Available online on 15.02.2026 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

Pharmacognostic, Physicochemical and Phytochemical Screening of *Enicostema axillare*

Brij Raj Singh [1]*, Rajib Kr. Singh [2], Jitendra Singh Yadav [3], Sursh Chandra [4]

[1] Department of Pharmacognosy, Malti Memorial Trust CSM Group of Institutions, Faculty of B.Pharmacy, 8th Milestone Rewa Road, Prayagraj 212111, U.P., India

[2] Department of Pharmaceutical Chemistry, Shakti College of Pharmacy, Balrampur, U.P., India

[3] Shakti College of Pharmacy, Balrampur, U.P., India

[4] Malti Memorial Trust CSM Group of Institutions, Faculty of B. Pharmacy, Prayagraj, U.P., India

Article Info:

Article History:

Received 19 Nov 2025
Reviewed 12 Jan 2026
Accepted 05 Feb 2026
Published 15 Feb 2026

Cite this article as:

Singh BR, Singh RK, Yadav JS, Chandra S, Pharmacognostic, Physicochemical and Phytochemical screening of *Enicostema axillare*, Journal of Drug Delivery and Therapeutics. 2026; 16(2):174-181 DOI: http://dx.doi.org/10.22270/jddt.v16i2.7579

For Correspondence:

Brij Raj Singh, Department of Pharmacognosy, Malti Memorial Trust CSM Group of Institutions, Faculty of B. Pharmacy, 8th Milestone Rewa Road, Prayagraj 212111, U.P., India

Abstract

Enicostema axillare is a perennial herb of the family Gentianaceae. The bitter-natured plant acts as a laxative, helps cure fever, rheumatism, and skin diseases, and is widely used in traditional medicine. Shade-dried whole plant material was subjected to Soxhlet extraction with Ethanol. The extracts were evaluated by chemical examination and chromatographic method for the presence of phytoconstituents. Morphological studies indicate Simple, and oblong to lanceolate leaves, sub-quadrangular or terete stems, and tubular roots. Microscopic studies indicate that leaves have diacytic and anisocytic stomata on the lower surface. Single-layered upper epidermis with thick-walled cuticle, and non- glandular trichomes in leaves, stem, and root. Root shows secondary growth; vascular tissue is in an ending arch condition. Phytochemical studies indicate that the plant contains carbohydrates, proteins, alkaloids, glycosides, flavonoids, tannins, steroids, terpenoids, and saponins. Percentage yield of ethanolic extract were 8%, alcohol -soluble extractive value 15.2%, water–soluble extractive value 18%, moisture content 5%, total ash 11.5%, acid-insoluble ash 6.5%, water-soluble ash were 7.5%, and in TLC analysis, Rf values were found to be 0.81,0.86,0.9.

Key words: Phytochemistry, Physicochemical, Microscopic, TLC

1. INTRODUCTION

The perennial glabrous medicinal herb *Enicostema axillare* A. Raynal belongs to the Gentianaceae family. The plant is widely distributed in Africa, America, and Asia and thrives in a variety of environmental, climatic, and soil conditions. Angola, Assam, Botswana, Cocos (Keeling) Islands, Ethiopia, Gulf States, India, Jawa, Kenya, South Africa, Lesser Sunda Islands, Malawi, Mozambique, Namibia, Oman, Pakistan, Saudi Arabia, Somalia, Sri Lanka, Sudan, Swaziland, Tanzania, Vietnam, West Himalaya, Yemen, Zambia, and Zimbabwe are among the countries that are native to the species. In addition, several other areas have seen the introduction and naturalization of *E. axillare*. It is well known for being used in traditional medicine to manage, treat, and cure a variety of illnesses, especially in rural areas where access to healthcare is a significant obstacle. Makgonatsohle, its local name in South Africa, means "omnipotent" because it is thought to be able to treat any ailment. The plant is used in Indian traditional medicine to treat helminthiasis, venereal infections, skin conditions, tumors, and intermittent fevers. It is also used as a blood purifier. Iron, potassium, sodium, calcium, magnesium, chloride, and vitamins B and C are among the minerals that are abundant in the plant[1, 2,3,4,5].

