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# ISOLATION AND CHARACTERIZATION OF PHYTOCONSTITUENT FROM METHANOL EXTRACT OF BARLERIA PRIONITIS LEAVES

**Pavankumar P. Wankhade[1*], Nitin B. Ghiware[2]**

[1]Ph.D Scholar, Department of Pharmacology, Centre for Research in Pharmaceutical Sciences, Nanded Pharmacy College, Nanded, Maharashtra, India

[2]Professor, Department of Pharmacology, Centre for Research in Pharmaceutical Sciences, Nanded Pharmacy College, Nanded, Maharashtra, India

*Corresponding Author; Email: : pavanw90@gmai.com

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**ABSTRACT**

The medicinal plants are believed to be a potential source of medicinal phytochemicals and have a great potential for discovery of new drug candidates. In the Ayurvedic medical system, *Barleria prionitis* Linn, also known as Kate Koranti, is a well-known medicinal plant. *Barleria prionitis* Leaves was exhaustively defatted using petroleum ether (BP-PE) and extracted successively with chloroform (BP-CH) and methanol (BP-ME) using Soxhlet apparatus. Methanolic extract was found potent hence fractionation of methanolic extract was done by column chromatography. Fraction of methanolic extract of Berleria prionitis were subjected to phytochemical screening for tannins, glycosides, steroids, terpenoids, flavonoids, and alkaloids. Isolation of phytoconstituent compound by using High Performance Thin Layer Chromatography (HPTLC). characterization of phytoconstituent was done by using FT-IR, IR, NMR, LC-MS and mass spectrum.

***Keywords*** : phytochemicals, *Barleria prionitis* Leaves, column chromatography, Isolation

## INTRODUCTION

Medicinal plants are used in the management of healthcare problems worldwide, and about 60-80% of the world's population still depends upon traditional herbal medicines (Bolta *et al*, 2000; Middleton *et al*, 2000; Dey *et al*, 2009) The global demand of herbal medicines is increasing rapidly because of their low cost and presumed safety of herbal medicines. Musayimi *et al*, 2008; Banerjee *et al*, 2012) The medicinal plants are believed to be a potential

source of medicinal phytochemicals and have a great potential for discovery of new drug candidates (Kayod *et al*, 2011, Hussain *et al*, 2012, Misra *et al*, 2012; Deshpande *et al*, 2014) Selected plant and its part leaves is less explored but is reported to possess important phytoconstituents of wide range which will be useful in respiratory disorders like asthma. Such constituents can be separated, fractionated and evaluated using modern tools for analytical, pharmacological and toxicological profile (Kayod *et al*, 2011) In the Ayurvedic medical system, Barleria prionitis Linn, also known as Kate Koranti, is a well-known medicinal plant. Due to its biological and pharmacological activities, it holds a significant position in ayurvedic medicine in India (Shendage *et al*, 2010) Part of the Barleria Prionitis Linn plant is used in the treatment of dental infections as well as stomach disorders, urinary affections, ulcers, fever, bronchial asthma, catarrhal affections, swellings, whooping cough, inflammations, toothaches, glandular swellings, fever, and gastrointestinal infections in Indian traditional medicine (Shendage *et al*, 2010, Banerjee *et al*, 2012, Khare, 2007, Khare 2004, Maji *et al*, 2011). Anti-arthritic (Choudhary *et al*, 2014), anti-inflammatory (Singh *et al*, 2003), hepatoprotective (Singh *et al*, 2005), antibacterial (Aneja *et al* 2010), immunomodulatory (Ghule *et al*, 2012) and gastroprotective (Choudhary *et al*, 2014) properties have been identified for the plant Barleria prionitis. Glycoside, phenolic substance, flavonoids and tannins were found in the extract of the whole plant of Barleria prionitis, according to a preliminary phytochemical investigation (Maji *et al*, 2011) It has been established that flavonoids has anti-inflammatory, smooth muscle relaxant, and vasodilator activities (Maji *et al*, 2011) Furthermore, there is a less published literature about the isolation of bioactive phytoconstituent. Hence, the present study was planned to Isolate and characterize phytoconstituent from methanol extract of Barleria prinitis leaves.

