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PHYTOCHEMICAL ANALYSIS AND ANATOMICAL STUDY OF TWO SPECIES OF *CESTRUM* FROM CHANDIGARH

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Keywords:

Phytochemicals, *Cestrum* species, Anatomical studies, HPLC

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ABSTRACT: Family Solanaceae is known for its tropane alkaloids which have many medicinal properties. In our study two *Cestrum* species namely *Cestrum diurnum* and *Cestrum nocturnum* which are also ornamental plants are studied as different parts of the plants possess anti-bacterial, anti-malarial, analgesic, anti-inflammatory, inhibitory effect on central nervous system and wound healing properties. The present study was undertaken to analyse the presence of various phytochemicals such as alkaloids, flavonoids, terpenoids, saponins, steroids through preliminary qualitative phytochemical screening of the leaf extract. HPLC(High Performance Liquid Chromatography) was used for the qualitative and quantitative active principle analysis which revealed the presence of nicotine and nornicotine as major alkaloids and comparative study of the two species showed the presence of these alkaloids in large quantity in *Cestrum diurnum*. The detailed anatomical studies of the part(s) used help in the correct easy identification of the commercial drug in its purest form.

INTRODUCTION: Plants are the richest source of medicine and have been used since ages for their therapeutic value. A large no. of new pharmacologically active agents which have been explored from natural resources and plants are one of them which lead to the discovery of many clinically useful drugs. The useful plant based drugs contain active principle ingredients which are secondary metabolites also called phytochemicals present in the plants in any part that can be leaves, roots, stem, seeds *etc*. These phytochemicals can be alkaloids, saponins, tannis, flavonoids and many more.

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Two plants namely *Cestrum diurnum* and *Cestrum nocturnum* have been selected for the present study from the different areas of Chandigarh. *Cestrum diurnum* L., (Day Jasmine), also known as "Din ka Raja" blooms during day time is a member of Solanaceae family native of West Indies and introduced to India as an ornamental plant. Because of its fragrant flowers and its moderate size, it is been planted as an ornamental plant [1].

The leaves contain many active constituents which have been used for tropical psoriasis therapy in many parts of the world. Ayurvedic practioners have also started to show interest in the plant. Its leaves are used externally to control itching, psoriasis and patches on skin. Its oil is effective in treating malaria in many African countries. Although leaves are toxic but they contain several active compounds which possess medicinal properties. The plant also shows larvicidal and cardioactive activities [2].

Another plant *Cestrum nocturnum* L., from the same family is an ornamental plant. Its flower blooms during night time calling it "Night Blooming jasmine", Night Cestrum and Lady of the Night [3]. It is native of tropical and subtropical regions of the world and is found in India too. Its leaves have been used traditionally for treating swellings and burns [4]. The oil is effective as its volatile and act as mosquito repellent and have been used in many African countries to cure malaria [5]. It is used to treat epilepsy too. Pharmacological studies have shown that it can be used to treat arterial hypertension, and has analgesic, diuretic, antiviral and abortive properties[6].

MATERIALS AND METHODS:

Plant Collection and Identification: Fresh plant samples were collected from two different localities. Identification of species was done by comparing with authenticated herbarium specimens, later confirmed with the help of diagnostic keys and morphological descriptions given in various floras. The useful parts such as leaves, stems *etc*. were separated and preserved for study.

1. Anatomical Study of the Part Used: The stems and the leaves of both the plants were fixed in F.A.A. (*i.e*. Formalin, acetic acid-alcohol 1:1:18) after trimming them to correct dimensions. Hand sections were cut using sharp blade. Thin transverse sections were stained in safranin and then fast green, passed through alcohol grades for dehydration and then mounted in D.P.X. Observations were taken from these sections using light microscope. These sections were also photomicrographed. Special identifying features of the plant part(s) were studied and identified.

2. Phytochemical Study: Leaves were washed with a solution of 5% mercuric chloride for 5min and then thoroughly washed with sterile distilled water in order to remove any impurities, shade dried and oven dried and fine powder was made. The solvent extracts were evaporated to dryness in rotary evaporator in distilled water, ethanol and methanol. The dried residues thus obtained were stored in screw capped vials at - 4°C. Phytochemical studies were carried out by following methods:

Test for alkaloids:

1. Hager's test: Test solution was treated with few drops of Hager's reagent (saturated picric acid solution). Formation of yellow precipitate showed the presence of alkaloids.

2. Mayer's reagent and Wagner's Reagent: The plant extract was warmed with 2% H_2SO_4 for two minutes, then it was filtered and few drops of reagents were added separately.

