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Extraction, Isolation and Characterization of Flavonoid Compound Quercetin from the *Rosa Centifolia* Roots

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ABSTRACT:
The present study was designed for isolation of flavonoid molecule quercetin from the roots of rosa centifolia and their subsequent characterization. Extracts of rosa centifolia roots were prepared using solvents such as ethanol. The plant extracts were subjected for phytochemical analysis and total flavonoid content. The extracts were subjected to column chromatography followed by TLC. Then the isolated compound was subjected to FT-IR, 1H NMR, 13C NMR and HPLC was studied. The ethanol extract showed the presence of higher flavonoid content when compared with other solvent extracts. The ethanol extract was subjected to fractionalization by column chromatography. The eluted fractions were run in TLC mobile phase with the different solvent ratio. The fractions showed rf value equal to standard quercetin in TLC were combined and crystallized. The characterization techniques confirmed that the isolated compound was found to be quercetin.

KEYWORDS: Rosa Centifolia roots, phytochemical analysis, Chromatography, UV, NMR, HPLC.

I.INTRODUTION

Medicinal plants are also called as herbal medicine, have been discovered and are used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemicals compounds for various functions, including defense and protecting against insects, fungi, diseases, and herbivorous mammals. A developed countries or even undeveloped countries are using herbal medicine in maintain human wellbeing and personal health condition (Hamburger & Hostettmann., 1991). Each plant consists of several important ingredients that can be involved in the development of different kind of drugs. Even today, the plants are not only indispensable in health care, but form the best hope of source for safe future medicines. Among the variety of modern medicines many of them produced indirectly from medicinal plants, for example: aspirin.

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. They have roles to protection of human health, when their dietary intake is significant. Phytochemicals accumulate in different parts of plants, such as primary or secondary constituents include common sugars, amino acids, chlorophyl etc. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, saponins, phenolics, flavonoids and glucosides (Gibson *et al.,* 1998). The secondary metabolites have an important role in defense compounds. Mostly, flavonoids are polyphenolic compounds that are ubiquitous in nature. More than 4000 flavonoids have been recognized. The flavonoids appear to have played a major role in successful medicinal treatments of ancient in times.

Flavonoids possess a number of medicinal benefits, including anti-cancer, anti-oxidant, anti-inflammatory and anti-viral properties. Researchers suggest that a diet rich in flavonoids could reduce your risk for cardio vascular disease, diabetes and some type of cancer. Quercetin is a most abundant poly phenolic bioflavonoid or flavonoid, which are generally classified as flavonol(Abirami & Vetrichelv., 2013). It is also known as phytoestrogens. Quercetin, comprising 3 rings and 5 hydroxyl groups, has more health beneficial effects like improvement of cardio vascular health and reducing the risk for cancer. Quercetin is mostly present in fruits and vegetables. And it is naturally present in glycone or carbohydrate conjugate in plants. Quercetin, a dietary flavonoid, is abundantly found in a several common foods like apples (*Malus domestica*); black and green tea (*Camellia sinensis*) onions (*Allium cepa*) (predominantly in the outer rings); broccoli (*Brassica oleracea*).

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It is found in several beneficial biological activities, like antioxidant, anti-inflammatory, anti-cancer, and anti-viral properties.

Rosa Centifolia roots are commonly known as cabbage rose; *Centifolia* belongs to the family of *Rosaceae*. It's native to Persia and is commercially planted in Bulgaria, Italy, Spain, France etc. The leaves, flowers and roots of rosa centifolia are used to treat cough, skin disease, cardiac disability, fever and general weakness. Medicinal uses of *Rosa Centifolia* are very useful to blood purifier, anti-inflammatory and aphrodisiac actions of this plant is effective in various health issues like in intestinal ulcer, diarrhea etc. The main chemical constituents present in *rosa centifolia* are citronella, generiol, neriol, stearopten, farnesol, phenyl ethanol, fat oil, tannins and organic acids.

II.MATERIALS AND METHODS

2.1 RESOURCES

Ethanol, Methanol, n-hexane, Chloroform, Ethyl-acetate were used resources in this study.