2. METHODOLOGY

2.1 Collection and authentication of whole plant

Enicostema axillare were collected in the month of October, from local place of the Allahabad district and were authenticated by Vinay Ranjan (scientists-E and Head of office), BSI Central Regional Center, Prayagrag U.P., India (Ref. no. **1904250001034 date 19/04/2025)**.

2.2 Morphological features[6, 7, 8]:

Morphology refers to the examination of an object's structure, while morphography involves the detailed description of that structure. This is applicable when the material is recognized to exist in a specific form. Botanical observations and evaluations were conducted on morphological characteristics as well as organoleptic attributes such as color, smell, flavor, shape, and dimensions.

2.3 Study of microscopical characters[6, 7, 8]

The sections were taken by placing the leaf portion cut along with the midrib in between the two flat surfaces of pith. Pith is usually a piece of potato (about 3x1x1 cm) in which the longitudinal slit of 2 cm deep is made, into which is then placed and sections are taken. Transferred the sections into watch glass containing water-glycerin solution and the sections were stained with phloroglucinol & hydrochloric acid; and then mounted in glycerine and observed under low power.

2.4 Physicochemical and Phytochemical Investigations[9, 10]

Alcohol soluble extractive value

5g of shade-dried *Enicostema axillare* powder was macerated with 100ml of ethanol in a closed flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly, taking precaution against loss of ethanol. Evaporate 25ml of filtrate to dryness in a tared flat-bottom shallow dish dried at 105^0C and weighed. Percentage ethanol soluble extractive was calculated with reference to the shade-dried plant powder.

Water soluble extractive value

Above procedure was adapted for its determination. Percentages of extractive values were calculated with reference to the shade-dried leaves powder.

Determination of foreign matter

Drug should be free from moulds, insects, animal fecal matter and other contaminations such as soil, stones and extraneous material.100 gm of drug sample to be examined was weighted and spread out in thin layer. The foreign material was detected by visual inspection, separated, weighted and the percentage was calculated.

Moisture content (WHO, 1998)

An accurately weighed quantity of the shade-dried of *Enicostemaaxillare*powder was taken in a tarred glass bottle and the initial weight was taken. The crude drug was heated at 105^0C in an oven and weighed. This procedure was repeated till a constant weight was obtained. The moisture content of the sample was calculated in percentage with reference to the shade-dried material.

Total Ash values (WHO, 1998; I.P, 1996)

3g of accurately weighed quantity of the shade-dried leaves powdered drug was taken in a tarred silica crucible and incinerated at a temperature not exceeding 450^0C until free from carbon, cooled and weighed.

Acid-insoluble ash

Total ash obtained was boiled for five minutes with 25 ml of dilute Hydrochloric acid. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited, cooled and weight. The percentage of acid insoluble ash was calculated with reference to shade-dried leaves powder.

Water-soluble ash

Total ash obtained was boiled for five minutes with 25ml of distilled water, cooled and collect the insoluble matter on an ash-less filter paper, washed with hot water and ignited for 15 minutes at temperature not exceeding 450^0C. The percentage of water-soluble ash was calculated with reference to Shade -dried powder.

2.5 Extraction

Alcoholic extraction

Shade dried *Enocostema axillare* whole plant pulverized and 100g of the crude drug powder was extracted with 95% ethanol in a Soxhlet extractor. The liquid extract was concentrated in a rotary flash evaporator. The residue was dried in desiccators over sodium sulfite.

2.6 Phytochemical screening[11, 12, 13, 14, 15]

The following qualitative chemical tests, for identifying various phytoconstituents present, were carried out on various extracts of *Enocostema axillare* whole plant (Harborne, 1984; Vogel, 1953; Turner, 1975).

Tests for Carbohydrates

Molisch's test:

Treat the extract solution with few drops of alcoholic α-napthol. Add 0.2 ml of concentrated H_2SO_4 slowly through the sides of the test tube, **purple to violet** color ring appears at the junction.

Benedict's test:

Treat the extract solution with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, **reddish brown** precipitate forms if reducing sugars are present.

Barfoed's test:

General test for monosaccharides. Heat the test tube containing 1ml reagent and 1 ml of extract solution in a beaker of boiling water; if **red cuprous oxide** is formed within two minutes, a monosaccharide is present. Disaccharides on prolonged heating (about 10min) may also cause reduction, owing to partial hydrolysis to monosaccharides.