A) Mayer's reagent: A creamy white colored precipitate showed the presence of alkaloids.

B) Wagner's Reagent: A reddish-brown precipitate appears which also confirmed presence of alkaloids in the extract.

- **Test for Tannins:** The extract was mixed with basic lead acetate solution and formation of white precipitate indicated the presence of tannins.
- **Test for Cardiac glycosides:** To the solution of extract in glacial acetic acid solution, few drops of $FeCl_3$ and conc. H_2SO_4 were observed for the reddish-brown coloration at the junction of two layers and bluish-green color in upper layer.
- **Test for saponins:** Frothing test: About 5 ml of filtrate was diluted with 20ml of water and shaken vigorously. A stable forth upon standing showed the presence of saponins.
- **Test for Steroids and Terpenoids:** 4mg of extract was treated with 0.5ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of H_2SO_4 was added and res violet color was observed for terpenoids and green - bluish color for steroids.

Test for Flavonoids:

- **Ferric-chloride test:** Few drops of $FeCl_3$ solution were added to the extract which formed the black color indicated the presence of flavonoids.
- **Lead-acetate solution test:** To the extract was added a few drops of lead acetate (10%) solution which resulted in the yellow precipitate thus indicating the presence of flavonoids.

C) High Performance Liquid Chromatography: The methanolic leaf extracts were analyzed using HPLC (Shimadzu, 2LC-10 ATVP pumps, SPD-10AVP, UV - visible detector, Rheodyne injector with 50 μL loop. The data was acquired and processed using Shimadzu LC- solution version 6.42 software. Phenomenex C18 column (250mm×4.6mm, I.D., 5μm) and 0.4% aqueous acetonitrile containing 0.1% (v/v) phosphoric acid buffered to pH 3.5 with triethylamine was used as mobile phase. The mobile phase was filtered through 0.22μm membrane filter and degassed by sonication for 10 mins. Injection volume was adjusted to 20μL and detection was made at 260nm.

RESULTS:

Anatomical Study of the Plants: The comparative account of anatomical features of plant parts such as leaf and stem are shown in the **Table 1** and **2**.

TABLE 1: COMPARATIVE ANATOMY OF STEM OF TWO SPECIES

Cestrum diurnum	*Cestrum nocturnum*
Epidermis: It is made up of single layer of uniseriate elongated rectangular row of cells. Cortex: It is made up of many layers of parenchymatous cells along with air cavities. Endodermis: Cells are compactly arranged in two three layers around the vascular tissues and forms circular rings around the vascular tissue. Vascular tissues: Secondary growth is clearly visible, outer and inner phloem are prominent. Secondary xylem is visible between phloem region and vessels are large and prominent. Secondary xylem forms an extensive compactly arranged region. Pith is prominent showing round parenchymatous cells. Calcium oxalate crystals are seen in patches in the pith	Epidermis: It is made up of multiseriate rows of small parenchymatous cells. Cortex: Epidermis is followed by cortex having patches of chollenchyma along with parenchymatous cells. Endodermis: It is not very prominent and forms a wavy ring around the vascular tissues. Vascular tissues: Secondary growth is visible with small outer and inner phloem region. Vessels are small and pith has compactly arranged small parenchymatous cells as compared to *C. diurnum*

TABLE 2: COMPARATIVE ANATOMY OF LEAF OF TWO SPECIES

Cestrum diurnum	*Cestrum nocturnum*
Epidermis: It is made up of single layer of cells usually small towards the centre and elongated towards the outside. Collenchyma: Patches of rounded cells below epidermis on both upper and lower sides. Parenchyma: A layer of parenchymatous cells surrounding the vascular tissues. Vascular bundles: An oval-shaped central ring of vascular bundle is present which has phloem on both the sides and xylem in the centre, *i.e.* bi-collateral and open vascular bundle is present. Lamina: Mesophyll is differentiated into spongy and palisade parenchyma. Palisade parenchyma is visible towards the abaxial side in 2-3 layers.	Epidermis: Uniseriate row of parenchymatous cells are present. Collenchyma: Single layer of collenchymatous cells is present on both upper and lower epidermis. Parenchyma: Parenchymatous cells are present around vascular tissues. Vascular bundles: Stele is crescent-shaped present in the centre, open vascular bundle is present Lamina: Mesophyll is differentiated into palisade and spongy parenchyma. Palisade parenchyma is visible

Cestrum diurnum Stem:

