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# ISOLATION AND IDENTIFICATION OF NEW C-FLAVANOL GLYCOSIDES FROM *ALLIUM CEPA*

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## ABSTRACT

*In this study, penta hydroxy flavonoid quercetin-4-glycoside was isolated from the ethanolic extract of Allium cepa which was then eluted in the solvents chloroform and methanol. The crude ethanolic extract was collected and subjected to column chromatography and sub fractionated with solvent chloroform and methanol. Isolate was further processed and selected sub fraction was subjected to fresh column chromatography. Washing was continued till TLC shows a single spot observed under UV at 254 nm and 366nm after spraying ANS reagent. Quercetin-4-glycoside was established on the chemical and spectral analysis and HPLC-PDA was developed for the standardization and estimation of compound. The results of analysis of the compound suggested that the compound may be used as a marker for standardization, and the developed isolation method is cost effective compared to all other existing methods reported in literature.*

***Keyword**: Allium cepa, Flavonoid, Quercetin-4-flavonoids.*

## 1. INTRODUCTION

Allium cepa has been cultivated and used as a nutrient for more than 6000 years[1].Onion are versatile and are often used as an ingredient in many dishes and are accepted by almost all traditions and culture[2,3]. The nutritional composition of onion is very complex. Onions are rich in two chemical groups that have perceived benefits to human health. These are the flavonoid and the alk(en)yl cystine sulphoxides (ACSOs).Two flavanoid subgroup are found in onion, the anthocyanins, which are impart a red/purple color to some varieties and flavanols such as quercetin and its derivative responsible for brown skin of many other varieties.

People detected therapeutic properties of the plant and used it in traditional and folk medicines. Compounds from onion have been reported to have a range of health benefits which include anti carcinogenic properties, antithrombotic activity and antibodies effect [4-6]. The use of plant extracts and *photochemical*, both with known antimicrobial properties have been identified by the WHO-2001.It is the necessities of the microorganism are increasingly acquiring resistance to currently available antibodies.

Active constitute are related to compounds in the plant which is directly responsible for therapeutic activity of the plant. The investigation of the bioactive natural products has assumed a greater sense of urgency in response to the expanding human population.

**1.1 Plant profile**

*Allium cepa* is weekly is known as bulb onion, garden onion and common has a rich history of use a rich history of use in India. It is a large herbaceous biennial, commonly found throughout the greater parts of the country. It has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutics properties.

The scientific classification of the plant is summarized below[7]:

Domain: EUKARYA

Kingdom: PLANTAE

Division: ANGIOSPERMS

Class: MONOCOTS

Order: ASPARAGALES

Family: ALLIACEAE

Genes: ALLIUM

Species: CEPA

The aim of the present study was to isolate, identify and standardize flavonoids of Allium cepa by chromatographic technique.

## 2. MATERIALS AND METHODS

**2.1 Materials**

The air dried raw material of *Allium cepa* bulb was collected from Natural Remedies Private Limited, Bangalore. All the reagents are obtained from Natural Remedies Pvt.Ltd. Methanol, Ethyl acetate, Petroleum ether, Acetonitrile and water (HPLC grade).