## MATERIALS AND METHODS

### Plant materials

On the basis of the literature survey leaves of *Barleria prionitis* Linn. *(Acanthaceae)* had been selected for present project. leaves of *Barleria prionitis* Linn. were collected from Shree Shail Medifarm, Nagpur.and identified on the basis of its morphological features with the help of taxonomist (Dr. Rajendra Kale). Herbarium of the plant specimen has been given for authentication to Dr. S. S. Bodke, Associate Professor & Head, Chairman, BOS in Botany, Department of Botany & Horticulture, Yashwant College, Nanded; which has been submitted to Nanded Pharmacy College Nanded, specimen no: H-05/NPC/Pharmacology/2016-17 and authenticated as *Barleria prionitis* Linn. leaves *(*family-Acanthaceae*).* The fresh leaves of plant

of *Barleria prionitis* was subjected to shade drying and further crushed to coarse powder, and then the powder is passed through the sieve no. 14. *Barleria prionitis* Leaves were shade dried, leaned and pulverized by hands made to obtain coarse powder of mesh size #40. Coarse powder (1000 g) of *Barleria prionitis* Leaves was exhaustively defatted using petroleum ether (60-80 ºC) (BP-PE) and extracted successively with chloroform (BP-CH) and methanol (BP-ME) using Soxhlet apparatus. All the extracts were collected, filtered through Whatman filter paper, concentrated and stored in tight desiccator and percentage yield was calculated.

**Fractionation of Extract:**

As per the results of obtained in preclinical evaluation, the Methanolic extract of *Barleria prionities* L. (MeBP) was further subjected to fractionation using column chromatography

**Column chromatography:**

**Preparation of solvent mixture:**

The solvents chloroform and methanol were used for the preparation of solvent mixture. The composition of solvent mixture was Chloroform: Methanol (50:50)

**Activation of silica**

Column grade silica (100-200#) was kept in oven at 110°C overnight (12 h) to remove all moisture content present in it. Weighed quantity (1500g) of activated silica was added to the beaker containing solvent mixture and stirred with glass rod to prepare the slurry, Column was packed with slurry.

**Packing of column:**

A clean and dry borosil glass column (height, 120 cm; diameter, 7 cm) was aligned in a vertical position with the help of clamps attached to metal stand. A piece of cotton soaked in solvent mixture was placed at the bottom of the column and gently tamped down with a glass rod. The solvent mixture was filled in the column up to around 1/3 level. The column was slowly and evenly filled to about 5/6 volumes full with gradual addition of silica gel slurry. Stopcock was opened to allow excess solvent mixture mobile phase to drain into the beaker. During the packing process, chromatographic column side was gently tapped with a cork for even & compact packing of silica gel. Stopcock opened gradually in between to remove additional solvent mixture. After the packing phase, the additional solvent mixture was drained until it reached to top level of silica stage (Ullah *et al*, 2020)

**Preparation & Application sample:**

BP-ME (30 g) combined with of solvent mixture (100 ml) and activated silica (100g) for slurry preparation. The sample slurry applied on the top of packed silica, and then stopcock was gradually opened to drain additional solvent mixture until it reached to top of sample. To prevent the interruption of the sample layer after addition of eluting solvent, cotton slab soaked in solvent mixture (column diameter) was placed on top of the bed. Column was allowed to stand overnight. After stability, column was eluted with eluting solvents. Fractions were collected and analyzed by TLC. Fraction showing similar bands were pooled together and analyzed by HPTLC.