2.2 PLANT COLLECTION AND MATERIALS

In this study, the roots of the medicinal plant "*RosaCentifolia*" were employed, and the roots were collected from the local markets in Trichy.

2.3 PREPARATION OF THE EXTRACT

"Rosa Centifolia" roots were collected, cleaned and dried in the shade. The 15gm of powdered sample was extracted by 100 ml ethanol by using hot percolation method. The extraction was carried out at room temperature for 24 hours with gentle shaking (Bharathi sambandam *et al*., 2016).

2.4 PHYTOCHEMICAL STUDY OF ETHANOLIC EXTRACT FROM *"ROSA CENTIFOLIA"* (KAVI MALAR & SARADHA., 2022)

TEST FOR TERPENOIDS

Add 2ml of ethanolic extract, then add few drops of chloroform and few drops of concentrated sulphuric acid. A reddish brown colour indicates the presence of terpenoids.

TEST FOR EMODIN

Take 2ml of ethanolic extract and then add few drops of ammonia solution and few drops of benzene. The red colour indicates the presence of emodin.

TEST FOR ANTHROQUINONE

Take 2ml of ethanolic extract and then add few drops of ammonia solution and few drops of benzene. The presence of anthroquinone is indicated by the production of a red colour.

TEST FOR FLAVONOIDS

A few drops of chloroform and few drops of concentrated sulphuric acid is added to the 2ml of ethanolic extract. The yellow colour was observed for flavonoid.

TEST FOR SAPONINS

Add 2ml of ethanolic extract and then add few drops of distilled water. The persistent foam indicates the presence of saponin.

TEST FOR TANNINS

A few drops of distilled water and 1-2 drops of ferric chloride is added to the 2ml of ethanolic extract. The green colour was indicates the presence of tannins.

TEST FOR ALKALOIDS

A few drops of glacial acetic acid and few drops of ammonia solution is added to the 2ml of ethanolic extract. A yellow colour was observed for alkaloids.

TEST FOR STEROIDS

Add 2ml of ethanolic extract, then add few drops of chloroform and few drops of concentrated sulphuric acid. The reddish-brown ring indicates the presence of steroids.

TEST FOR GLYCOSIDES

Add 2ml of ethanolic extract and then add few drops of chloroform and few drops of glacial acetic acid. The presence of glucosides is indicated by changing the colour from violet to blue or green.

TEST FOR PHLOBATANNINS

A few drops of conc. HCl is added to the 2ml of ethanolic extract. The production of red precipitate confirms the existence of phlobatannins.

TEST FOR PROTEIN

A few drops of concentrated sulphuric acidis added to the 2ml of ethanolic extract. The white precipitate confirms the presence of protein.

TEST FOR COUMARIN

A few drops of sodium hydroxide is added to the 2ml of ethanolic extract. The yellow colour indicates the presence of coumarin.

TEST FOR ANTHOCYANIN

A few drops of concentrated HCL and few drops of ammonia solution is added to the 2ml of ethanolic extract. The presence of anthocyanin is confirmed by the pinkish red colour.

TEST FOR CARBOHYDRATE

A few drops of distilled water, then add few drops of concentrated sulphuric acid and a pinch of α-napthol is added to the 2ml of ethanolic extract. The creation of a reddish violet ring at the junction confirms the existence of carbohydrate.

TEST FOR LEUCOANTHOCYANIN
Take 2ml of ethanolic extract and then add few drops of isoamyl alcohol. The presence of leucoanthocyanin is indicated by the red colour.
TEST FOR CARDIOGLYCOSIDES
Take 2ml of ethanolic extract and then add few drops of glacial acetic acid, few drops of ferric chloride and few drops of concentrated sulphuric acid. A violet brown ring is indicated the presence of cardio glycosides.
TEST FOR XANTHOPROTEIN
A few drops of ferric chloride is added to the 2ml of ethanolic extract. The black colour shows the presence of xanthoprotein.
TEST FOR PHENOL
Take 2ml of ethanolic extract and then add few drops of ammonia solution. The reddish brown colour indicates the existence of phenol.