**2.2 Extraction and Fractionation**

**2.2.1 Preparation of column-1**

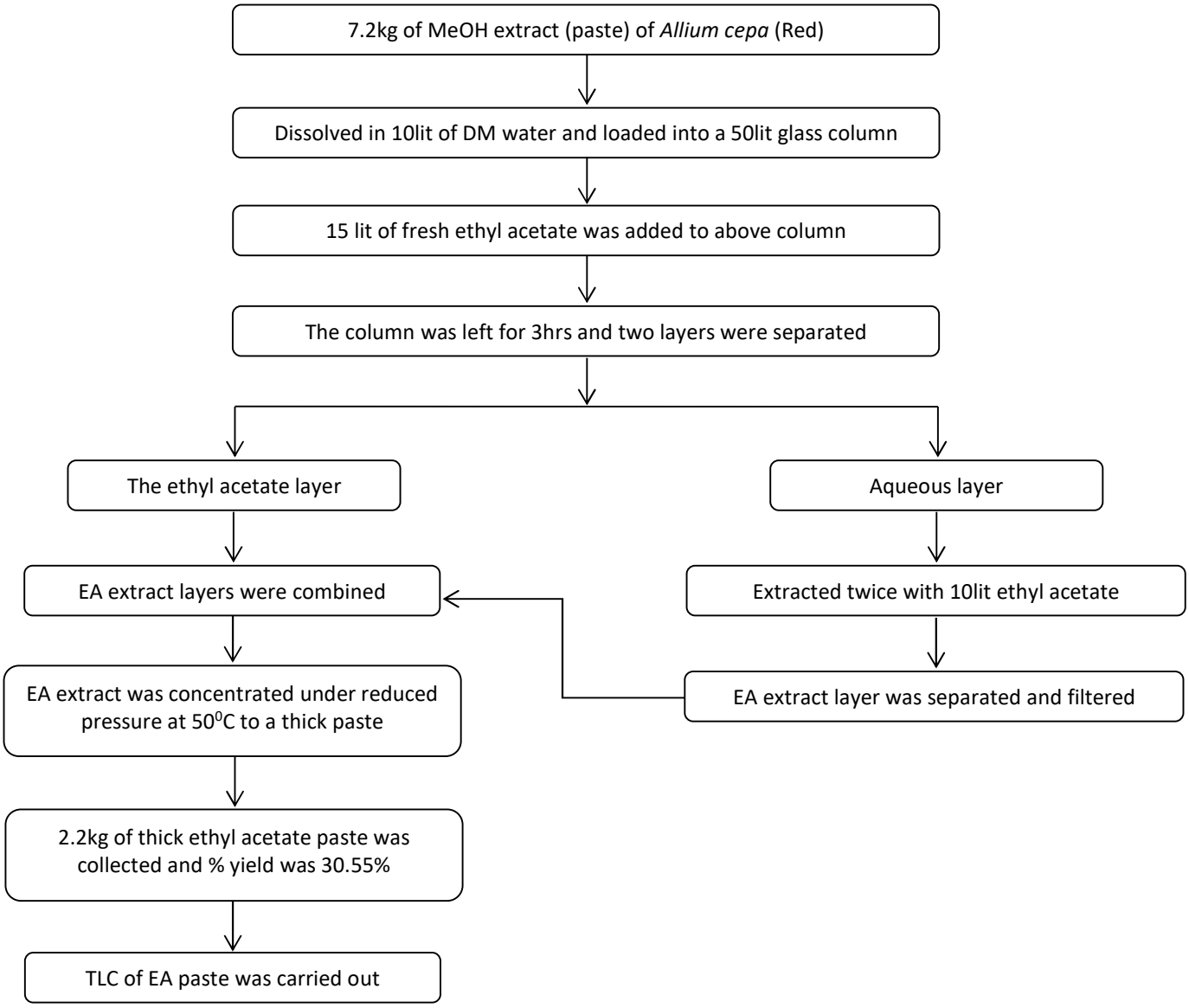
2.2kg of ethyl acetate extract was mixed with 2.2kg of silica gel (#120-200) by addition of pet. ether to form a paste and it was dried on water bath to form powder which was used to charge the **column 1.** A cotton layer was placed at the bottom and the glass column was filled with 5lit of pet. ether. 400gm of silica gel (#120-200) was added slowly. Then the solvent was eluted slowly during silica gel packing to remove the air bubbles. Then the solvent was eluted up to the level of the column bed and the dried ethyl acetate extract was charged in the column. Another layer of cotton was placed over the charged ethyl acetate extract paste to prevent the disturbance of the extract bed while pouring the eluting solvent from the top.

The charged column was left overnight for complete saturation and removal of air bubbles to make the bed static. All the fractions collected were dried in rotavapour (Buchi R-114). The fractions collected from the column 1 were shown in **Flow chart no. 1**. All the fractions were subjected to TLC for the identification of the phytochemical constituents.

