

**SEPARATION AND IDENTIFICATION OF ACTIVE CONSTITUENTS OF CALOTROPIS GIGANTEA LATEX, BY HPLC, FTIR, UV-VISIBLE AND CLASSICAL TECHNIQUES.**

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### ABSTRACT

*Calotropis gigantea* is a well known Indian medicinal plant, distributed in most parts of India. Medicinally important parts of this plant are flower, terminal leaf pairs, root with root bark and latex. Out of that the most significant part which drew attention of researchers is latex because of its medicinal properties. Some parts of plant have laxative property and beneficial in skin diseases, promotes digestive power. It is a very good purgative, in ancient ayurvedic medicine, the plant *Calotropis gigantea* is known as “Sweta Arka” and *Calotropis procera*

as “Raktha Arka”. Both of them are often similar in their botanical aspects and also have similar pharmacological effects. In present work Isolation and Characteration of calotropis gigantea latex, were done by HPLC, FTIR, UVVisible and classical techniques.

**KEYWORDS:** *Calotropis gigantea*, sweta arka, terminal leaf pairs, akado, latex.

### INTRODUCTION

The milky latex contains hydrocarbons, fatty acids, sterols and terpenels. Their active principles are calactin, calotropin, calotoxin, and uscharidin.<sup>[1]</sup> The flowers, leaves, rootbark, and milky juice of calotropis are extensively used in the form of poultice, tincture, powder, and snuff. Latex is a natural plant polymer secreted by highly specialized cells known as laticifers. Latex is milky fluid secreted by ducts of laticiferous tissue.<sup>[2]</sup> Mainly flow inside laticifers including roots, stems, leaves and fruits of all flowering plants.<sup>[3]</sup> It is an emulsion

like sticky material that exudes from various plant parts after having a small tissue injury. In most plant species latex is squirt out as white glue from bark of plants. It is a complex mixture of proteins, alkaloids, starch, sugars, oils, tannins, resins and gums.<sup>[4]</sup> In most plants, latex color is normally white, yellow, orange, or scarlet but its colour changes after an air exposure.

## MATERIAL AND METHODS

*Calotropis gigantea* plants latex from leaves were aseptically collected into the three different beakers having the solvents methanol, ethanol, and water respectively for the extraction of glycosides and alkaloids and other material which is subjected to further study.<sup>[5]</sup> After 4-5 hours ethanol and methanol dissolved samples were filtered through the Whatman filter paper, and sample dissolved in water were filtered after 7-8 hours through the Whatman filter paper, filtrated ethanol and methanol samples were concentrated by evaporating the solvents. The water dissolved sample is extracted by separating funnel by using the diethyl ether, organic layer of extracted sample of diethyl ether were concentrated and shaken with the aqueous acid (10% HCL) to get separation of alkaloids and glycosides. Solvents used for extraction and purification were from MERCK, HPLC grade.

### Thin layer chromatography

In thin layer chromatography silica gel is coated on the glass plate. A solvent or mixture is selected and kept in a wide mouth container having a top cover to it. The plate is placed in this container such that the liquid solvent does not touch the line marked with spot with a pencil. The number of spots that come on the silica plate depends on the solvent used, and number of compounds present in the dissolved liquid to be spotted on the line on the plate. Selection of the solvent is done keeping in mind the polarity and non-polarity of the solvent. The Rf value was calculated in different phases, some are mentioned below.

**Water Extract** Solvent system: chloroform: ethanol (7:2.5) Rf values obtained are 0.70; 0.83; 0.96; for Uscharidin, Calotoxin, Calactin respectively.

**Methanol extract** Solvent system: chloroform: ethanol (7:3). Rf values obtained are 0.77; 0.86; 0.94; for Uscharidin, Calotoxin, Calactin respectively.

**Ethanol extract:** Solvent system: chloroform: ethanol (7:2.5) Rf values obtained are 0.74; 0.94; 0.96; for Uscharidin, Calotoxin, Calactin respectively.

