CHAPTER 4

CHROMATOGRAPHIC PROFILING OF THE ISOLATED COMPOUNDS

4.1 INTRODUCTION

Column Chromatography, Thin Layer Chromatography (TLC), Gas Liquid (GLC) and High Performance Liquid Chromatography (HPLC) are the major tools used for the separation, identification, purification, and quantification of chemical compounds. Of late computers and automation are added to the convenience of sophisticated HPLC systems and other Chromatographic techniques. HPLC techniques are much faster and accurate than the conventional Column and TLC techniques. We have used the HPLC techniques for our work to confirm the identity of the respective components in the extract(s) / fraction(s) of the plants.

Although HPLC is widely considered to be a technique mainly for biotechnological, biomedical, and biochemical research as well as for the pharmaceutical industries, these fields currently comprise only about 50% of HPLC users\(^1\). Currently HPLC is used by a variety of fields including cosmetics, energy, food, and environmental industries. The total HPLC system is shown in figure 4.1.

Chemical Separations can be accomplished using HPLC by utilizing the fact that certain compounds have different migration rates in a given particular column and mobile phase. Thus, the chromatographer can separate compounds from each other using HPLC; the extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase\(^2\).

Purification\(^3\)\(^4\) refers to the process of separating or extracting the target compound(s) from other (possibly structurally related) compounds or contaminants in a
mixture. Each compound should have a characteristic peak under certain chromatographic conditions. Depending on what needs to be separated and how closely related the samples are, the chromatographer may choose the conditions, such as the proper mobile phase, to allow adequate separation in order to collect or extract the desired compound as it elutes from the stationary phase.
The migration of the compounds and contaminants through the column need to differ enough so that the pure desired compound can be collected or extracted without incurring any other undesired compound.

Identification of compounds by HPLC is a crucial part of any HPLC assay. In order to identify any compound by HPLC, a detector must first be selected. Once the detector is selected and is set to optimal detection settings, a separation assay must be developed. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the
detection levels, which the assay will be performed. To alter the retention time of a compound, several parameters can be manipulated. The first is the choice of column; another is the choice of mobile phase, temperature and last is the choice in flow rate.

A sample of a known compound as reference must be utilized in order to assure identification and confirmation of the unknown compound. Identification of compounds can be assured by combining two or more detection methods.

Quantification of compounds by HPLC is the process of determining the unknown concentration of a compound in a known solution. It involves injecting a series of known concentrations of the standard compound solution onto the HPLC for detection. The chromatograph of these known concentrations will give a series of peaks that correlate to the concentration of the compound injected. (Figure 4.2)

Using the area of a triangle equation ($A = \frac{1}{2}bh$) to calculate the area under each peak, a set of data is generated to develop a calibration curve. This is done by graphing peak area vs. the concentration of the sample solution. From the graphing software, a best-fit line can be derived, and the equation of that line can be determined. This equation of a line, $y = mx + b$, generated by the data, is the calibration curve equation. (Figure 4.3).
The equation of the line is then used in the following manner: On injecting a sample of unknown concentration $x$ (x-axis of calibration curve) onto the HPLC, the chromatograph gives a peak output of area $y$ (y-axis of the calibration curve). The area $y$, is then in the equation of a line $y = mx + b$ from the calibration curve, and the concentration is found by solving the equation for $x$. Now a days, several sophisticated software are used in the HPLC system to calculate and quantify the actual concentrations of the unknown sample automatically from the data generated.

4.2 PRESENT WORK

The fruits of the plant *Chisocheton paniculatus* (*Meliaceae*) & *Cudrania javenensis* (*Moraceae*) have been selected and three compounds, viz. MK-001 and MN-01, MN-02 have been isolated from *Chisocheton paniculatus* and *Cudrania javenensis* respectively.

The HPLC profiling of the selected plant extracts, for identification of the signals of the compounds of our interest in the chromatogram have been undertaken.
4.3 EXPERIMENTAL

The collected cleaned fruits of *Chisocheton peniculatus* are first shed dried and finely powdered. The powdered fruits are extracted with Methanol in a soxhlet apparatus. The extract on concentration under reduced pressure, yielded a gummy material (F₁), which then, is fractionated with Petroleum ether (60°-80°). The petroleum ether extract is concentrated in a rotavopur at low pressure and temperature to obtain the white-crystalline solid, 6α-acetoxy azadirone⁶· m.p. 192°C, (MK-001) from this plant¹,⁷-¹⁰.