**2.2.2 Preparation of column-2**

30gm of fraction-5 of column-1 was mixed with 70gm of flash silica gel (#60-120) by addition of chloroform to form a paste and it was dried on water bath to form powder which was used to charge the **column-2**. A cotton layer was placed at the bottom and the glass column was filled with 1.5lit of chloroform. 280gm of silica gel (#120-200) was added slowly. Then the solvent was eluted slowly during silica gel packing to remove the air bubbles. Then the solvent was eluted up to the level of the column bed and the dried fraction-5 of column-1 was charged in the column-2. Another layer of cotton was placed over the charged fraction-5 of column-2 to prevent the disturbance of the extract bed while pouring the eluting solvent from the top. The charged column-2 was left overnight for complete saturation and removal of air bubbles to make the bed static. All the collected fractions were dried in rotavapour (Buchi R-114). The

fractions collected from the column-2 are shown in flow chart no. 2. All the fractions were subjected to TLC for the identification of the phytochemical constituents (Refer Fig.-1, Fig.2 and Fig. 3).




**Extraction process of methanolic extract**

**2.2.3 Purification of fraction-8, 9 and 10 of column-2:** Fraction-8, 9 and 10 showed a single spot in TLC with little color which was decolorized by crystallization with methanol. We got brick red colored compound. After purification again TLC was carried out to get single spot and TLC was shown in figure-4. The isolated compound was UV sensitive also visualized after spraying with ANS reagent. The purity of the compound was analyzed by HPLC method. The characterization of the compound was done by Melting point, FTIR, $^1$H-NMR, $^{13}$C-NMR and Mass spectroscopy respectively. This compound was identified as Quercetin 4'-glycoside.

**2.3 HPLC Analysis**

One miligram of compound was dissolved in 1ml of HPLC grade methanol and analyzed using chromatographic parameters mentioned in table-1.

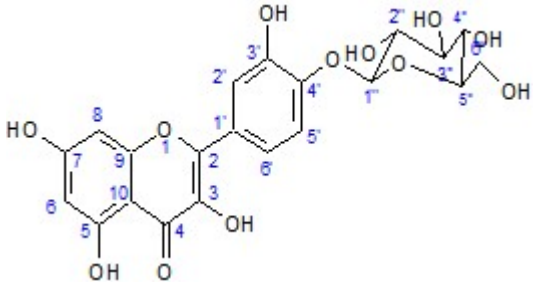
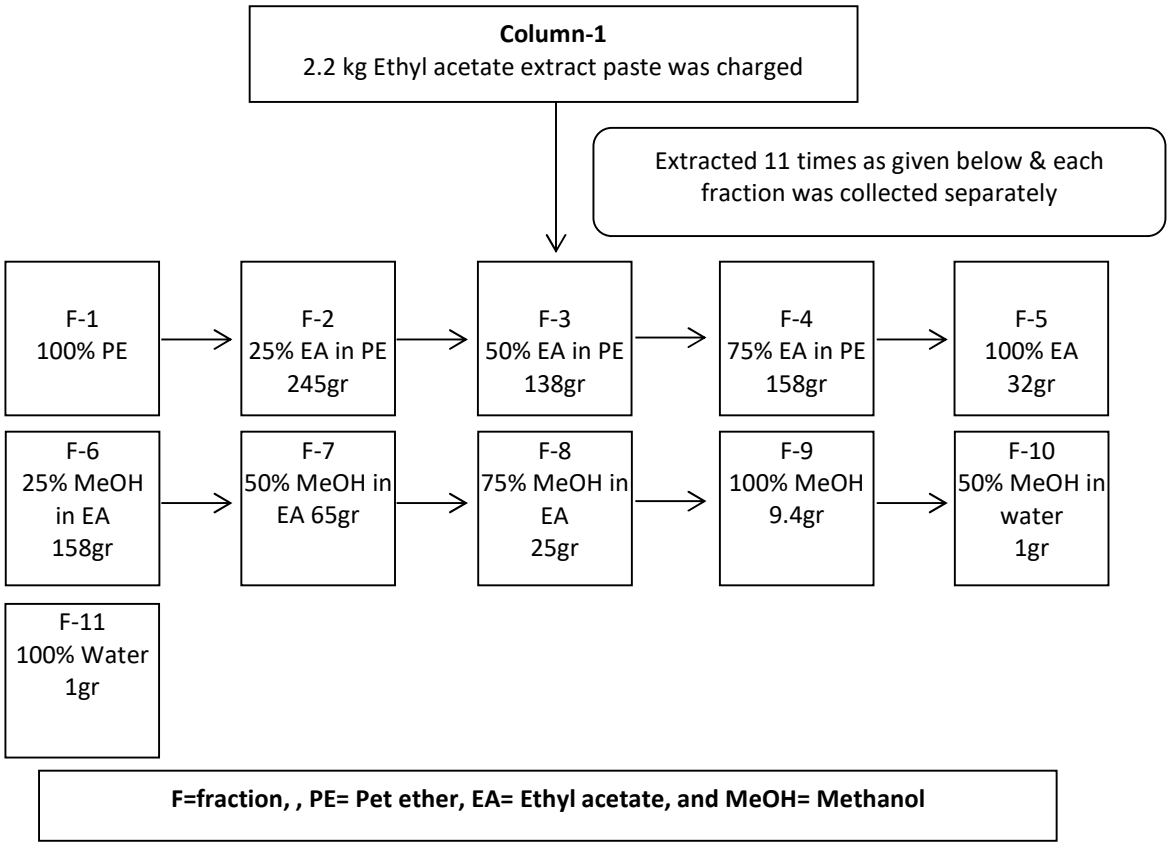


