standardisation Unit, O.U. B.32, Road No.4, Habsiguda, ²Department of Botany, Osmania University, Hyderabad, ³Drug Standardisation Section, Central Council for Research in Homoeopathy, New Delhi, India

Address for correspondence:

Dr. P. Padma Rao,
Pharmacognosy Section, Drug Standardisation Unit,
O.U.B. 32, Road No. 4, Habsiguda,
Hyderabad - 500 007, India.
E-mail: drpprdsu@gmail.com

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temporary treatment of wounds. The plant is a tree growing up to 20 to 40 feet, with spreading branches. The leaves are ovate or oblong ovate, serrate, 2-3" long; petioles ½ to 1" in length. The flowers are large, fragrant, pale rose in colour and borne in sub umbellate corymbs. Fruit is a pome and edible.^[3]

Chemically the plant is reported to contain^[4,5] patulin, campesterol, stigmasterol, β - sitosterol, nicotinic acid, phloretin 2- O-glucoside, phlorizin, Gibberellin A 62, melibiose, ostreasterol, 1,28-octacosanediol, pomonic acid and 1,24-tetracosanediol.

The earlier studies on *Pyrus malus* L, pertaining to Pharmacognostic and physico-chemical parameters in general and homoeopathic perspective in particular is not available. Hence, the authors have carried out detailed pharmacognostic and physico- chemical studies on leaves and young aerial parts as per the protocols suggested by Central Council for Research in Homoeopathy (CCRH).

MATERIAL AND METHODS

Pharmacognosy

Pyrus malus L. was collected and identified by Survey officer, Survey of Medicinal plants and Collection Unit (SMPCU), Nilgiris and given specimen No. 8582 dated 06.06.2011, deposited in the Herbarium, SMPCU, Nilgiris, T.N. The leaves and young aerial parts of *Pyrus malus* L. were boiled and fixed in F.A.A. (Formaldehyde: Acetic acid: Alcohol) and processed for microtomy (Paraffin method) sectioned, stained and permanent slides prepared following Johansen.^[6] The epidermal peels of leaf were obtained by gently scraping and peeling with razor blade. The microtome sections (TS and Longitudinal section (LS)) were obtained at 6-8 μ m thickness on Leica RM 2155 microtome. The powder microscopy characters were studied by boiling the powder drug in distilled water, stained in Safranin and mounted with glycerine. Photomicrography was done with Olympus BX 53 Research trinocular microscope.

Physico-Chemical

The air dried young aerial parts of the drug were coarsely powdered to 10/44 (sieve size) and subjected to determination of moisture content (loss on drying at 105°C), total ash, water soluble ash, acid insoluble ash, extractability in different solvents, physico-chemical constants, thin layer

chromatography (TLC) and Ultra violet (UV) aspects of mother tincture following recommended official methods.^[7] Mother tincture was prepared as per Homoeopathic pharmacopoeia of India (HPI). In this method, 100 g coarse powder of the drug was suspended in 520 ml of 95% alcohol and 500 ml of purified water for 24 hours at room temperature. It was filtered and made up to 1000 ml using same solvent ratio. Percolation method was used for the preparation of mother tincture.^[7]

High Performance Thin Layer Chromatography Analysis

Mother tincture was used for the high performance thin layer chromatography (HPTLC) study. The mother tincture was diluted in methanol and the same was spotted in the form of band width 4 mm with a Camag microlitre syringe on pre-coated silica gel aluminium plate 60F-254, (5 × 10 cm with 0.25 mm thickness; Merck, Darmstadt, Germany) using a Linomat IV sample applicator (Camag, Muttenz, Switzerland, supplied by Anchrom technologists, Mumbai). A constant application rate of 6 mL/sec was followed. The slit dimension was kept at 4 × 0.45 mm and 20 mm/sec scanning speed was employed. The mobile phase consisted of n-butanol: methanol: water (3:1:1 v/v/v) and 10 mL of mobile phase was used for chromatography. Linear ascending development was carried out in a 10 × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland), saturated with the mobile phase at room temperature for 20 minutes. The length of the chromatogram run was 9 cm. Subsequent to the development, the TLC plates were dried in a current of air with the help of hot air dryer in a wooden chamber with adequate ventilation. Derivatisation was done using anisaldehyde-sulfuric acid reagent with subsequent heating for 5 minutes at 105 ± 5°C. The plate was scanned using (Camag TLC scanner III) at visible light (540 nm) by reflectance scanning and operated by cats 4 software (v 4.05, Camag) resident in the system.^[8-10]

OBSERVATIONS AND RESULTS

Macroscopy

Leaves 2-3" long 1.5-2" wide, ovate or oblong ovate, margins serrate, acute, glabrous above and tomentose beneath, petiole short, 0.5-1" long; young stem greenish, rough and hairy.