**Test for alkaloids** Mayer's Test (Reagent):\_ (potassium mercuric iodide solution) to the 2 ml extract in a test tube, picric acid solution was added after some minute, orange colour was developed. It indicates the presence of alkaloids in the sample.

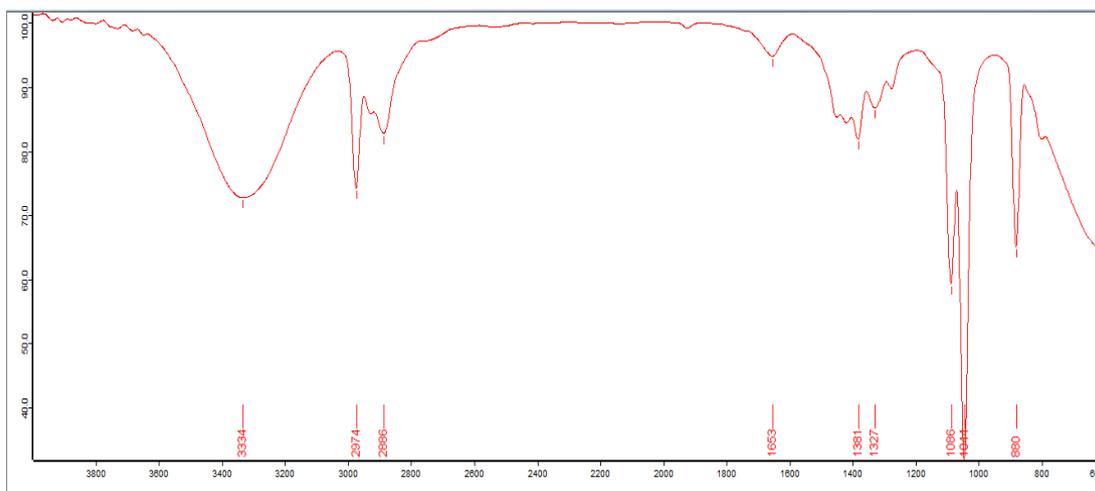
**Hagerac's Test** To the 1 ml of extract in a test tube 3ml of reagent (saturated aqueous solution of picric acid), were added, yellow precipitate indicates the presence of alkaloids.

**Test For Tannins** 0.5 g (powdered sample of *Calotropis* dried extract) was boiled in 20 ml water in a test tube, to its filtrate 0.1 % of ferric chloride were added, which gives a brownish green colour, shows the presence of Tannin.

**Tannins** are generally found in woody plants. Tannins are of two types hydrolyzed tannins or condensed tannins. This property of tannins to bind proteins, is used in leather industry for tanning of leather.

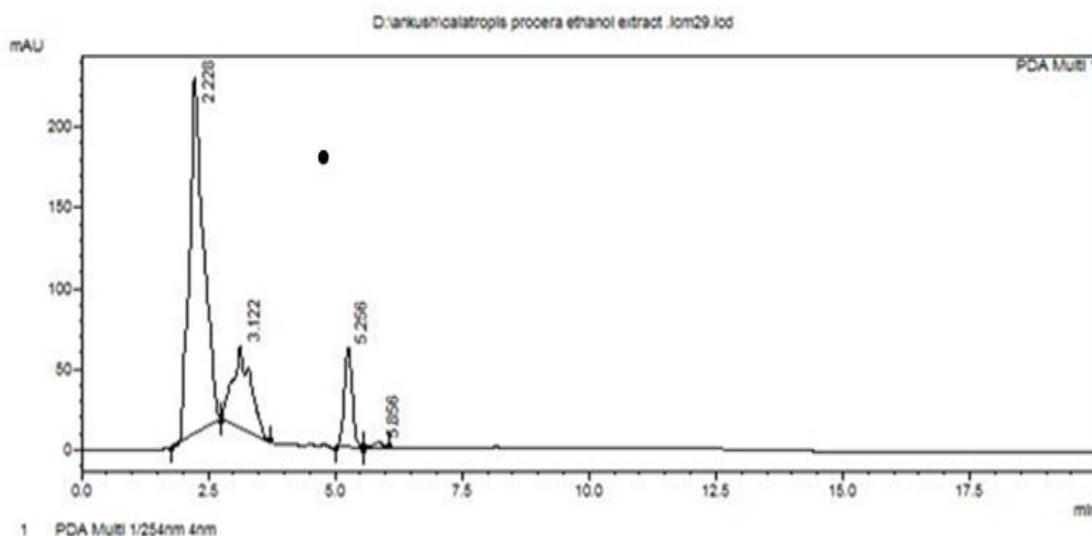
**Test For Cardiac Glycosides** To the 5 ml of the extract filtrate 2 ml of glacial acetic acid and 1 drop of ferric chloride solution were added, followed by 1ml conc. Sulfuric acid along the side of test tube, this gives a brown ring at the interface which changes to greenish after some time in acetic acid. This proves presence of cardiac glycosides.