**Fig. 1(a):** Structure of Quercetin 4'-glycoside

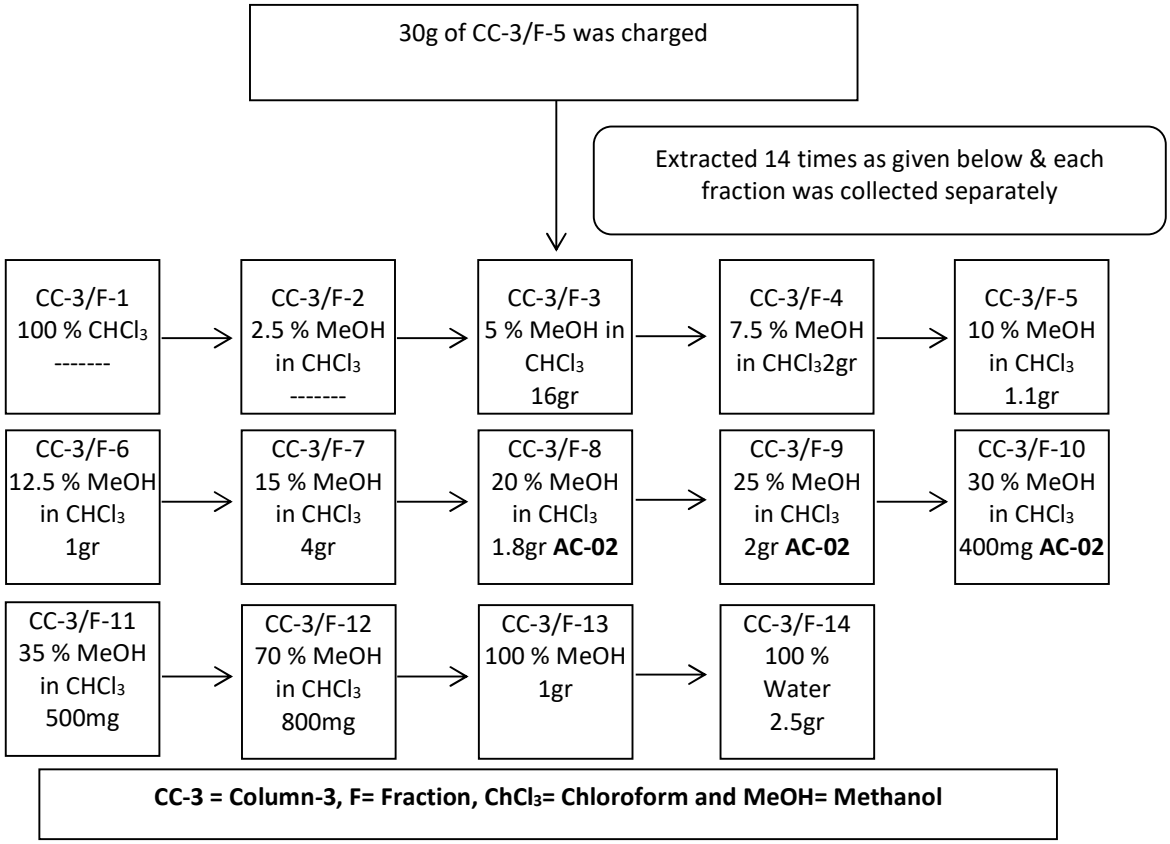
**Table-1:** HPLC Chromatographic conditions