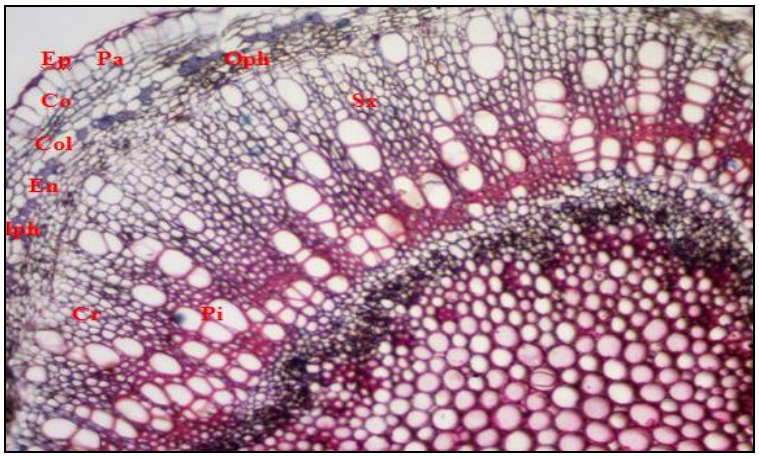

FIG. 1: T. S. STEM *CESTRUM DIURNUM* SHOWING Ep-Epidermis, Co-Cortex, Col- Collenchyma, Pa-Parenchyma, En-Endodermis, Oph- Outer phloem, Sx- Secondary xylem, Iph- Inner phloem, Pi- Pith

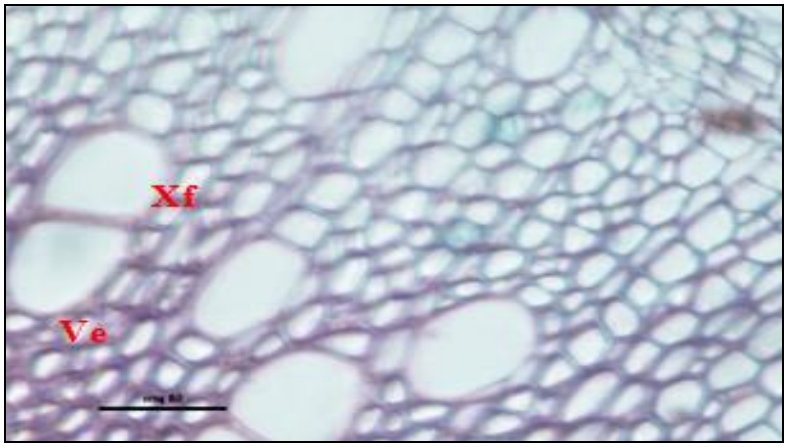

FIG. 2: T.S. STEM *CESTRUM DIURNUM* SHOWING VESSELS AND XYLEM FIBRES

Cestrum diurnum Leaf:

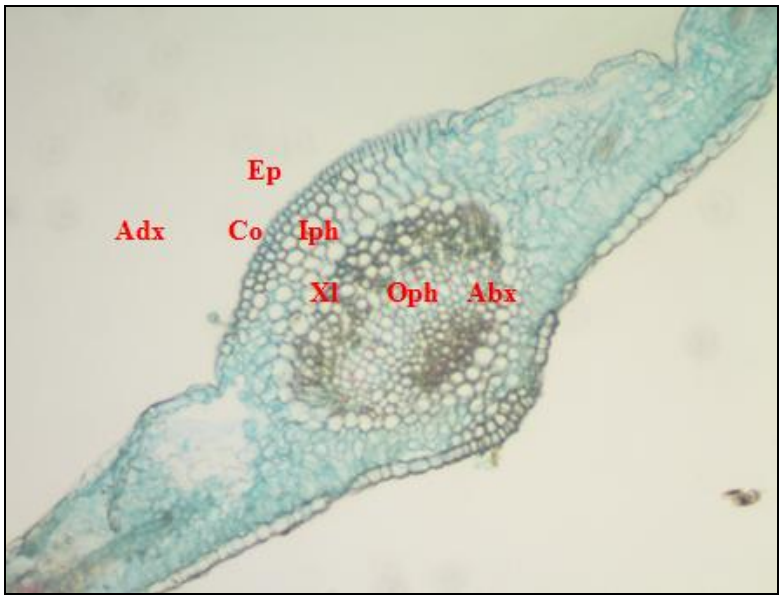

FIG. 3: T.S. *CESTRUM DIURNUM* LEAF MIDRIB SHOWING Adx- adaxial side, Ep- Epidermis, Co- Collenchyma, Iph- Inner phloem, Xl- xylem fibres, Oph-Outer phloem and Abx- Abaxial side.

