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Research Article

# ISOLATION AND CHARACTERIZATION OF A FLAVONOID FROM ETHANOLIC EXTRACT OF *Portulaca quadrifida* LINN.

**Mrinmay Das*[1], Ashok Kumar D[2], Jyotirmoy Deb[3] and Durga Srinivasa Rao[3]**

[1] PRIST University, Vallam, Thanjavur, Tamilnadu-613403

[2] Department of Pharmacy, Pratistha Institute of Pharmaceutical Sciences, Suryapet Dist, Andhra Pradesh-508214.

[3] Department of Pharmacy, S. Chaavan College of Pharmacy, Jangalakandriga (Vi), Nellore Dist, Andhra Pradesh-524346.

**ABSTRACT**

The current study aims to verify the best method for a rapid and efficient extraction of flavonoids from *Portulaca quadrifida* Linn. and characterization of the flavonoids by using several chromatographic and analytical methods. Dried aerial part of the plant was extracted by using 95% ethanol in Soxhlet apparatus. The ethanolic extract was used for isolation. TLC was performed to find the no of constituents present in the extract. Column had done to separate the constituent. The fractions giving same spot in TLC were collected, mixed together and crystallized. It was purified and recrystalized for analytical studies (EEPQ-I). FTIR, NMR and MASS had performed for characterizing about the compound EEPQ-I. IR study support the presence of hydroxyl, methoxyl group and saturated ring. The NMR study proved the presence of methine proton in different position of the structure, ring character and no of groups present in the structure. The MASS study gave EI-MS spectrum of molecular ion peak at m/z 272, corresponding to the molecular formula $C_{16}H_{16}O_4$. The available data and on comparison data from literature confirmed the compound as 3´-methoxy -4´-hydroxy flavan-3-ol.

**Key words:** Flavonoids, Chromatography, FTIR, NMR, MASS.

## INTRODUCTION

Phytochemistry is the branch of chemistry that study the relationship between natural products and organic chemistry. The study of Phytochemistry is very important and relevant because it helps to impact the knowledge of various plants constituent which can be tested for their pharmacological activity.

Phytochemicals such as flavonoids, saponins, tannins, glycosides etc. are very essential nutrients that are required by human body for sustaining life. There are many phytochemicals and each works differently. Most phytochemicals have anti-oxidant activity and protect cells against oxidative damage and reduce the risk of developing certain types of cancer e.g. Flavonoids. So for knowing the importance of phytochemicals in biological studies it is necessary to isolate the active metabolites. Different processes are adopted for this purpose viz. Chromatography, Spectroscopy etc [1].

The process of separation of the individual components of a mixture based on their relative affinities towards stationary and mobile phases is called as chromatography. The identification, separation and purification of plant constituents are mainly carried out using one or a combination of chromatographic techniques.

The IR region is divided into three regions: the near, mid, and far IR. Infrared radiation is absorbed by organic molecules and converted into energy of molecular vibration. The wave numbers (sometimes referred to as frequencies) at which an organic molecule absorbs radiation give information on functional groups present in the molecule [2].

Nuclear magnetic resonance, induces changes in the magnetic properties of certain atomic nuclei, notably that of hydrogen. NMR spectroscopy is used to investigate the properties of organic molecules and provide detailed information about the structure, dynamics, reaction state and chemical environment of molecules [3].

Mass spectrometry is a powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample, and to elucidate the structure and chemical properties of different molecules. The complete process involves the conversion of the sample into gaseous ions, with or without fragmentation, which are then characterized by their mass to charge ratios (m/z) and relative abundances [4].