**IR spectra:** The absorption bands at 1100 – 1000  $\text{cm}^{-1}$  in the fingerprint region indicate several modes such as C-H deformation or C-O or C-C stretching, pertaining to carbohydrates. The stronger the relative intensity of the band, the higher the chemical constituents. From the IR obtained, figure.1, it shows that the bands 3434  $\text{cm}^{-1}$  (O-H) stretching, 2974  $\text{cm}^{-1}$  C-H Stretchig planer due to ring ( $\text{CH}_3\text{CH}_2$ ), 2886  $\text{cm}^{-1}$  C=O-H, 1663  $\text{cm}^{-1}$  (C=O), 1391  $\text{cm}^{-1}$  (C-O), 1086-1386  $\text{cm}^{-1}$  Stretching (C-O,C-C) and 1044  $\text{cm}^{-1}$  Stretching (C-O) confirm the presence of Calotropogenin and Calotropin.



**Figure 1: IR Spectra of Calotropis gigantea.**

**HPLC :** Solvent system used were acetonitrile water (60:40), flow rate 1 ml/min, run time 10 min. pump pressure max 380 psi, column- C18, detector PDA, model Shimadzu, solvent quantity 20 micro liters. The sample of latex dissolved into the ethanol were filtered through the Whatman filter paper no.1 and injected for HPLC analysis, the following peaks were obtained figure. 2.

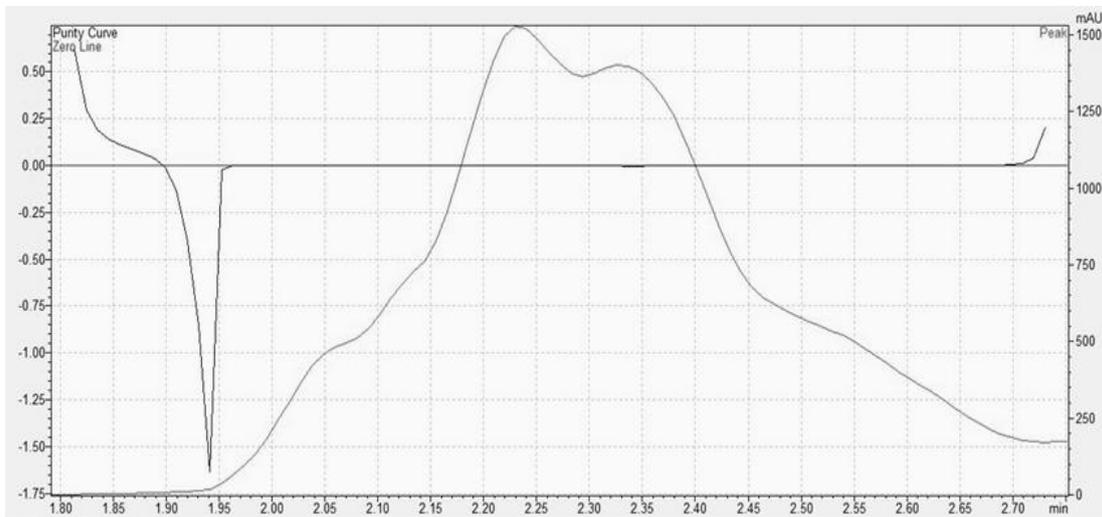


**Figure 2. HPLC Retention Time Peaks**

The peaks with retention times in ethanol extract is found to be 2.2, 3.1, 5.2 respectively Table1. These are the peaks of *Calotropis Gigantea*, which may be calatoxin, calotropin, calactin. The peak impurities and peak purity is shown in fig. 3 and 4.