Fehling's test:

Equal volumes of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) reagents are mixed along with a few drops of extract solution, boiled, and a brick red precipitate of cuprous oxide forms if reducing sugars are present.

Tests for Proteins & Amino acids

Millon's Test:

Extract solution + 2 ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) **white precipitate** appears, which turns **red** upon gentle heating.

Ninhydrin Test:

Amino acids and proteins, when boiled with 0.2% solution of Ninhydrin (Indane 1, 2, 3 trione hydrate), produce a violet colour.

Tests for Sterols and Triterpenoids

Libermann-Burchard test: Extract treated with few drops of acetic anhydride, boil and cool, concentrated sulphuric acid is added from the side of the test tube, A brown ring at the junction of two layers and the upper layer turns green indicates the presence of sterols and formation of deep red color indicates the presence of triterpenoids.

Salkowski's test: Treat the extract with chloroform and a few drops of concentrated Sulfuric acid; shake well and allow to stand for some time. A red colour in the lower layer indicates the presence of sterols, and a yellow-coloured lower layer indicates the presence of triterpenoids.

Tests for Glycosides

Test I:

Extract 200 mg of the drug by warming in a test tube with 5 ml of dilute (10%) sulphuric acid on a water bath at 100^{o}C for two minutes, centrifuge or filter, pipette out supernatant or filtrate. Neutralize the acid extract with 5% solution of Sodium hydroxide (noting the volume of NaOH added). Add 0.1 ml of Fehling's solution A and B until alkaline (test with pH paper) and heat on a water bath for 2 minutes. Note the quantity of red precipitate formed and compare with that formed in Test II.

Test II:

Extract 200 mg of the drug using 5 ml of and boil on water bath. After boiling add equal volume of water to the volume of NaOH used in the above test. Add 0.1 ml of Fehling's A and B until alkaline (red litmus changes to blue) and heat on water bath for two minutes. Note the quantity of the red precipitate formed.

Compare the precipitates of Test II with Test I. If the precipitate in Test-II is greater than in Test-I, then Glycoside may be present. Since Test I represent the amount of free reducing sugar already present in the crude drug, whereas Test-II represents the Glycoside after acid hydrolysis.

Tests for Alkaloids

Mayer's test: (Potassium mercuric iodide solution).

To extract/sample solution, add few drops of Mayer's reagent, creamy white precipitate is produced

Dragendroff's test: (Potassium bismuth iodide solution).

To extract/sample solution, add a few drops of Dragendroff's reagent, and a reddish brown precipitate is produced.

Wagner's test: (Solution of Iodine in Potassium Iodide).

To extract/sample solution, add a few drops of Wagner's reagent, and a reddish brown precipitate is produced.

Hager's Test: (Saturated solution of Picric acid)

To extract/sample solution, add a few drops of Hager's reagent, and a yellow precipitate is produced.

Tannic acid test:

To extract/sample solution, add a few drops of Tannic acid solution, buff precipitate is produced.

Tests for Phenolic Compounds

Ferric chloride test:

The extract solution gives a blue-green colour with a few drops of $FeCl_3$

Shinoda Test (Magnesium Hydrochloride reduction test)

To the extract solution, add a few fragments of magnesium ribbon and concentrated Hydrochloric acid dropwise, yellowish; yellow-orange occasionally orange colour appears after a few minutes.

Zinc-Hydrochloride reduction test:

To the extract solution, add a mixture of Zinc dust and conc. Hydrochloric acid. It gives a yellowish, yellow-orange, or occasionally orange colour after a few minutes.

Tests for Flavonoids

Shinoda Test (Magnesium Hydrochloride reduction test)

To the extract solution, add a few fragments of magnesium ribbon and concentrated Hydrochloric acid dropwise, pink scarlet, crimson red or occasionally green to blue colour appears after a few minutes.

Zinc-Hydrochloride reduction test:

To the extract solution, add a mixture of Zinc dust and conc. Hydrochloric acid. It turns red after a few minutes.

Alkaline reagent test:

To the extract solution, add a few drops of Sodium hydroxide solution; the formation of an intense yellow colour that turns colourless on addition of a few drops of dilute acetic acid indicates the presence of flavonoids

Tests for Tannins

Gelatin test:

Extracting with 1% gelatin solution containing 10% sodium chloride gives a **white** precipitate.