*Corresponding author: Mrinmay Das / E-mail: jyotirmoydev@gmail.com

*Portulaca quadrifida* Linn. belongs to the family Portulacaceae. It is a small diffused, succulent, annual herb found throughout the tropical parts of India. It is used as a vegetable and also used for various curative purposes. It is said to be useful in asthma, cough, urinary discharges, inflammations and ulcers. In Rajasthan, the leaves are used in preparing bread by mixing with Bajra. In Tamilnadu, leaves and tender shoots cooked and eaten as greens. A poultice of the plant is applied in abdominal complaints, erysipelas and haemorrhoids [5]. In Indo-China the juice of leaves is applied to abscesses and used as a collyrium; a decoction is given in dysentery. In Nigeria the leaves are used as a local application to swellings [6].

Preliminary phytochemical analysis of different extracts shows positive results for tannins, phenolic compounds, flavonoids and triterpenoids in petroleum ether extract. Chloroform extract showed positive test for tannins only, ethanolic extract exhibited positive test for alkaloids, flavonoids, triterpenoids, glycosides, tannins, amino acids and saponins where as aqueous extract was found to be positive for flavonoids, alkaloids, carbohydrates, glycosides, amino acids and saponins. These secondary metabolites are the active constituents of *Portulaca quadrifida* Linn. may be responsible for its pharmacological activities [7]. In the present study an attemyt had been taken to isolate secondary metabolites from ethanolic extract of *Portulaca quadrifida* Linn. aerial parts and tried to characterize the compound by using several analytical tools.

## MATERIAL AND METHODS:

### *Plant Material*

The plant was identified by the botanists of the VR College, Nellore, Andhra Pradesh. After authentication, fresh aerial parts of the young and matured plants were collected in bulk from the rural belt of Jangalakandriga, Nellore, Andhra Pradesh, India during early summer, washed, shade dried and then milled in to coarse powder by a mechanical grinder.

### *Preparation of plant extract (EEPQ)*

The powdered plant material (400gm) was defatted with petroleum ether (60-80$^0$C) and then extracted with 1.5 liter of ethanol (95%) in a Soxhlet apparatus. The solvent was removed under reduced pressure, leaving a greenish-black sticky residue (yield: 11.6% w/w with respect to dried plant material). The dried ethanolic extract (EEPQ) was stored in a desiccators till needed.

### *Identification of phytoconstituents by TLC*

In thin layer chromatography technique the extract was dissolved in solvent and mixed thoroughly. The mixture was then used for spotting in the TLC plates. The plates are prepared by using adsorbent like Silica gel G. A fine capillary had been used for spotting. The spot was done on the TLC plate near about 1cm above from the bottom of the plate. The plate was then dried and kept in developing chamber containing suitable solvent systems. After a proper running period the plate(s) were removed and dried in the air and spraying reagent was used to locate the spot. The $R_f$ value was calculated. Different solvents were used in different ratios and TLC had been carried out to confirm the presence of different mixtures of phytoconstituents in the extract [8-10].

$$R_f\ value = \frac{Distance\ travelled\ by\ solute}{Distance\ travelled\ by\ solvent\ front}$$

### *Separation of Phytoconstituents by Column Chromatography*

In this technique the stationary phase is solid and the mobile phase is liquid. The separation takes place when the component of two or more compound mixture is more strongly adsorbed than the other by the solid stationary phase. The isolation of active constituent was performed by using the Column chromatography technique [11].

The adsorbent was dissolved in chloroform to make slurry poured in to a column up to ¾$^{th}$ level. The solvent were continuously run to get proper packing. Then the sample was packed as slurry with the same solvent [12]. Mobile phase was poured on the column bed to make the column to settle properly. Then sample was mixed with chloroform and poured in to the column. Different solvent systems of n-Hexane, Benzene, Chloroform and Ethanol in different ratios were used for the elution of phytoconstituents. The fractions of 200 ml were collected each time. Detection of the component was done by monitoring each fraction by TLC. The fraction details are tabulated in Table 1.