Microscopy

Leaf-surface

Adaxial epidermal cells 5-7 sided, polygonal isodiametric and anisodiametric, sides thin, straight to curved, few sinuate, contents slightly dense, 2687 per sq.mm. Abaxially cells are similar except sides wavy to sinuate; 5560 per sq.mm.

Stomata confined to abaxial, anomocytic, subsidiaries 4 or 5, indistinct, 760 per sq.mm. Stomatal Index (SI) 12.02; sizes 22-33 μm long and 11-22 μm wide.

Trichomes are of two types (i) uniseriate, macroform, flagellate, conical hairs, occur all over and more on veins on abaxial and (ii) unicellular, filiform, cylindrical hairs, occur all over on abaxial [Figure 1a-c].

Transection

Epidermis is 1-layered, cells adaxially larger on lamina, barrel shaped, tabular, oval to spherical, contents dense in few, abaxially narrower, interspersed with stomata, contents dense. Epidermal cells over midvein oval to oblong and spherical, papillated and covered by thick cuticle. Collenchyma is hypodermal as a group of cells in midvein adaxial and 1-2 layered on abaxial, lamellar. Ground parenchyma in midvein 15-20 celled on adaxial and 10-12 layered on abaxial, cells polygonal to spherical, 8-32 μm in diameter on adaxial and 22-63 μm in diameter on abaxial. Palisade 2-layered,

closely packed, cylindrical, cells extending into midvein. Palisade ratio is 7-11.5. Spongy tissue nearly half the area of lamina with oval to oblong, cylindrical and dumbbell shaped loosely dispersed cells [Figure 2a].

Midvein is shield like, flat or indistinctly ridged on adaxial and conspicuously ridged on abaxial, 900-1360 μm (1090) in diameter, lamina dorsiventral, 130-270 μm (179) thick, undulated and grooved over smaller bundles [Figure 2b].

Vascular tissue of midvein consists of single large arc shaped vascular bundle, 680-790 μm (730) long and 420-560 μm (490) wide, endarch, conjoint, collateral with a cambium between xylem and phloem. Xylem is extensive, arranged in radial rows. Tracheary elements in L.S. are mostly helical and few scalariform thickened. Phloem is external, precocious and surrounded by discontinuous patches of sclerenchyma [Figure 2b].

Petiole

In TS shield like with shallow groove on adaxial and two slender ridges on either sides; 792-1330 μm (1129) long and 736-1189 μm (988) wide.

Epidermis is 1-layered, cells small, oval to spherical, papillated and covered by thick cuticle. Hypodermis is 1-2 layered, collenchymatous, angular or lamellar thickened. The peripheral cortex is aerenchymatous, 8-15 layered, cells polygonal to spherical, 11-49 μm in

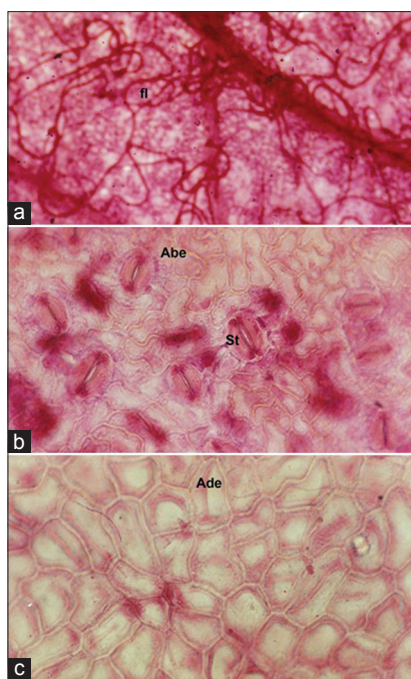


Figure 1: *Pyrus malus* (leaf). (a) Epidermis in surface with flagellated hair $\times 47$. (b) Epidermis of abaxial surface $\times 600$. (c) Epidermis of adaxial surface $\times 634$ (Fl: Flagellated hair, Abe: Abaxial epidermis, St: Stomata, Ade: Adaxial epidermis)