Ferric chloride test:

The extract solution gives a blue-green colour precipitate with $FeCl_3$.

Vanillin Hydrochloride test: Extract solution when treated with a few drops of Vanillin Hydrochloride reagent gives a purple-red colour.

Alkaline reagent test:

Extracting with sodium hydroxide solution gives a yellow to red precipitate within a short time.

Metchell's test:

With iron and ammonium citrate, or iron and sodium tartarate, tannins form a water-soluble iron-tannin complex that is insoluble in ammonium acetate solution.

Tannins precipitate from solution when treated with heavy metals.

Tannins yield a bulky precipitate with phenazone, especially in the presence of sodium and phosphate.

3. RESULTS:

3.1 Morphological evaluation of *Enicostema axillare*

Figure 1: Whole plant and morphology of Leaf

Table 1: Organoleptic features of fresh leaf

S. No.	Features	Observations
01	Upper surface	Darkish green (rough)
02	Lower surface	Light green (smooth)
03	Odour	Distinct , but not strong
04	Taste	Bitter
05	Shape	Lanceolate or elliptic
06	Size	Length 3-6 cm, width 0.5-0.7cm

Table 2: Botanical evaluation of leaf

Leaf portion	Observations
Margin	Entire, wavy
Apex	Acute or obtuse
Lamina, leaf blade	
Shape	Linear, lanceolate, or narrowly oblong
Composition	Simple, genkwanin, swertisin
Incision	Nil
Venation	3-nerved from the base
Surface	Glabrous (smooth)
Base	Petiole
Petiole	
Size	0.5cm
Shape	Narrow-oblong, lance- shaped or linear to elliptic
Sessile leaves	No present petiole

3.2 Microscopic observations

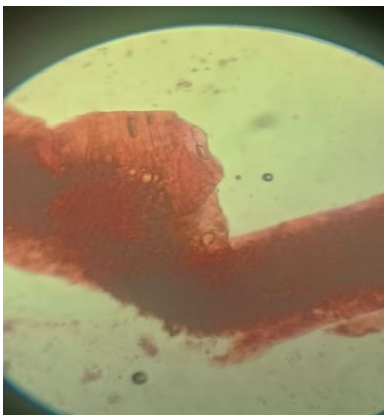


Figure 2: T.S. of the mid-rib

Figure 3: T.S. of stem

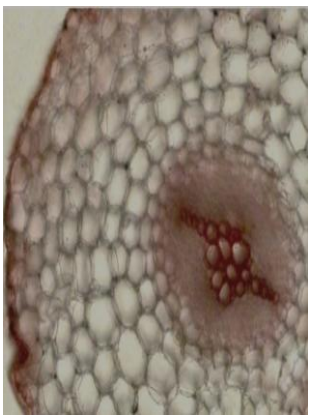


Figure 4: T.S. of root

Table 3: Transverse section of leaf (midrib)

S. no.	Features	Observations
1	Trichome	Multicellular and non- glandular
2	Upper epidermis	Single layered, covering by a thick, waxy cuticle,
3	Lower epidermis	Single layered, covering by a thick, waxy cuticle
4	Vascular bundle	Xylem and phloem, midrib
5	Calcium oxalate crystal	Persent
6	Parenchyma	Thin-walled, number of vascular bundles

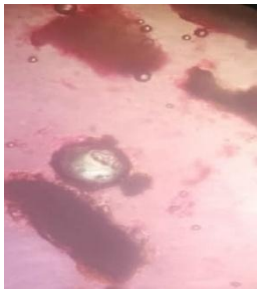
Table 4: Transverse section of stem

S. no.	Features	Observations
1	Epidermis	Outer layered, covered by thick cuticle and unilayered ,compactly arranged cell
2	Cork	Thin –walled
3	Cortex	2-5 layered, parenchymatous cells, containing wing-like structur
4	Vascular tissue	Collateral and open xylem & phloem ring
5	Pith	Cental pith cell found and Sphaeraphides (calcium oxalate crystals