### *Confirmation of constituents by using Thin Layer Chromatography*

The fractions were collected and the residue of fraction was obtained each time by evaporating the solvent by using rotary vacuum evaporator and tested for the components by using Thin Layer Chromatography. TLC spot was identified by spraying 5% w/v alcoholic solution of $H_2SO_4$ as a spray reagent. The sprayed plates were heated at 100°C for 5-10 min and the numbers of constituents present in the each fraction were found.

***Isolation of phytoconstituents from EEPQ***

The Chloroform-Ethanol 50:50 ratios gives the fractions 98 to 103, were found to be similar and showed a single spot. Thus they were mixed and crystallized from ethanol as pale yellow powder; melting point 274-276$^0$C (104 mg), on TLC, a purple spot was observed under UV light and darkened when exposed to ammonia. It gave dark green colour with ferric chloride, pink colour in Shinoda's test suggesting that it was a flavone. The isolated compound was designated as **EEPQ-I**. The general physical properties of the compound were tabulated in Table 2 and solvent system for TLC and $R_f$ value of the isolated compound tabulated in the Table 3.

**CHARACTERIZATION OF ISOLATED COMPOUND:**

***IR spectrum of EEPQ-I***

The IR region is divided into three regions: the near, mid, and far IR. The mid IR region is of greatest practical use to the organic chemist. This is the region of wavelengths between 3 x $10^{-4}$ and 3 x $10^{-3}$ cm. In wave numbers, the mid IR range is 4000–400 $cm^{-1}$. Infrared radiation is absorbed by organic molecules and converted into energy of molecular vibration.

The IR spectrum of isolated compound EEPQ-1 had shown absorption bands at 3524.0 to 3294.53 (O-H, free hydroxyl group), 2966.6 (Cyclic C-H, stretching), 2864.3 (Ali- C-H, stretching), 1708.9 (C=O stretching), 1554.6 (C-C ring stretching), 1454.3 (C-C ring stretching), 1238.3 to 1192.0 (C-C stretching), 1028.0 to 962.0 (O-H, out of plane bend). The FT-IR spectrum of EEPQ-I had shown in Fig 1. The spectral data of the compound EEPQ-I and their functional group assignments were tabulated in Table 4.