**Elution:**

Eluting solvents were added to top of column and by gravity elution at the flow rate 2ml/min was carried out. Initially elution was carried out with chloroform (200 ml) and then followed by methanol (200 ml) in increased order polarity. In amber-coloured bottles, fractions (20 ml each) were collected. All fractions were analysed by HPTLC and fractions showing similar compounds were pooled together and labelled as BP-Ch & BP-Me

**Fig- 1 Column Chromatography for fraction collection of ME-BP**

**Isolation by using High Performance Thin Layer Chromatography**

**Instrumentation:**

HPTLC (CAMAG, Muttena, Switzerland), Anchrom Enterprises (1) Pvt. ad, Mumbai, comprise of sample applicator (Linomat 5), twin trough chamber with lid (10-10 cm, CAMAG, Muttenz, Switzerland), UV cabinet (Aetron, Mumbai) with dual wavelength (254/366 nm) was used for study.

**HPTLC plates**

Prated aluminium support silica gel 60 $F_{254}$ HPTLC plates (20x 20cm) were cut to required size (10 x 10 cm) and used for the sample application.

**Chromatographic Conditions**

The plate was prewashed with methanol and activated at 110 $^0$C for 5 minutes, prior to chromatography. The sample (Methanolic Extract of *Barleria prionitis* leaves) was applied in the form of band of width 6 mm with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 $F_{254}$ (5 × 10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The optimized chamber saturation time for mobile phase was kept 20 min. The length of chromatogram run was 8 cm. HPTLC plate was dried in a current of air with the help of a hair dryer and scanned. The slit dimensions of 5 × 0.45 mm and scanning speed of 20 mm/sec were employed in analysis (Agrawal *et al* 2011)

**Mobile phase:**

The composition of mobile phase was chloroform: Methanol (9.5: 1.5)

**Calculation of Rf Values:**

Plate was observed in the daylight, under UV light (254 and 366 nm). After each observation the central points of spots appeared on chromatogram were marked with needle. Retention factor (Rf) was calculated by following formula

Rf = A/B

A = distance between point of application and central point of spot of material being examined.

B = distance between the point of application and the mobile phase front.

**Isolation of active constituent from active fraction Instruments used:**

IR spectra were recorded using KBr on FT-IR (IR Affinity-1S- Shimadzu-00694) instrument by DRIFT method. H-NMR spectra were recorded in CDCl3 solution on "FTNMR VARIAN

MERCURY YH- 300" using tetramethyl silane (TMS) as internal standard. Mass Spectra were recorded on "Shimadzu GC-MS QP-5050" instrument by direct injection method. (Sharma *et al*, 2005)

## RESULT AND DISCUSSION

**Table 1 : Preliminary phytochemical analysis of fraction**

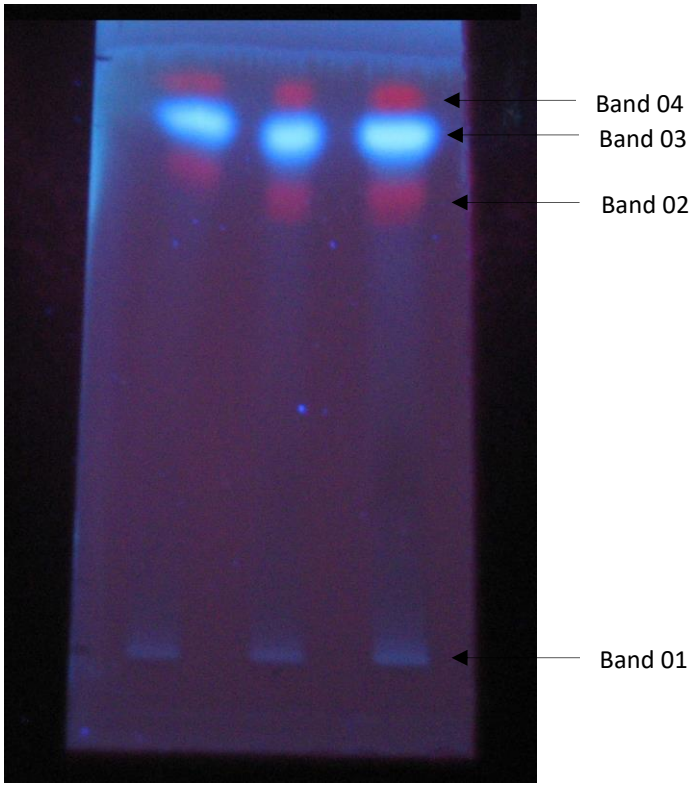
| Fraction | Flav | Alka | Carb | Sap | Tan | Gly | Ster | Trtrp |
|---|---|---|---|---|---|---|---|---|
| Chloroform fraction | + | + | - | - | + | - | - | - |
| Methanol fraction | + | + | - | - | + | + | - | - |