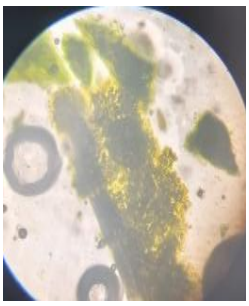
Table 5: Transverse section of root

S.no.	Features	Observations
1	cork	4-6 row of thin-walled, phelloderm, a layer of thicker-walled cells
2	cortex	2-5 layers, loosely packed with thin- walled, parenchymatous cells
3	Stone cells	Have thick, lignified walls or in smaal groups
4	Medullary rays	Radial extension of the cortex in the vascular cylinder
5	Vascular tissues	Cental, stele- like sturucture with xylem and phloem

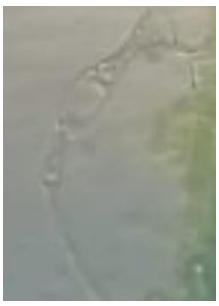
3.3 Powder Microscopy:



Calcium oxalate crystal



lignified fibre



covering trichome

anisocytic stomata

3.4 Powder characteristics

Table 6:

S. no.	Features	Observation		
		Leaf	**Stem**	**Root**
1	**Nature**	Coarse powder	Coarse powder	Coarse powder
2	**Colour**	Light brown	grey	Reddish-white
3	**Odour**	Pungent	characteristics	characteristics
4	**Taste**	Very bitter	Bitter	Bitter

3.5 Fluorescence analysis of powder

Table 7:

Treatment	Visible	Long U.V. 254 nm	Short U.V. 365 nm
Powder	Green	Brownish green	Light green
Powder + % KOH	Green	dark green	Black blueish
Powder + 5%NaOH	Greenish black	Dark green	Black
Powder + $FeCl_3$	Greenish yellow	Green	Light green
Powder + con. H_2SO_4	Yellow	green	Not visible
Powder + dil. H_2SO_4	light –brown	yellowish	light green
Powder + con. HCl	Yellowish green	Green	Black
Powder + dil. HCl	Yellowish green	yellowish	Light green
Powder + con. HNO_3	brown	blue	green
Powder + dil. HNO_3	Yellowish brown	blackish-green	Not visible
Powder + dil. NH_3	cream	yellowish	green
Powder + Ethanol	Yellowish green	Green	Black

3.6 Phytochemical Investigations

Physico-chemical analysis of whole plant of *Enicostema axillare*

Table 8:

Physico-chemical parameter	Percentage
Foreign matter	Nil
Percentage extractive	
A) Ethanol soluble	15.2%
B) Water soluble	18%
Ash value % W/W	
A) Total ash	11.5. %
B) Acid-insoluble ash	6.5%
C) Water- soluble ash	7.5%
Moisture content	5%
Total alkaloids	0.16%

3.7 Qualitative chemical analysis of various extract of whole plant of *Enicostema axillare*

Table 9:

Chemical constituents	Ethanol extractive
Carbohydrates	-ve
Alkaloids	+ve
Glycosides	+ve
Tannin	+ve
Phenolic	+ve
Steroids	+ve
Terpenoids	+ve
Proteins	+ve
Saponin	+ve

+ Ve Present, - ve absent

3.8 TLC evaluation of leaf ethanolic extracts

Table 10:

Extract	Mobile phase	Evaluation of the chromatogram			
		Visualization	No. of spots	Rf values	
Ethanol	Ethyl acetate:Methanol :Water (9:0.8:0.2)	UV light and after Vanillin or Anisaldehyde staining	3	0.81, 0.86, 0.9	

4. SUMMARY AND CONCLUSION

The present study includes a standardised limit of Pharmacognostic and Phytochemical investigation of the whole plant of *Enicostema axillare*.

Microscopic examination of the powdered whole plant indicated the presence of helical, pitted-shaped lignified vascular tissue, covering trichome- and rosette-shaped calcium oxalate crystals, as its diagnostic characteristics. Histochemical analysis indicates a crescent shape outline in the petiole; straight and wavy-walled epidermis, Diacytic and anisocytic stomata in the leaf; secondary growth and vascular tissue ring in the stem and roots. Leaf surface data and fluorescence analysis of powder were also determined.

In physicochemical analysis, moisture content, ash values, extractive values were determined. The moisture content plays an important role in drug storage, and as it varies, so does the extractive value, which further affects drug dose.