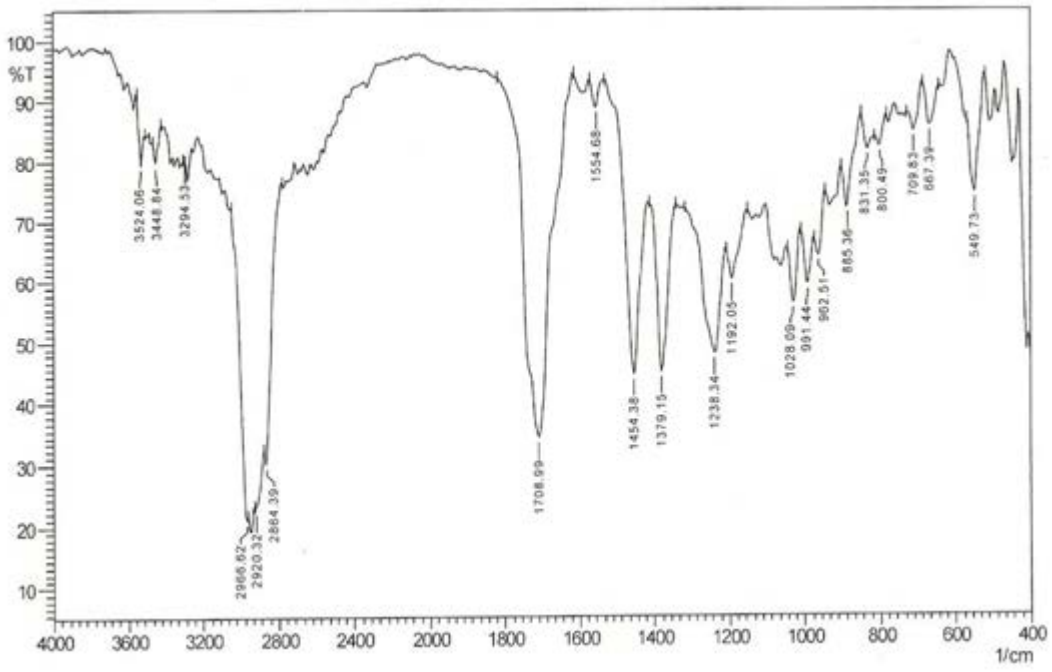


**Fig 1: FT-IR Spectrum of Isolated Compound EEPQ-I**

***Nuclear Magnetic Resonance Study***

Nuclear magnetic resonance, induces changes in the magnetic properties of certain atomic nuclei, notably that of hydrogen. Hydrogen atoms in different environments can be detected, counted and analyzed for structure determination.

$^{13}$C NMR spectroscopy is the most powerful and in dispensable technique provide information about intricate nature of the carbon skeleton of a compound such as, the total number of carbon, number of oxygenated carbons and the number of carbon present in the sugar moiety and exhibits carbon resonance signal extending over 200 ppm.

***$^1$H-NMR spectrum of EEPQ-I***

The $^1$H-NMR spectrum of the isolated compound EEPQ-I had displayed the characteristic signals at $\delta_H$ 7.27(OH-3, s), 5.55 (OH- 4', s), 5.138 (H- 5',d), 4.881 to 3.430 (H- 5, 6, 7 & 8, m), 3.269 (OMe-3', s), 2.175 (H- 4, d), 1.67 to 1.33 (H- 5', 6', & 2', s) . The $^1$H-NMR spectrum of EEPQ-I had shown in Fig 2 and data were tabulated in Table 5.