Similarly, the collected fruits of *Cudrania javenensis* are first washed with cold water and then shed dried and powdered. Finely powdered fruits are extracted exhaustively with petroleum ether (60-80°C), Chloroform and Methanol in cold condition and the extracts are concentrated under reduced pressure to yield a dark coloured material (F₂). The dried methanol extract is then partitioned between the following solvents separately.

a) Petroleum ether (60-80°C) and b) Chloroform

The isolated compound of *Cudrania javenensis* (MN-01) & (MN-O2) and these the fraction are analyze by HPLC method¹,⁷-¹⁰.

The HPLC(high performance liquid chromatography) analysis of the isolated compound and the extract fraction of different solvent are undertaken Perkin-Elmer 200(column c-18)quadrople pump system(run-in to binary condition),auto-injector,uv-diode array detector with flow rate .8ml/min at room temperature

The solvent used in the HPLC analysis were acetonitrall and .1% phosphoric acid.
4.4 RESULTS AND DISCUSSION

4.4(a) Fruits extract of *Cudrania javenensis*

In the HPLC analysis and spectral data (total analysis time 15 minute) of the plant fruits *Cudrania javenensis* for isolated and different solvent fraction are display in figure 4.4: (MN-01), figure 4.5: (MN-02), figure 4.6 (water), figure 4.7: (petroleum) figure 4.8: (chloroform) and figure 4.9: (methanol)

On studying the spectral data their peak it is agree that the isolated compound (MN-01) in figure 4.4 the peak time 4.05 min and the compound (MN-02) in figure 4.5 the peak time 3.33 min

Again on studying other spectral peak and data for different fraction of the extract in figure 4.6 (water) it is seen three major peaks at 3.33;3.39 and 4.04 min which shows that in extract of water fraction both the compound(MN-01) peak time 4.04 and the compound (MN-02) peak time 3.33 are present.

In the petroleum fraction in figure 4.7 there is only one major peak present at 3.43min, in which represent the compound (MN-02). In chloroform fraction in figure 4.8 there is also one major peak present which is represent the compound(MN-02).Lastly in the methanol fraction in figure 4.9 there are three major peak at 3.34 , 3.4.01 and 7.84 min. First two peaks of them represent the isolated compound (MN-02) & (MN-01) respectively.

4.4(b) Fruits extract of *Chisocheton peniculatus*

In the HPLC analysis spectra and spectral data (total analysis time 8 minute) of the plant fruits *Chisocheton peniculatus* for isolated and different solvent fraction are display in figure 4.10(MK-001), figure 4.11 (petroleum) figure 4.12(methanol)
On studying the spectral data their peak it is agree that the isolated compound (MK-001) in figure 4.10 the peak time 2.82 min.

Again on studying other spectral peak and spectral data for different fraction of the extract in figure 4.11 the petroleum fraction there is only one major peak present at 2.82 min, in which represent the isolated compound (MK-001). Lastly in the methanol fraction in figure 4.12 there are three major peaks at 2.86, 5.28 and 6.51 min. First one peak of them represents the isolated compound (MK-001).
Analysis of flavone

Analysed by G. N. Deka, D. F. S.

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Figure 4.4: Isolated compound MN-01
Analysis of flavone

Analysed by G.N.Deka, D.F.S.

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**Figure 4.5**: Isolated compound MN-02
Analysis of flavone

Analysed by G.N. Deka, D.F.S.

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Figure 4.6: Extract: Cudrania javenensis, fraction-water
Figure 4.7: Extract: *Cudrania javenensis*, fraction-petroleum
### Analysis of flavone

**Analysed by G.N.Deka, D.F S**

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**Figure 4.8:** Extract: *Cudrania javenensis*, fraction-chloroform
Analysis of flavone

Extract: *Cudrania javenensis*, fraction-methanol

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**Figure 4.9:** Extract: *Cudrania javenensis*, fraction-methanol
### Analysis of steroid

Analysed by G.N Deka Drugs & Narcotic Division, F.L. Assam

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**Figure 4.10:** Isolated compound MK-001
### Analysis of steroid

**Analysed by G N Deka Drugs & Narcotic Division, F.L.Assam**

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**Figure 4.11:** Extract: *Chisocheton peniculatus* fruits, fraction-petroleum
Analysis of steroid

Analysed by G N Deka Drugs & Narcotic Division, F.L Assam

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Figure 4.12: Extract: *Chisocheton peniculatus* fruits, fraction-methanol
REFERENCES: 4


**********
PART-III

MOLECULAR & SINGLE CRYSTAL STRUCTURE ANALYSIS