Ash values are useful for assessing the quality and purity of the crude drug, especially in the powdered form. Total ash reflects the care taken in its preparation, with all traces of organic matter removed during ash formation, and usually consists of carbonates, phosphates, and silicates of sodium, potassium, calcium, and magnesium. A higher acid-insoluble ash limit indicates the presence of silica or a very high calcium oxalate content in the drug. The water-soluble ash is used to detect the presence of material exhausted by water.

The extractive value of crude drugs is useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other method. Further, these values indicate the nature of the constituents present in the crude drug.

In the fluorescence analysis, some of the constituents exhibit varying degrees of brightness when exposed to radiation. This phenomenon of emitting visible wavelengths in response to radiation of a different wavelength is known as fluorescence.

The qualitative chemical examination of various extracts revealed the presence of alkaloids, glycosides, tannins, triterpenoids, saponins, phenolic compounds, steroids, amino acids and carbohydrates and their presence was substantiated by thin-layer chromatographic studies.

Conflict of Interest: The authors declare no potential conflict of interest concerning the contents, authorship, and/or publication of this article.

Author Contributions: All authors have equal contributions in the preparation of the manuscript and compilation.

Source of Support: Nil

Funding: The authors declared that this study has received no financial support.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Ethical approval: Not applicable.

REFERENCES

1. Srivastava, A., Bhatt, N., Patel, T., Dadheech, N., Singh, A., & Gupta, S. Anti-apoptotic and cytoprotective effect ofEnicostemma littoraleagainst oxidative stress in Islets of Langerhans. Pharmaceutical Biology, 2016;54(10):2061-2072. https://doi.org/10.3109/13880209.2016.1141222 PMid:26974043

2. Rajamani Saranya, Thirunavukkarasu Thirumalai, Munisami Hemalatha, Ranganathan Balaji, Ernest David, Pharmacognosy of Enicostemma littorale: A review,Asian Pacific Journal of Tropical Biomedicine, 2013;3(1):79-84,ISSN 2221-1691, https://doi.org/10.1016/S2221-1691(13)60028-3 PMid:23570022 PMCid:PMC3609395

3. Garad, M. C., Upadhya, M. A., Kokare, D. M., & Itankar, P. R. Aerial parts of Enicostemma littorale Blume serve as antipyretic and antacid: in vivo and in vitro evaluations. screening, 2012;13:16. https://doi.org/10.5530/pc.2012.3.9

4. Paivera J, Nogueira I. Encostemmalittorale herbal summary. (Gentianaceae: Potalieae: Faroinae: E. Littorale) Flora Zambesica. 1990;7(4)

5. Gupta S, Dadheech N, Singh A, Soni S, Bhonde RR. Enicostemma littorale: A new therapeutic target for islet of neogenesis. Int J Int Bio. 2010;9(1):50.

6. Evans WC, Trease. Text Book of Pharmacognosy; 15th ed.; ELBS London. 2002.

7. Khandelwal. Practical Pharmacognosy. Nirali Publications, 1st edition. 1995.

8. World Health Organization. Quality control Methods for Medicinal Plant Materials. Geneva. A.I.T.B.S.Publishers & Distributors, Delhi. 1998.

9. Indian Pharmacopoeia. Vol. II: Appendix 3.23, A47. 1996.

10. Harborne JB. Phytochemical Methods. 2nd ed; London New York, Chapman and Hall. 1984. https://doi.org/10.1007/978-94-009-5570-7

11. Vogel AI. A text book of Macro and semi micro qualitatative inorganic analysis. Longman Green & Co. Ltd. London. 1953;489 - 563.

12. Turner RA. Screening Methods in Pharmacology. Academic press, New York. 1965;100-116. https://doi.org/10.1016/B978-1-4832-3266-9.50012-8

13. Stahl Engon. Apparatus and General Techniques in TLC. Thin layer chromatography edited by Egon Stahl. George Allen & Unwin Ltd. London. 1969;52-86. https://doi.org/10.1007/978-3-642-88488-7_3

14. Wagner H, Bladet S, Zgainski EM. Plant Drug Analysis, A TLC Atlas; 1st ed. Springer Verlag Berlin Heidelberg New York.1994.

15. Wallis TE. Textbook of Pharmacognosy; 5th ed. CBS Publishers & Distributors, New Delhi.1985.