2.5 QUANTITATIVE ANALYSIS OF ETHANOLIC EXTRACT FROM *ROSA CENTIFOLIA* (SHELCIYA *ET AL*., 2022)
TEST FOR FLAVONOIDS
Add 3ml of methanol in ethanolic extract and thesolution was filtered through a filter paper. The filtrate was transferred to a crucible and evaporated to dryness over a water bath before being weighed.
TEST FOR TANNINS
Add 3 or 5 ml of distilled water in ethanolic extract. It should be filtered. And then add 1drop of ferric chloride and potassium ferro cyanide. The absorbance should be measured at 300 nm within 10 minutes.
TEST FOR SAPONINS
Add 3ml of ethanol in ethanolic extract. It should be filtered. And then add 1ml of diethyl ether. It became a double layer after a vigourous shaking filter. Remove the top layer and add 1 drop of n-butanol to it. Then, in a water bath, heat it up. After evaporation, the sample was dried in a crucible to a consistent weight.
TEST FOR ALKALOIDS
To the ethanolic extract, add 10% acetic acid in ethanol [1ml acetic acid + 2ml ethanol]. It should be filtered. And then add few drops ammonia solution to it.
TEST FOR PHENOL
Add 3 or 5ml of distilled water in ethanolic extract. It should be filtered. And then add 3 drops of ammonia solution and 2 drops of isoamyl alcohol to it.
TEST FOR TERPENOIDS
Add 3ml of ethanol in ethanolic extract. It should be filtered. And then add 3 drops of petroleum ether.

2.6 DETERMINATION OF TOTAL FLAVONOID CONTENT (TFC)
We used Aluminium Chloride method to measure the total flavonoid content in ethanolic extract. We mixed ethanolic extract with 1ml of ethanol and added 500µl of $NaNO_3$. After 10 minutes in the dark, we added 150µl of $AlCl_3$ and waited for another 5 minutes. Then we added 500µl of NaOH and 1000µl distilled water. We prepared a set of standard solutions of quercetin with concentrations ranging from 20 to 100µl/ml. We calculated the total flavonoid content from the calibration curve and expressed it as milligram of quercetin equivalent (QE)per gram of extracts (Singh Khushbu & Patel Dinesh., 2021).

2.7 ISOLATION OFFLAVONOID BY COLUMN AND TLC (CHANDRAPPA *ET AL*2014)
Column chromatography:
The condensed ethanol extract of the rhizomes (50g) of the sample was subjected to column chromatography over TLC grade silica gel. Elution of the column first with n-hexane, increasing the amounts of ethyl acetate in n-hexane and finally with methanol. There are 22 fractions were obtained in this study. The compound wasdetected on TLC plates by spraying with LibermannBurchard reagent and heated at 100□ for 10 minutes.

2.8 PREPARATIVE THIN-LAYER CHROMATOGRAPHY (TLC)
The isolated pure compound was dissolved in the appropriate solvents. 5µl of isolated compounds (red fraction) were applied to silica gel plates, Merck (Germany) 20×20 cm, 0.25 mm in thickness. Plates were developed using the solvent system Ethyl acetate: Methanol(1:9v/v) for quercetin. The separated zones were visualized with freshly prepared LibermannBurchard reagent and heated at 100□ for 10 minutes. Chromatograms were then examined under daylight within 10 minutes.

2.9 CHARACTERIZATION AND STRUCTURAL ELUCIDATION STUDY OF ISOLATED COMPOUND (NIKITA SANGHAVI *et al*., 2014)
Different spectroscopic methods including UV, FTIR and ^{13}C NMR were used to elucidate the structure of isolated compounds.
UV:
The UV spectrum of the isolated compounds in ethanol was recorded using a Shimadzu 160A UV-visible spectrophotometer.

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FTIR:
The Fourier Transform Infrared (FTIR) spectra of isolated compound was recorded with a nominal resolution of 4 cm^{-1} and a wave number range from 400 to 4000 cm^{-1} using the KBr pellet technique.