**Table1. Retention time**

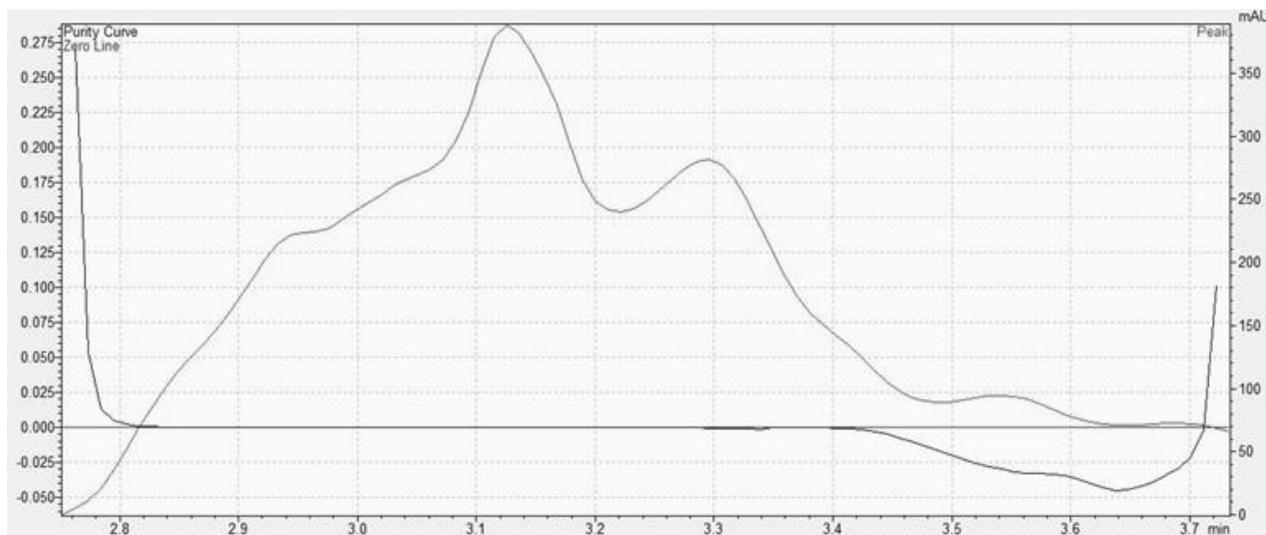
Sr. No.	Peak	Retantion Time	Concentration	Area	Height
1	1	2.234	67.77041	3138948	167628
2	2	3.122	17.31792	802121	29445
3	3	5.256	14.03909	650254	61151