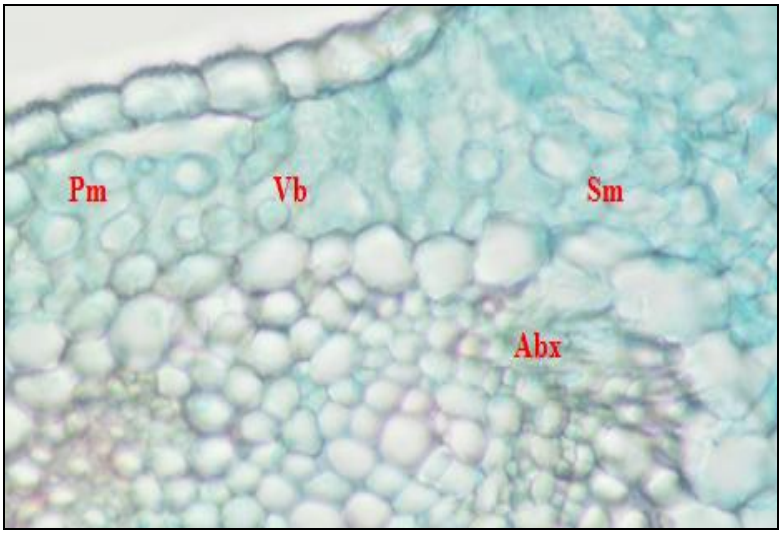

FIG. 4: T.S. *CESTRUM DIURNUM* LEAF LAMINA SHOWING Adx- adaxial side, Ep- epidermis, Pm- palisade mesophyll, Vb- vascular bundles, Sm- Spongy mesophyll and Abx- abaxial side.

***Cestrum nocturnum* Stem and Leaf:**

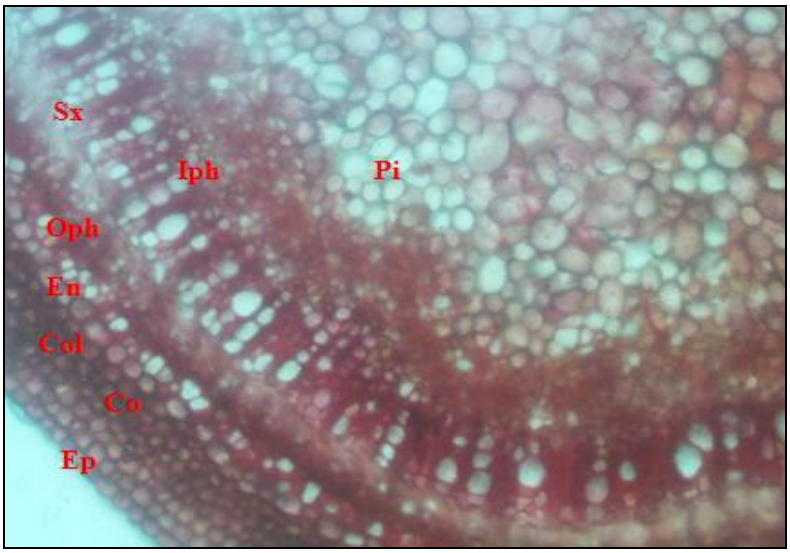

FIG. 5: T.S. STEM *CESTRUM NOCTURNUM* SHOWING Ep-Epidermis, Co-Cortex, Col- Collenchyma, Pa-Parenchyma, En- Endodermis, Oph- Outer phloem, Sx- Secondary xylem, Iph- Inner phloem, Pi- Pith