(-) = Absent., (+) Present

The phytochemical analysis of the fraction reveals that methanolic fraction exhibited presence of more number of biological active constituents like gylocosides, flavonoids, alkaloids, tannins etc over chloroform fraction

### Chromatographic Analysis and isolation and characterisation of MeBP (HPTLC)

High Performance Thin Layer Chromatographic analysis of MeBP



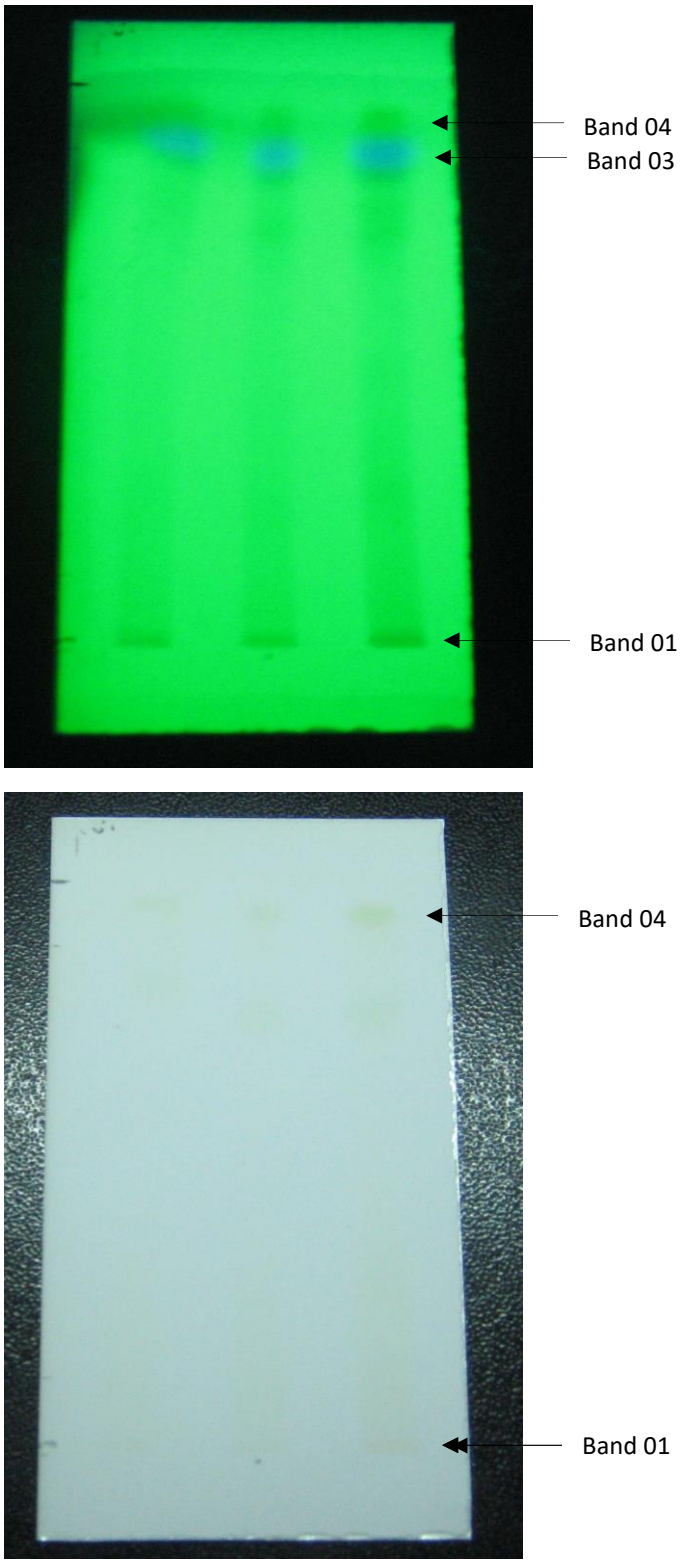



**Figure 2: Methanolic Extract of Barleria prionitis leaves A) at 366 nm and B) at 254 nm and C) Visible light, Volume applied 10 ⌈l in triplicate. The Band 3 at Rf Value 0.82 was scratched and subjected to structure elucidation**

**FT-IR Spectrum of compound in MeBP (Rf.0.82)**

The Band 3 at Rf Value 0.82 was scratched and subjected to structure elucidation. FT-IR, NMR were recorded at Department of Chemistry, North Maharashtra University, Jalgaon. LC-MS was carried out at Venture Centre, Pune. IR spectra was recorded using KBr on "JASCO FT-IR 460 plus" by DRIFT method. $^{1}$H-NMR spectra was recorded in $CDCl_3$ solution on "FTNMR VARIAN MERCURY YH-300" using tetramethyl silane (TMS) as internal standard. Purity checking and Mass Spectra recording was carried by liquid chromatography–electrospray ionization mass spectrometry (LC–ESI/MS) with accurate mass measurements upto four decimal. It was recorded on Agilent LC-MS Q – TOF (6200 series TOF/6500 series) (5301 Stevens Creek Blvd, Santa Clara, CA 95051, United States) equipped with a dual AJS ESI with improved sensitivity [ AJS – ESI: Agilent Jet Stream Electro spray Ionizer] and Q-TOF B.05.01software version.