**Figure 3: Purity Index of sample.**

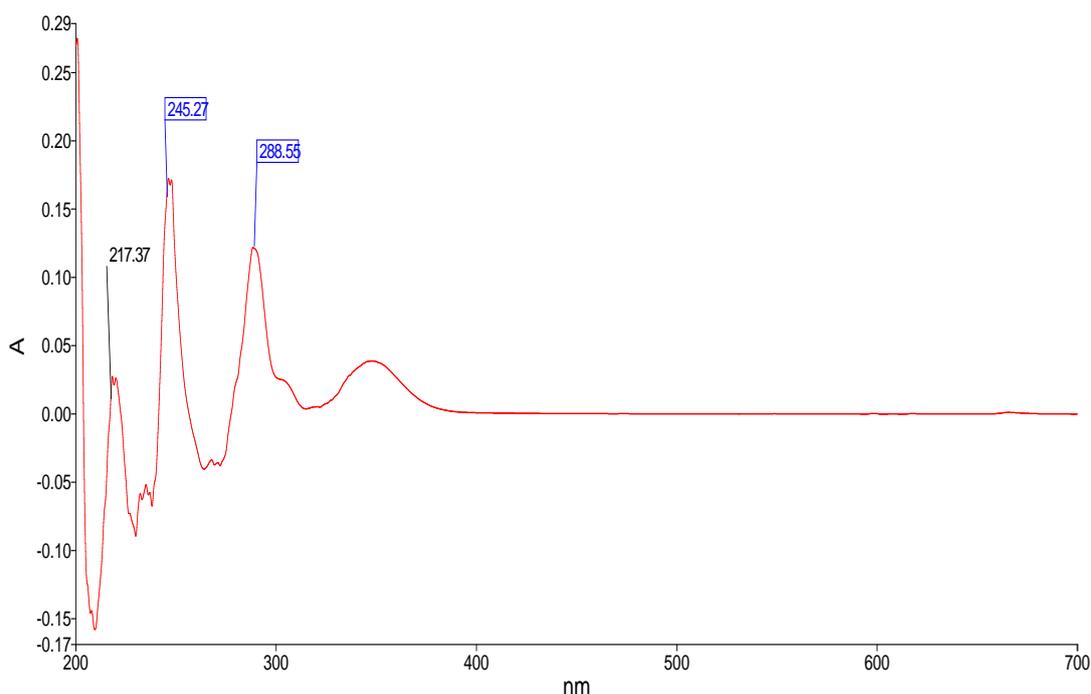
Purity index shows, detected at 1.94minute, pick purity index is -0.709386, single point threshold 0.922612 and minimum purity index is -1631998, figure. 3.

Impurity index shows, detected at 3.64 minute, pick purity index is 0.946405, single point threshold 0.991337 and minimum pick purity index is -44931 fig.4



**Figure 4: Impurity Index of sample.**

**UV-Visible:** The sample of *calatropis gigantea*, were analyzed by uv visible spectrophotometry of Perkin Elmer at the Government Institute of Forensic Science Aurangabad. The sample was prepared in ethanol, the absorbance and the lambda max of the alkaloids and glycosides containing sample is obtained. The UV of *Calatropis Gigantea* lambda max is found to be at 217.37 nm, 245.27nm and 288.55nm. The lambda max of *Calatropis Gigantea* glycosides indicates the presence of glycosides or alkaloids in the extracted sample. these may be calactin, calotropin, calotoxin Figure. 5.



**Figure 5: UV-Visible absorption.**

## RESULT AND DISCUSSION

The active constituents of *Calotropis Gigantea* such as alkaloids are studied by different instrumental techniques and spot tests. These glycosides or alkaloids are hazardous to human health when it is used by any intension such as to kill the person or blind the person.<sup>[6]</sup> The studied active principle has great potential for many reasons. It will establish various actions on the body when it is applied to the skin or eyes. When the juice is applied on the skin, it becomes red with formation of blisters which excoriate later, applied into eyes, it reduces conjunctivitis which may result in permanent impairment of vision. When ingested, it acts as a gastrointestinal tract and cerebrospinal poison.<sup>[7]</sup> There is an acrid better taste along with nausea, vomiting and diarrhea. Pupils are dilated and there may be titanic convulsion. Circulatory collapse and death may occur.

## CONCLUSION

The result of phytochemical screening of water, methanol and ethanol extracts of *Calotropis Gigantea* latex revealed the presence of alkaloids, such as calactin, calotropin, and calotoxin. Which is confirmed by colour test, TLC Rf values, HPLC retention time data, UV-absorbance and IR spectra. Though *Calotropis gigantea* has various medicinal applications, but it is the need of hour to explore its medicinal values at molecular level with the help of various biotechnological tools and techniques. Further studies should be conducted to elucidate the molecular mechanism of interaction of various plant based drugs with human in different diseases.

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