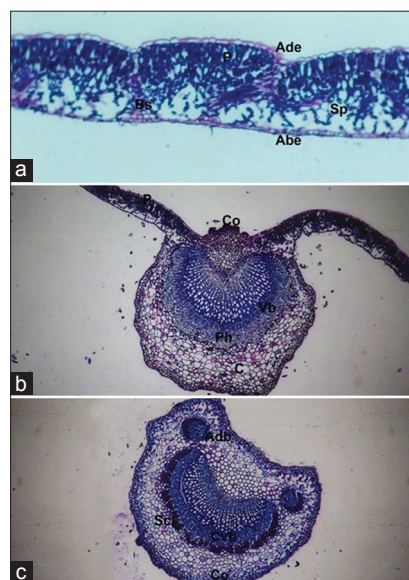


Figure 2: *Pyrus malus* (leaf). (a) T.S. of leaf lamina $\times 138$. (b) T.S. of leaf at midvein $\times 43$. (c) T.S. of petiole $\times 55.5$ (Ade: Adaxial epidermis, P: Palisade, Sp: Spongy parenchyma, Abe: Abaxial epidermis, Bs: Bundle sheath, Co: Collenchyma, Vb: Vascular bundle, Ph: Phloem, C: Cortex, Avb: Adaxial vascular bundle, Sc: Sclerenchyma, Cvb: Central vascular bundle)

diameter. Contents scanty, in few dense [Figure 2c].

The vascular tissue is made of a large arcuate bundle at centre besides two small spherical adaxial bundles. The central vascular bundle is laterally 529-842 μm (678) long and 216-432 μm (313) wide, endarch, conjoint, collateral with a cambium in between xylem and phloem. The vascular bundle is enclosed by a thick layer of sclerenchyma. The adaxial bundles are 48-82 μm (128) in diameter and enclosed by sclerenchymatous sheath [Figure 2c].

Young Stem

In T.S. stem is oval to spherical, 1.8-2 mm in diameter. The outermost epidermis is 1-layered. Collenchyma is 1-3 layered, cells oval to spherical and tabular, lamellar or angularly thickened. Cortex is 12-15 layered with peripheral cells containing chloroplasts. The vascular tissue is in the form of continuous cylinder. The xylem is in transition state with secondary growth in some vascular bundles. Vessels/tracheids mostly isolated and few in radial rows of 2-3. Medullary rays are 1-3 seriate. The phloem is external and interspersed with prismatic crystals. Vascular cambium is also present. Discontinuous patches of sclerenchyma encloses the phloem. Secretory canals are arranged in a ring. Pith is abundant at the centre [Figure 3a-d].

Powder Microscopy

1. Fragments of leaf with unicellular conical and cylindrical hair
2. Flagellate hairs, many
3. Crystals of calcium oxalate, few
4. Fragments of epidermis of lower surface with anomocytic stomata
5. Pieces of tracheary tissue, with fibers, vessels and attached ray parenchyma
6. Fragments of cortical parenchyma with dense contents.

Organoleptic Characters

Colour: Brownish yellow.

Touch: Smooth.

Odour: Pungent.

Taste: Bitter.

Physico-Chemical Studies

The determined data under the physico-chemical study for the raw drug is summarized in Table 1 and that of mother tincture preparation and its standardisation in Tables 2 and 3 respectively.

Qualitative Phytochemical Tests

Preliminary phytochemical study indicates the presence of alkaloids, steroids, saponins, and tannins. Moisture content reveals the presence of water in the plant and also some volatile organic matter. Results of physico-chemical studies are summarized in Tables 1-3.

HPTLC Finger Printing

The profile of chromatographic separation scanned in visible light shown in Figure 4, reveals six spots. Among them 5 and 4 spots possess maximum composition with R_f at 0.81 and 0.47 respectively. It is clear that

Table 1: Standardisation of raw drug

Parameters	Quantitative values (% w/w)
Moisture content (Loss on drying at 105°C)	Not more than 6.34
Total ash	Not more than 3.6
Acid insoluble ash	Not more than 0.43
Water soluble ash	Not more than 2.45
Alcohol soluble extractive	Not less than 17.0
Water soluble extractive	Not less than 17.9
Extractive values in	
Hexane	Not less than 0.93
Chloroform	Not less than 2.15
Methanol	Not less than 21.5

Table 2: Formulation of mother tincture (Percolation technique was used)

Alcohol	50% v/v (MEV*)
Drug strength	1/10
Preparation	
<i>Pyrus malus</i> in coarse powder	100 g
Strong alcohol	520 ml
Purified water	500 ml
To make 1000 ml of the mother tincture	

*MEV: Maximum extractive value

Table 3: Standardisation of mother tincture

Parameters	Observations
Organoleptic profile	
Appearance	Clear, non viscous, foam on shaking
Colour	Dark brown
Odour	Pleasant and fruity
Sediments	Absent
Weight per ml	Not more than 0.93 g
Total solids	Not less than 1.26% w/v
Alcohol content	47-50% v/v
pH	4.5-5.0
λ max	223, 287, 327 nm

these are characteristic for the studied drug, which will help in identification and authentication of the mother tincture. These are considered as valuable standards in pharmacopoeia. The chromatogram scanned at visible light (540 nm) reveals 6 spots, appearing at R_f 0.12, 0.32, 0.41, 0.47, 0.81 and 0.91 [Figure 4].