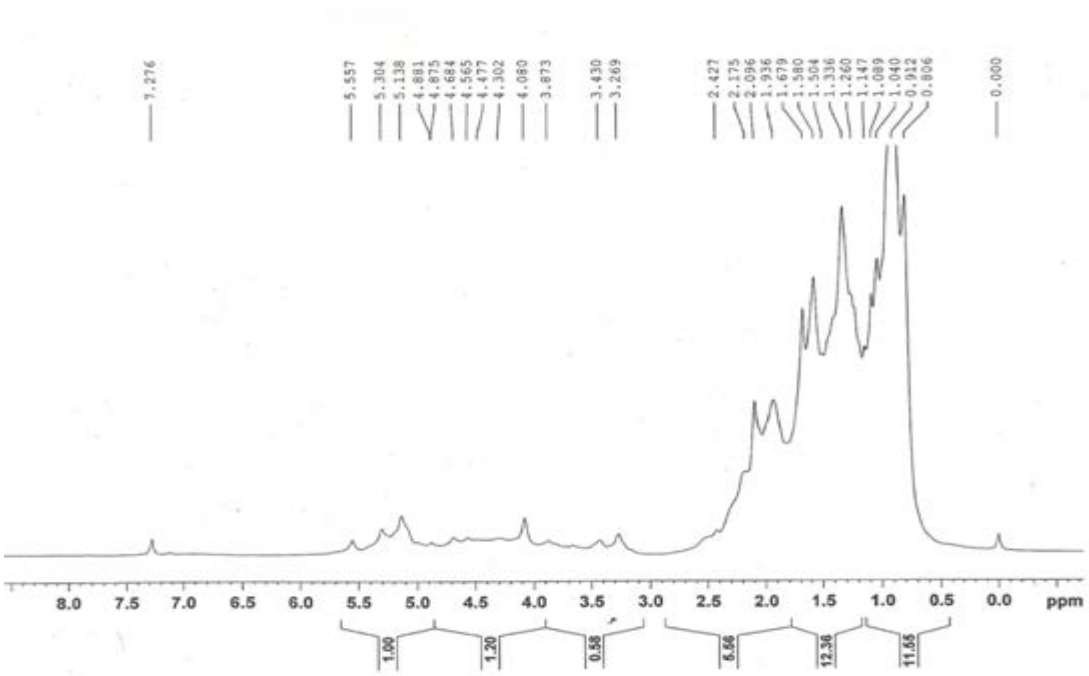


**Fig 2: $^{1}$H-NMR spectrum of isolated compound EEPQ-I**

***$^{13}$C NMR spectrum of EEPQ-I***

The $^{13}$C-NMR spectrum of isolated compound EEPQ-I had shown the characteristic signals at $\delta_H$ 2-77.41, 3-130.29, 4-50.41, 5-76.99, 6-73.42, 7-76.57, 8-70.62, 3´-139.41, 4´-131.99, 1´-170.39, 2´-121.69, 5´-123.09, 6´-123.50. The carbon signals indicated the presence of 13 carbons due to the flavones skeleton. The hydroxylated C-2, C-3, C-4, C-5, C-6, C-7 and C-8 resonate at δ ppm. The $^{13}$C-NMR spectrum had shown in Fig 3 and the spectral data of EEPQ-I and corresponding signal assignments were tabulated in Table 6.

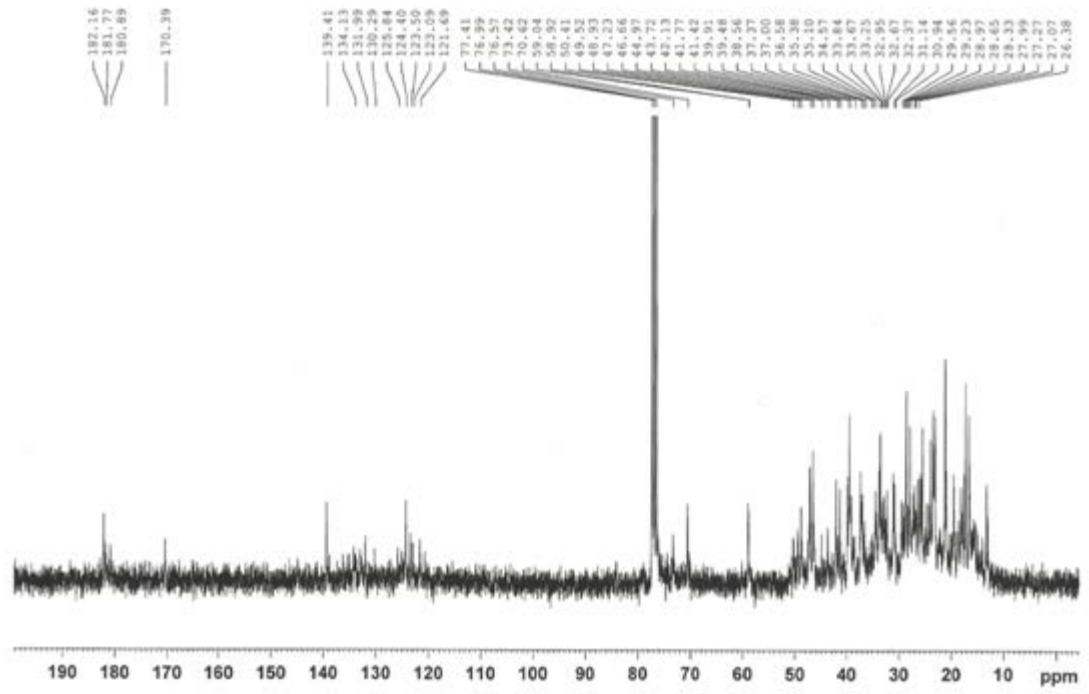


**Fig 3: $^{13}$C-NMR spectrum of isolated compound EEPQ-I**

***MASS spectrum of EEPQ-I***

Mass spectrometry is a powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample, and to elucidate the structure and chemical properties of different molecules. The complete process involves the conversion of the sample into gaseous ions, with or without fragmentation, which are then characterized by their mass to charge ratios (m/z) and relative abundances.