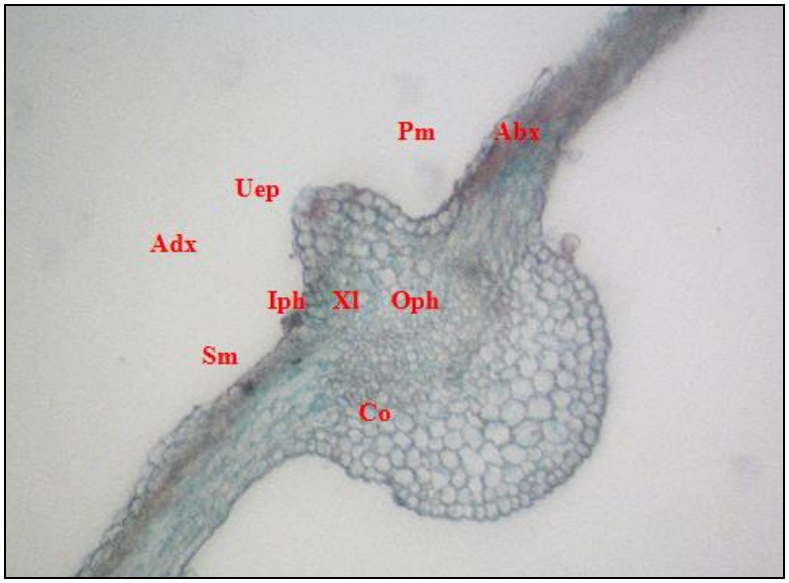

FIG. 6: V.S. *CESTRUM NOCTURNUM* LEAF MIDRIB SHOWING Adx- Adaxial side, Ue- upper epidermis, Pm- palisade mesophyll, Iph- inner phloem, Xl- xylem, Oph- Outer phloem

Phytochemical Studies: Different phytochemical constituents analysed from two species of Cestrum are given below: The qualitative analysis revealed the presence of alkaloids, glycosides,saponins and flavonoids in all three extracts in both the species.

TABLE 3:PHYTOCHEMICAL STUDIES ON *CESTRUM DIURNUM* LEAF EXTRACT

Phytochemicals	Aqueous	Ethanol	Methanol
Alkaloids	+	+	+
Glycosides	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Terpenoids	-	+	+
Tannins	+	+	+
Steroids	-	+	+

+ (Present), - (absent)

TABLE 4:PHYTOCHEMICAL STUDIES ON *CESTRUM NOCTURNUM* LEAF EXTRACT

Phytochemicals	Aqueous	Ethanol	Methanol
Alkaloids	+	+	+
Glycosides	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Tannins	-	+	-
Steroids	+	+	+

Active Principle Analysis By HPLC:

HPLC profiles of both the species: In the present study, it has been observed that in case of *Cestrum diurnum* maximum peak area was observed as 20530 with retention time 4.345 whereas in case of *C.nocturnum* the maximum peak area was observed at 808 with retention time 4.340. The peaks were observed by comparing with the reference standard which showed the maximum retention time of 4.578. Hence from the chromatograms it is clearly observed that *Cestrum diurnum* has maximum peak area with more amount of alkaloid content present as compared to *C. nocturnum*. One more peak has been identified which is of nornicotine but its has less retention time *i.e.* 3.796 and 3.604 in *C. diurnum* and *C. nocturnum* respectively. The chromatograms of HPLC profile of standard, and both the species have been given in the **Fig. 7, 8** and **9** respectively.

TABLE 5: PEAK TABLE

Sample ID	Area	Concentration	Nicotine present(µg/ml)
Cestrum diurnum	20540	2.52290582	5.045812
Cestrum nocturnum	808	0.78206373	1.564127

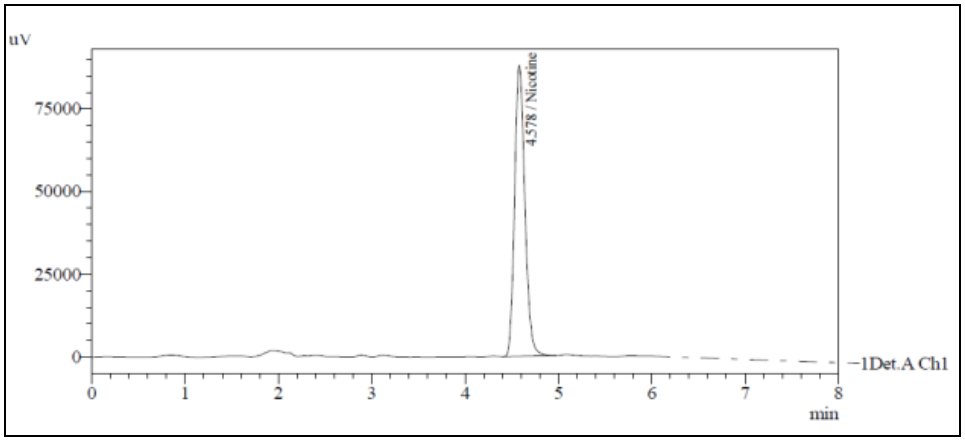

FIG. 7: HPLC CHROMATOGRAM OF NICOTINE(STANDARD)