Infrared Spectrum

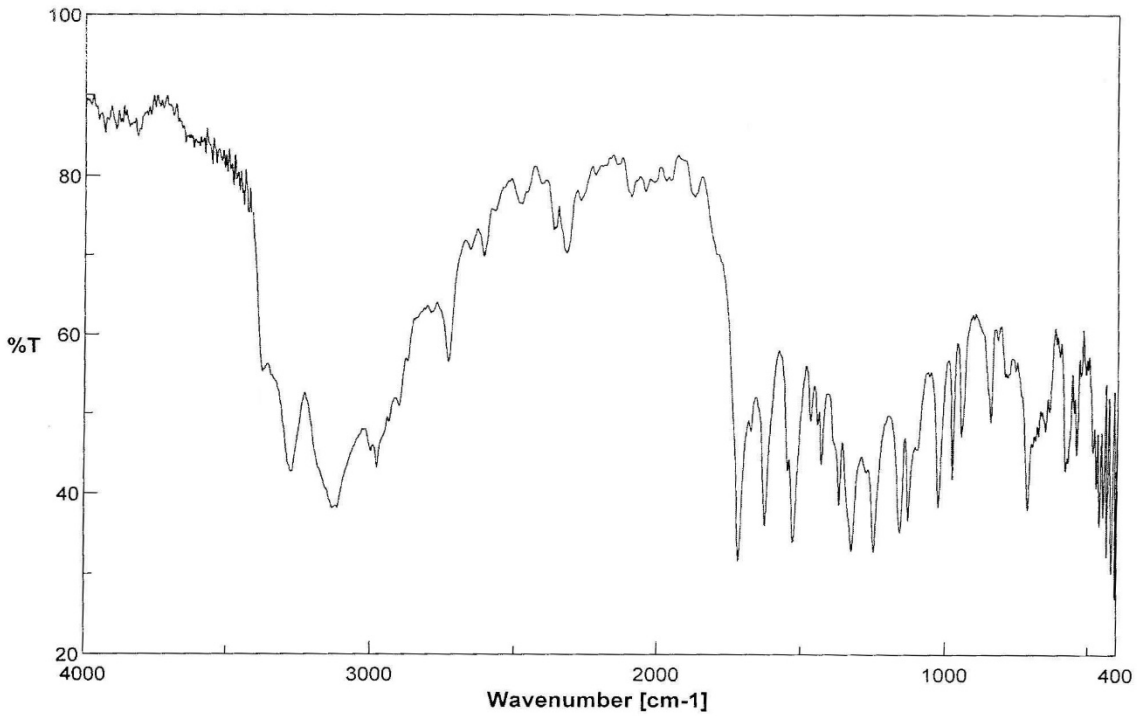


**Figure 3 : FT-IR Spectrum of compound (Rf:0.82)**

**Interpretation of IR spectrum**

Table 1 : Interpretaion of IR Spectrum

| Sr. No. | Part of molecule | Vibration | Frequency $cm^{-1}$ | Standard Ranges $cm^{-1}$ |
|---|---|---|---|---|
| 1 | Aliphatic $CH_2$ | a) C-H str<br>b) C-H bend | 2995<br>1442 | 2850-3000<br>1300-1470 |
| 2 | -C(O)-$CH_2$ - | a) C=O str | 1718 | 1700-1750 |
| 3 | -C=C- ring<br>(Furan ring) | a) C=C str<br>b) C-O str | 1585<br>1368 | 1430-1600<br>1250-1400 |
| 4 | Ar ring | a) C-H str<br>b) C=C str<br>c) C-H bend | 3097<br>1517<br>726 | 3000-3100<br>1430-1600<br>700-900 |
| 5 | -OH groups | a) O-H str | 3372 | 3300-3600 |