DISCUSSION

Pyrus malus L. popularly known as 'apple' is a tree growing up to 20-40 ft. high belonging to the family Rosaceae. Morphologically the leaves are 2-3" long, ovate or oblong ovate, serrate, glabrous above and tomentose beneath. Petiole is up to 1" long and young stem is greenish, rough and hairy.

Epidermal cells in surface on adaxial possess sides thin, straight to curved, few wavy to sinuate walls and 2687 per sq.mm. while those on abaxial have wavy to sinuate walls and 5560 per sq.mm. Leaf is hypostomatic with anomocytic type of stomata. Stomatal frequency 760 per sq.mm and SI is 12.02. Uniseriate flagellate conical and unicellular cylindrical hairs occur on abaxial side. The palisade ratio is 7-11.5.

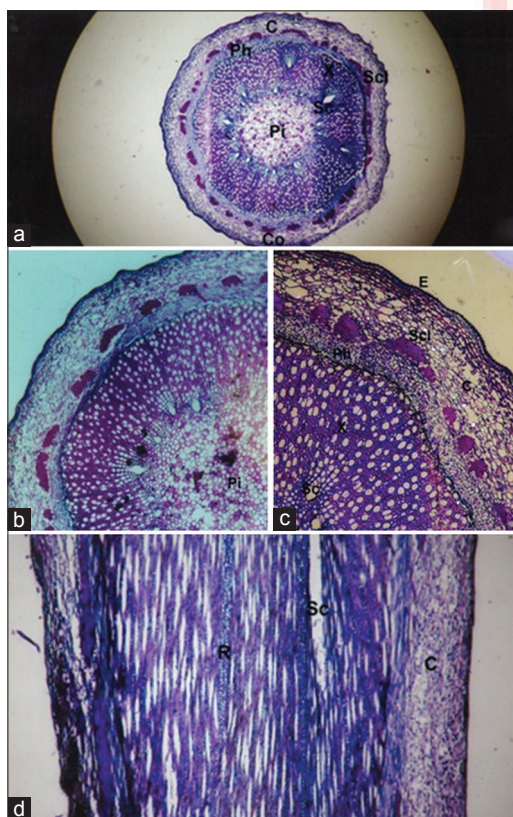


Figure 3: *Pyrus malus* (young stem). (a) T.S. of young stem \times 25. (b) T.S. of young stem, a portion \times 56. (c) T.S. of young stem, a portion \times 43. (d) LS of young stem \times 43 (Ph: Phloem, Scl: Sclerenchyma, Pi: Pith, Co: Collenchyma, E: Epidermis, Sc: Secretory canal, R: Rays)

The midvein in TS is shield like, 900-1360 μ m in diameter and conspicuously ridged on abaxial. Lamina is dorsiventral, 130-270 μ m thick and undulated.

Epidermis is 1-layered with larger cells on adaxial. The epidermal cells over the midvein are papillated with thick cuticle. Palisade is 2- layered and extend into the midvein. Spongy tissue occupy half the area of mesophyll and with loosely dispersed cells.

A single large arcuate bundle, endarch, collateral with a vascular cambium is present in the midvein. A layer of discontinuous patches of sclerenchyma is present enclosing the vascular bundle.

Petiole

Shield like with a shallowed groove on adaxial. Centrally a large arcuate bundle and with two small spherical bundles in adaxial ridges. Peripheral cortex is aerenchymatous. A sclerenchymatous sheath encloses the vascular bundles.

Young Stem

Oval to spherical in outline and up to 2 mm in diameter. The cortex is 12-15 layered. The vascular tissue is in the form of a continuous cylinder. The xylem also shows secondary growth in the vascular bundles. Medullary rays are 1-3 seriate. The phloem is external and interspersed with prismatic crystals of calcium oxalate. Secretory canals are present towards centre. Pith is abundant.

The powder microscopic features and organoleptic characters along with the anatomical studies are diagnostic and establish the standards for the drug.