| | | | |
|---|---|---|---|
| **Column** | KROMOCIL C-18 (250*4.6*7µ) | | |
| **Flow rate** | 1.5ml/min | | |
| **Detection** | 254nm | | |
| **Mobile phase** | Pump A (136mg $KH_2PO_4$+ 0.5ml $H_3PO_4$+ HPLC water q.s. to 1000ml)<br>Pump B ( Acetonitrile) | | |
| **Gradient time program** | Time | B conc. | A conc. |
| | 0.01 | 5 | 95 |
| | 18.00 | 30 | 70 |
| | 25.00 | 55 | 45 |
| | 28.00 | 55 | 45 |
| | 35.00 | 30 | 70 |
| | 45.00 | 5 | 95 |
| | 52.00 | Stop | - |
| **Injection volume** | 20µl | | |
| **Detector** | SPD-M10AVP photodiode array detector | | |

**Flow chart- 1 : Fractions collected from Column chromatography - 1**



**Flow chart- 2 : Fractions collected from Column chromatography - 2**




## 3. RESULTS AND DISCUSSION

The isolated portion from ethyl acetate fraction of the Allium cepa Linn was chromatographic over silica gel to afford flavanoid. The presence of the compound Quercetin-4-glycoside was established by using IR, NMR and MASS spectroscopy. Rf value (0.06) was obtained and melting point 238-240ºC uncorrected which coincided with the standard. The UV- Visible max 254,355nm; FTIR: 3375 $cm^{-1}$ (OH, str.), 1661.75 $cm^{-1}$ (C=O str.), 1615$cm^{-1}$ (Ar. C=C, str.) 1358.91 $cm^{-1}$ (C-OH; deformational vbr,) and 1245 $cm^{-1}$ (C-O-C; str.). TMS served as internal standards for $^{1}H$ NMR.$^{13}C$-NMR spectra was collected on commercial instruments with complete proton decoupling. NMR spectra of Quercetin 4'-glucoside showed peak at δ9.536 (s, 1H, OH at $C_3$) and δ9.325 (s, 1H, OH at $C_{3'}$) but it didn't show OH proton at $C_{4'}$ in Quercetin 4'-glucoside. Therefore it shows that the glycoside linkage at $C_{4'}$ through oxygen.

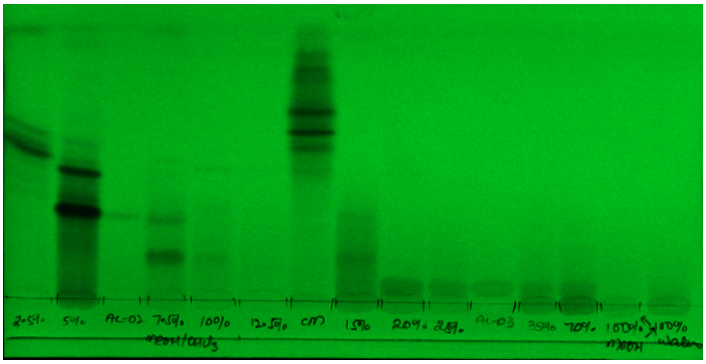


**Figure 1(b) :** TLC of all fractions 1-14 of column-02

Visualization of TLC of fractions 1-14 of column-3 at 254nm; solvent system: Toluene: Ethyl acetate: Formic acid (6:4:1).