**Interpretation of NMR Spectrum of compound of MeBP (Rf. 0.82)**

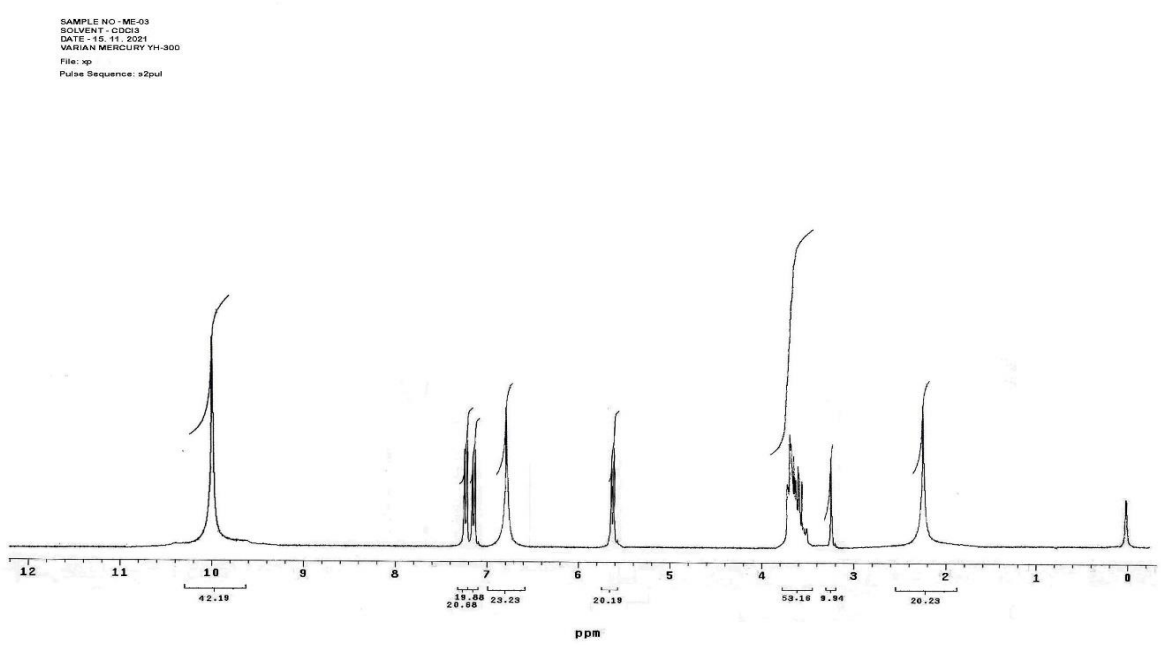
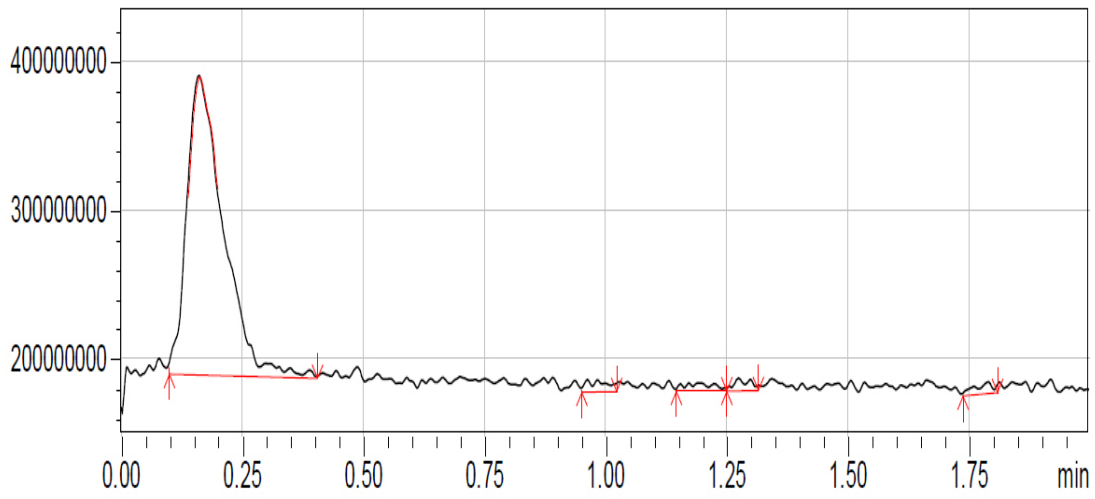



Figure 4 NMR spectrum of compound

| Sr No. | ™ | Protons (multiplicity) | Type |
|---|---|---|---|
| 1 | 2.216 | 2 (s) | OH Protons |
| 2 | 3.214 | 1 (s) | CH Protons on ring of fused Benzopyran ring |
| 3 | 3.418-3.862 | 5 (m) | Protons on the pyran ring |
| 4 | 5.626 | 2 (d) | $CH_2$ Proton near oxygen linkage of the pyran ring |
| 5 | 6.792 | 2 (s) | Aromatic Protons on ring of fused Benzopyran ring |
| 6 | 7.126-7.243 | 2 + 2 (d) | Protons on Benzene ring attached to Benzopyran ring |
| 7 | 10.012 | 4 (s) | OH Protons on pyran ring |