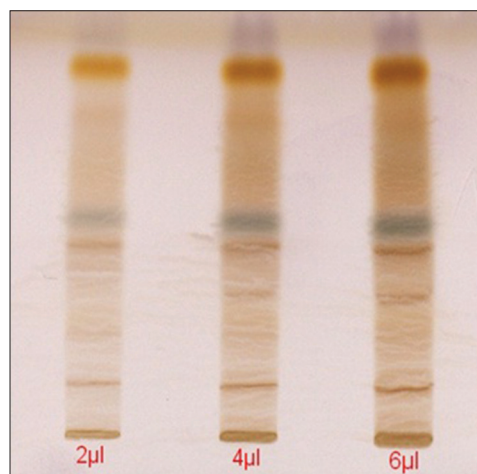


Figure 4: HPTLC finger printing of *Pyrus malus* mother tincture under visible light

Physico-Chemical

The values of total ash, water soluble ash and acid insoluble ash fall within acceptable range. The results of HPTLC finger printing and UV Spectrophotometric study exhibits (maximum absorption) three prominent peaks which will be taken as characteristic standards for the drug. The HPTLC photo-documentation provides for its characteristic finger printing image viz., number of constituents present, R_f values, along with colors and percentage composition. It is helpful for identification, authentication and differentiation of adulterants from the genuine samples and ensures purity, quality, batch to batch uniformity, safety and efficacy. Hence, pharmacopoeia has included this as fingerprinting tool.

The determined physico-chemical data, macro and microscopical characters and methodology employed in the study will help in identification, authenticity and ensures quality, purity and efficacy of the drug.

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सारांश: पायरस मालस लिनसमानार्थमालससिलवेस्ट्रीसलिन., जोआमतौरपर 'सेब' या 'जंगलीसेब' के नामसे जाना जाता है। यह गुलाबपरिवारसे संबंधित एक प्रसिद्ध पेड़ है। इसकी पत्तियां और नव-वायुविषयक भाग को होम्योपैथी में दवा के रूप में इस्तेमाल किया जाता है। इसकी पत्तियां प्राकृतिक एंटीबायोटिक होती हैं जिसे पीस कर साफ़ करने के पश्चात घाव पर इस्तेमाल की जाती है। भेषजज्ञान और भौतिक रासायनिक अध्ययन में सही प्रजातियों और मानक कच्चे माल के उपयोग की जानकारी मिलती है। पत्तियां आयताकार, दन्तुर किनारा, नुकीले, ऊपर की तरफ कम्बल की तरह का रोएदार और एक छोटी डंठल के साथ अंडाकार आकार की होती हैं। इसकी पत्तियां अल्प-रंध्र के साथ एनोमोसायटिक होती है। इसकी पत्तियाँ एकपंक्तिक, बृहद-प्रकार, मजबूत, शंक्वाकार और कोशिकीय, तन्तुरूप, बेलनाकार बाल जैसी होती हैं। अनुप्रस्थ अनुभाग (टीएस) में, मध्यशिरा अपाक्ष में सुस्पष्ट रूप से उभरता है। बाह्यत्वचा बड़ी कोशिकाओं के साथ एक परत वाली अपाक्ष है। मध्यशिरा पर कोशिकाएं अधिक अन्कुरक होती है। स्तंभपंक्ति दो परत वाली होती है। मध्यशिरा पर संवहनी बंडल एकल, बड़ी, और चाप के आकार की होती है। डंठल के अनुप्रस्थ अनुभाग के मध्यतल की ओर एक नाली की तरह ढाल होती है। प्रांतस्था मध्यतल की ओर वायुतक होती है। संवहनी कोशिका में एक बड़ी धनुषाकार बंडल और दो छोटे मध्यतल की ओर बंडल होते हैं। छोटी डंठल गोल या अंडाकार होती है। संवहनी कोशिका अविरत बेलनाकार होती है। जाइलम में 1&3 पंक्तिबद्ध किरणें मौजूद होती है। केंद्र की ओर मौजूद जाइलम नाल में व्यवस्थित स्राव चक्र होते हैं। पाउडर सूक्ष्म सुविधा और ज्ञानेन्द्रिय-सुग्राह्य वर्ण प्रदान की जाती है। मूल औषधि के भौतिक रासायनिक मापदंडों अर्थात मदर टिंचर के लिए निष्कर्षण मूल्यों, राख मूल्यों, प्रति मिलीलीटर भार सूत्रीकरण, कुल ठोस, उच्च निष्पादन पतली परत क्रोमैटोग्राफी (HPTLC) के साथ औषधि सामग्री और अल्ट्रा वायलेट दृश्य (यूवी विज) का अध्ययन किया जाता है।

खोजशब्द: होम्योपैथी, HPTLC, भेषजज्ञान, भौतिक रसायन, पायरस मालस लिन, मानकीकरण।