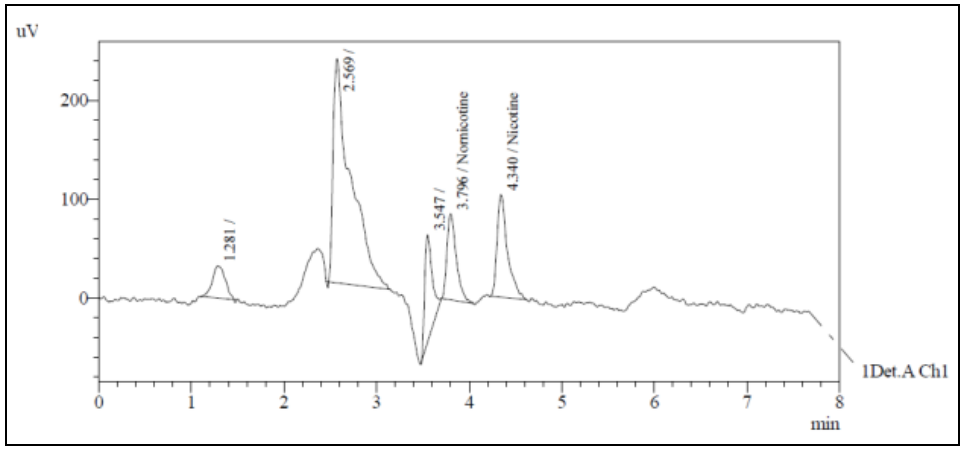

FIG. 8: HPLC CHROMATOGRAM OF *CESTRUM DIURNUM* LEAF EXTRACT

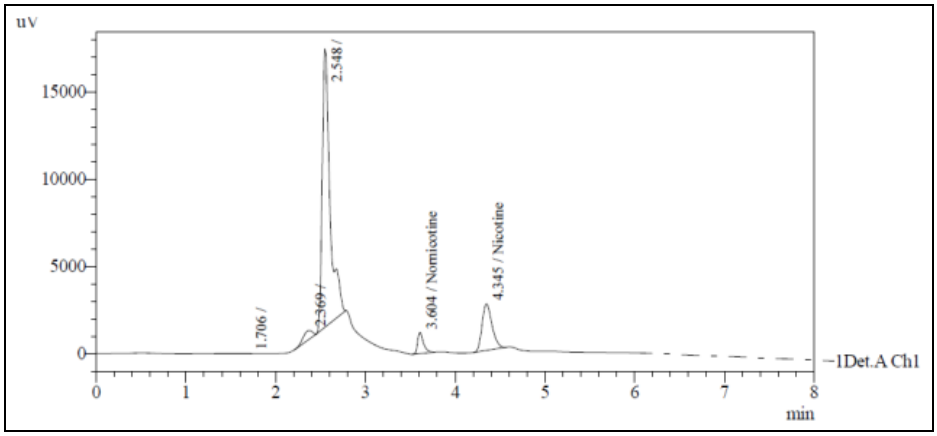

FIG. 9: HPLC CHROMATOGRAM OF *CESTRUM NOCTURNUM* LEAF EXTRACT

DISCUSSION: Qualitative screening of phyto-chemical constituents reveal the presence of alkaloids, flavonoids, saponins, glycosides in all the extracts in the **Table 3** and **4** whereas tannins are absent in aqueous and methanolic extracts of *Cestrum nocturnum* and steroids and terpenoids are absent in aqueous extracts in *Cestrum diurnum*. Pharmacological studies have demonstrated that the leaf extracts possess nicotine as major alkaloid along with nornicotine which is found to be maximum in *Cestrum diurnum* as shown in the **Fig. 8** and **9** through its peak area when studied through HPLC.These alkaloids possess wide range of medicinal properties such as antibacterial, antiinflammatory, antispasmodic, expectorant and vermifuge properties [8]. The anatomical studies of the part(s) used help to identify the distinguishing features. Since the drug is primarily obtained from leaves these have been studied in detail to prevent adulteration of commercial drug by users. Certain diagnostic features of morphology and anatomy have been found useful for the correct identification of species of *Cestrum* plant in the field to obtain the drug in its purest form.

CONCLUSION: Approaches like screening, phytochemical profiling of these plants helps to find out elite species. The correct identification of the herbal drugs of commerce help to check piracy of these drugs and hence make available true botanicals to consumers and manufacturers of drugs for its correct use as medicine which depends on the exact amount needed to make it more efficient source of medicine for its use in the best possible way.

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CONFLICTS OF INTEREST: Nil

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