**Interpretation of NMR spectrum -**

**LC-MS and mass spectrum of compound**




LC-MS Spectra

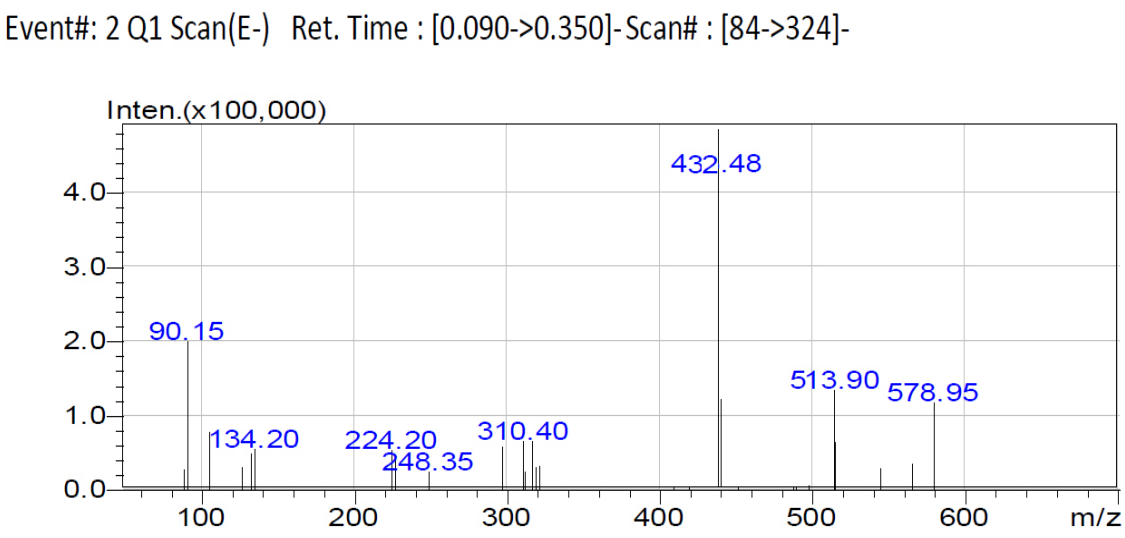



Figure 6 mass spectrum of the compound (rf – 0.82)

Probable structure of the compound from IR and NMR and LC-MS found to be

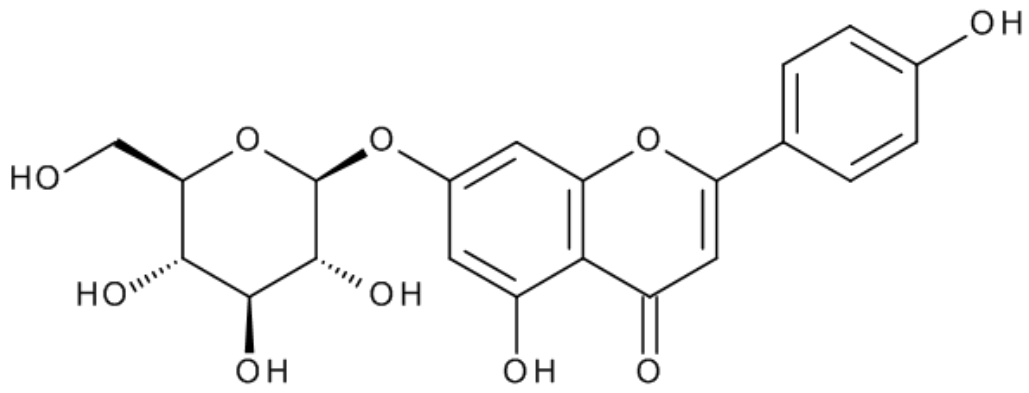



Fig No 7 Isolated Compound Apigenin-7-o-glucoside

**Conclusion**

With respect to previous published data and the phytochemical studies of MtBP, HPTLC analysis of fraction carried out. The results indicated the most prominent band Rf value data matching with Apigenin-7- o-glucoside. Hence it was taken for further structural elucidation. IR, LC-MS & NMR studies have revealed that Apigenin-7- o-glucoside, is most prominent phytochemical (flavone) which is present in methanolic fraction

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