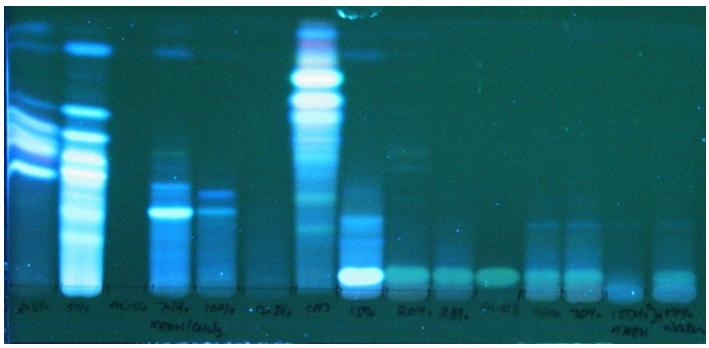


**Figure 2:** Visualization of TLC of fractions 1-14 of column-3 at 366nm; solvent system: Toluene: Ethyl acetate: Formic acid (6:4:1).

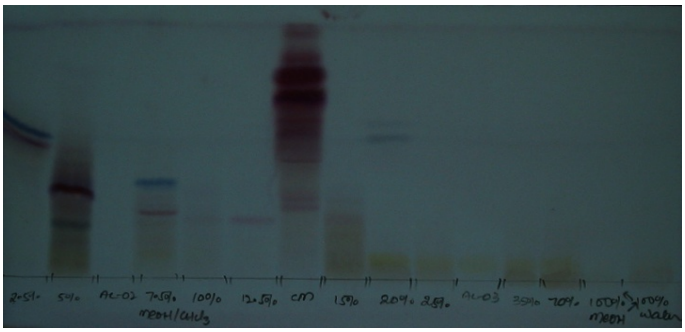


**Figure 3:** Visualization of TLC of fractions 1-14 of column-3 after ANS spray; solvent system: Toluene: Ethyl acetate: Formic acid (6:4:1).

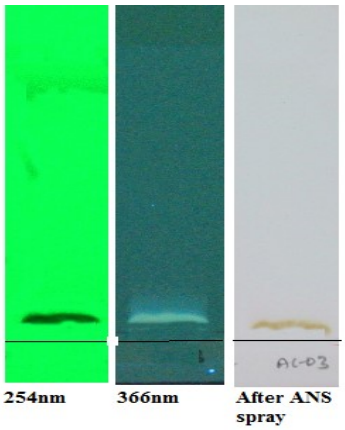



**Figure 4:** TLC of isolated compound

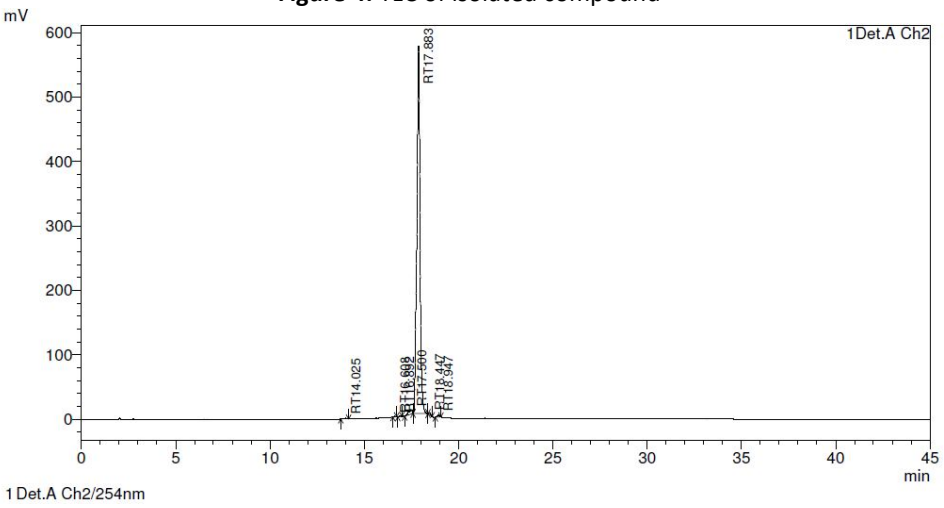



**Fig. 5:** Representative HPLC chromatogram

## 4. CONCLUSION

The simplicity of isolation and analysis of the compound suggest that the compound may be termed as marker for standardization, and its cost effective method compared to all other existing isolating method.

## 5. ACKNOWLEDGEMENT

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