The mass data of isolated compound EEPQ-I had shown the m/z = 272 indicative of $C_{16}H_{16}O_4$, m/z = 254 indicative of $C_{16}H_{14}O_3$, m/z = 240 indicative of $C_{15}H_{12}O_3$, m/z = 230 indicative of $C_{14}H_{14}O_3$, m/z = 216 indicative of $C_{13}H_{12}O_3$, m/z = 196 indicative of $C_{10}H_{12}O_4$, m/z = 132 indicative of $C_9H_8O$, m/z = 98 indicative of $C_5H_6O_2$, m/z = 81 indicative of $C_5H_5O$ and m/z = 43 for $C_2H_3O$. The mass data were in decreased sequence due to the absence of different parts on the compounds. The MASS spectrum of isolated compound EPQ-I had shown in Fig. 4 and the EI-MS spectrum of EEPQ-I exhibited the molecular ion peak at *m/z* 272.

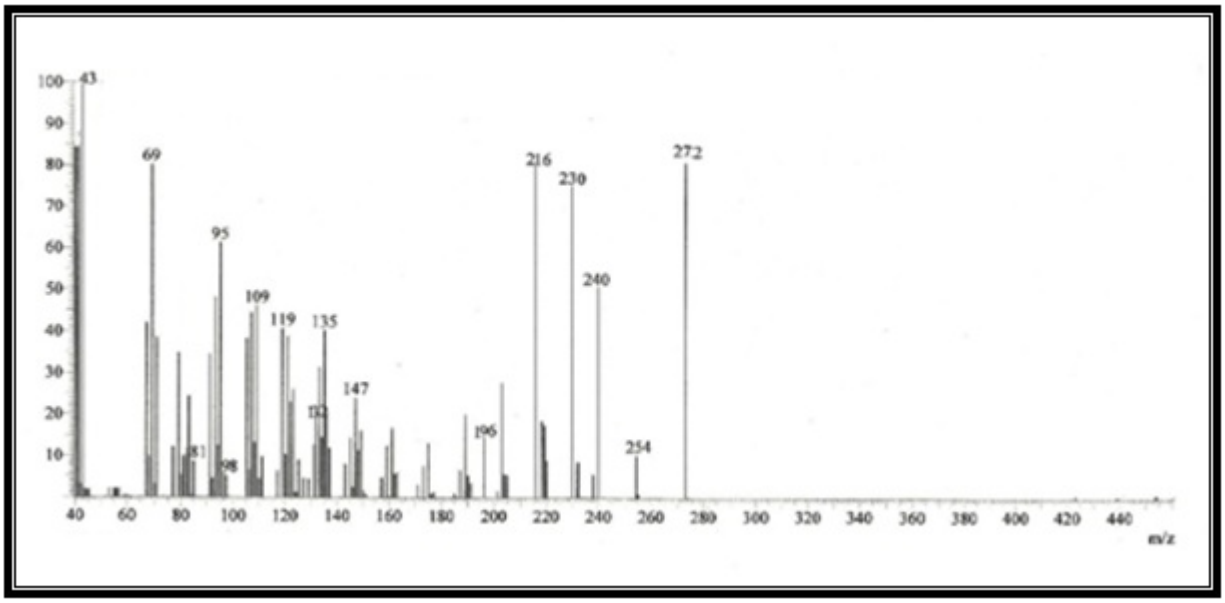


**Fig 4: MASS spectrum of isolated compound EEPQ-I**

*Structure of Isolated Compound*

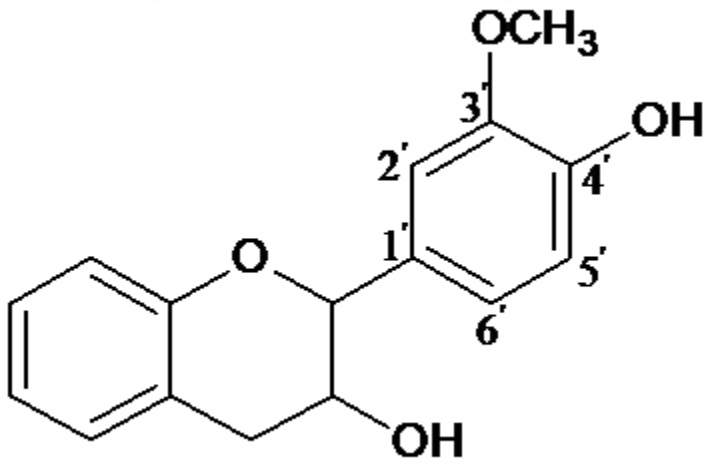


**Fig 5: 3´-methoxy-4´-hydroxy-flavan-3-ol**

**RESULT:**

**Table: 1 Chromatographic fractions of ethanolic extract of *Portulaca quadrifida* Linn.**

| Sl. No. | Elution Composition | Fractions | Compounds |
|---|---|---|---|
| 1. | n-Hexane (100%) | 1-8 | Oily |
| 2. | n-Hexane: Benzene (90:10) | 9-14 | Oily |
| 3. | n-Hexane: Benzene (80:20) | 15-21 | Waxy |
| 4. | n-Hexane: Benzene (50:50) | 22-27 | Waxy |
| 5. | n-Hexane: Benzene (30:70) | 28-32 | Intractable gum |
| 6. | Benzene (100%) | 33-38 | Intractable gum |
| 7. | Benzene: Chloroform (95:5) | 39-46 | Intractable gum |
| 8. | Benzene: Chloroform (75:25) | 47-56 | Intractable gum |
| 9. | Benzene: Chloroform (50:50) | 57-61 | Intractable gum |
| 10. | Chloroform (100%) | 62-70 | Intractable gum |
| 11. | Chloroform: Ethanol (98:2) | 71-76 | Intractable gum |
| 12. | Chloroform: Ethanol (95:5) | 77-82 | Intractable gum |
| 13. | Chloroform: Ethanol (85:15) | 83-87 | Intractable gum |
| 14. | Chloroform: Ethanol (75:25) | 88-91 | Intractable gum |
| 15. | Chloroform: Ethanol (65:35) | 92-97 | Intractable gum |
| 16. | Chloroform: Ethanol (50:50) | 98-103 | Single Spot |
| 17. | Chloroform: Ethanol (30:70) | 104-111 | Intractable gum |
| 18. | Ethanol (100%) | 112-116 | Intractable gum |

**Table 2: Properties of isolated compound EEPQ-I**

| Sl. No. | Property | Observation |
|---|---|---|
| 1. | Appearance | Pale yellow powder |
| 2. | Melting Point | 274 – 276$^0$C |
| 3. | Solubility | Ethanol, Methanol, Chloroform |

**Table: 3 $R_f$ value of the isolated compound EEPQ-I**

| Sl. No. | TLC Solvent System | $R_f$ Value |
|---|---|---|
| 1. | Chloroform : Ethanol = 4 : 1 | 0.62 |

**Table 4: FT-IR spectral data of compound EEPQ-I**

| Sl. No. | Wave Number (cm$^{-1}$) | Type of Vibration | Functional group assigned |
|---|---|---|---|
| 1. | 3524.0, 3448.84 & 3294.53 | O - H | Free hydroxyl group |
| 2. | 2966.6 | Cyclic C - H, str | Aromatic Hydrocarbon |
| 3. | 2864.3 | C - H, str | Aliphatic Hydrocarbon |
| 4. | 1708.9 | C = O, str | Ketone |
| 5. | 1554.6, 1454.3 | C = C, ring stretch | Aromatic Nuclei |
| 6. | 1238.3, 1192.0 | C – C, str | Aliphatic Hydrocarbon |

**Table 5: $^{1}$H-NMR spectral data of compound EEPQ-I**

| Sl. No. | Chemical shift value (δ ppm) | Signal Assignment - H |
|---|---|---|
| 1. | 7.27 | 3 – OH, s |
| 2. | 5.55 | 4′ - OH, s |
| 3. | 5.138 | 5′ - H, d |
| 4. | 4.881 – 3.430 | 5, 6, 7 & 8 – H, m |
| 5. | 3.269 | 3′ - $OCH_3$, s |
| 6. | 2.175 | 4 – H, d |
| 7. | 1.67 – 1.33 | 5′, 6′ & 2′ - H, s |

**Table 6: $^{13}$C-NMR spectral data of isolated compound EEPQ-I**

| Sl. No. | Chemical shift value (δ ppm) | Signal Assignment - C |
|---|---|---|
| 1. | 77.41 | C-2 |
| 2. | 130.29 | C-3 |
| 3. | 50.41 | C-4 |
| 4. | 76.99 | C-5 |
| 5. | 73.42 | C-6 |
| 6. | 76.57 | C-7 |
| 7. | 70.62 | C-8 |
| 8. | 139.41 | C-3′ |
| 9. | 131.99 | C-4′ |
| 10. | 170.39 | C-1′ |
| 11. | 121.69 | C-2′ |
| 12. | 123.09 | C-5′ |
| 13. | 123.50 | C-6′ |

**DISCUSSION:**

Thin layer chromatography was the first attempt taken to find out the presence phytoconstituents presents in the extracts. TLC was performed by using stationary phase as silica gel G and mobile phase as chloroform: ethanol in the ratio of 4: 1 for ethanol extract of *Portulaca quadrifida* Linn. The TLC plate showed mixture of compounds with yellow colour spot. This was separated by column chromatography.

The ethanolic extract of *Portulaca quadrifida* Linn. was packed on column chromatography with silica gel G 60-120 mesh size and mobile phase was eluted as per increasing polarity. The fractions were collected and tested for the components by using Thin Layer Chromatography. TLC spot was identified by spraying 5% w/v alcoholic solution of $H_2SO_4$ as a spray reagent. The sprayed plates were heated at 100°C for 5-10 min and the numbers of constituents present in the each fraction were found. The Chloroform-Ethanol 50:50 ratios gives the fractions 98 to 103, were found to be similar and showed a single spot. Thus they were mixed and crystallized from ethanol as pale yellow powder; melting point 274-276$^{0}$C (104 mg), on TLC, a purple spot was observed under UV light and darkened when exposed to ammonia. It gave dark green colour with ferric chloride, pink colour in Shinoda's test suggesting that it was a flavone. The compound was designated as **EEPQ-I**.

The isolated compound EEPQ-I had gone through the different possible spectral analytical processes and gave EI MS spectrum of molecular ion peak at m/z 272, corresponding to the molecular formula $C_{16}H_{16}O_4$, supported also by spectroscopic analysis. In the $^{1}$H NMR spectrum, the double doublets were observed for methine proton at 5, 6, 7, 8, 2', 5' & 6' position of ring. The singlet for proton of hydroxyl group was found at 3 & 4' while for methoxy at 3' position of carbon, and these signals are typical of a flavonoid nucleus with an

unsubstituted ring. The double doublets protons at 2', 3', 5' & 6' of carbon suggested that third ring was saturated. This splitting pattern was due to the coupling between the H-2 axial proton and the H-3 geminal protons. The above data suggested a flavone nature for compound. From the above data of $^{13}C$ NMR of δ supported the presence of two hydroxyl group, methoxyl group and while other were CH and $CH_2$ groups. The data of IR supported the presence of hydroxyl group, methoxyl group and saturated ring. Structures were elucidated on comparison with data in the literature and confirmed the compound as **3´-methoxy-4´-hydroxy flavan-3-ol.**

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