NMR:
^{13}C NMR spectra of isolated compound were acquired on Bruker WP 200 SY and AM 200 SY instruments (1H, 200.13MHz; ^{13}C, 50.32 MHz) using Tetramethyl silane (TMS) as internal standard and Deuterated chloroform (CDCl3) as solvent.

2.10 PURIFICATION OF ISOLATED COMPONENTS FROM HPLC (ASIH IMULDA HARI PURWANI *ET AL* 2022)

The analytical HPLC system (Shimadzu) was equipped with adiode array detector, a 20µl loop, 200 × 4.6 mm ^{18}C column, methanol (HPLC grade, 0.2 mm filtered) used as the mobile phase. The isolated quercetin compound was separated by using a mobile phase of methanol: water (75:25v/v) at a flow rate of 1.0 ml/min, column temperature 30□. The injection volume was 40µl and detected at 346 nm.

III.RESULT AND DISCUSSION

3.1 QUALITATIVE ANALYSIS

Presence of different phytochemical compounds via, terpenoids, flavonoids, saponins, tannins, alkaloids, steroids, glycosides, phlobatannins, protein, coumarins, emodin, anthraquinone, anthocyanins, carbohydrates, leucoanthocyanin, cardiacglycosides, Xanthoprotein and phenols were analysed in ethanolic extract of Rosa centifolia.

The ethanolic extract of *Rosa centifolia* indicated the presence of all phytochemical chemical compounds mentioned in the above.

TABLE 3.1: Qualitative analysis of phytocompounds from the roots of *rosa centifolia*.

S.NO	COMPOUNDS	OBSERVATION
1	Terpenoids	+++
2	Flavonoids	+++
3	Saponin	+++
4	Tannins	+++
5	Alkaloids	+++
6	Steroids	+++
7	Glycosides	+++
8	Phlobatannins	++
9	Proteins	+++
10	Coumarins	+++
11	Emodin	+++
12	Anthroquinone	+++

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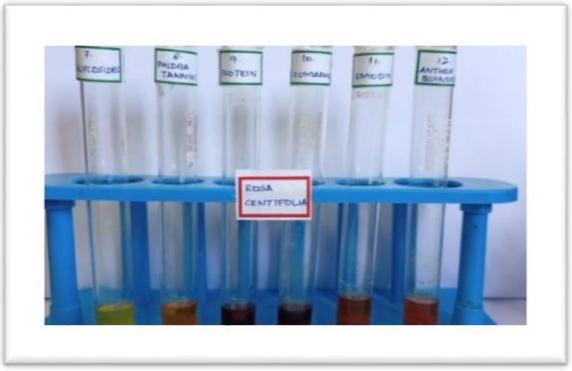
13	Anthocyanin	+++
14	Carbohydrate	+++
15	Leucoanthocyanin	+
16	Cardiacglycosides	+++
17	Xanthoprotein	+++
18	Phenol	+++

+++ - Strongly Present
++ - Moderately Present
+ - Slightly Presesnt
- - Absent

FIGURE: 3.1 Qualitative analysis of phytocompounds fromthe ethanolic extract of *rosa centifolia*



3.2 QUANTITATIVE RESULTS:

Qualitative analysis of important phytochemicals in the ethanolic extract of *Rosa centifolia* contains these phytochemicals in varying amounts in the roots. The phytochemical with the highest quantity was flavonoid followed by tannins, saponins, alkaloids, phenol and terpenoids respectively, as shown in (table 2). The highest concentration of flavonoids (0.031mg/g), tannin (0.25mg/g), saponin (0.007mg/g), alkaloids (0.004mg/g), phenol (0.015mg/g), terpenoid (0.005mg/g) respectively. Flavonoid has the higher concentration as compared to other phytochemicals. So, it indicates the presence of quercetin in ethanolic extract.

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FIGURE: 3.2 Quantitative analysis of ethanolic extract of roots of *Rosa Centifolia*

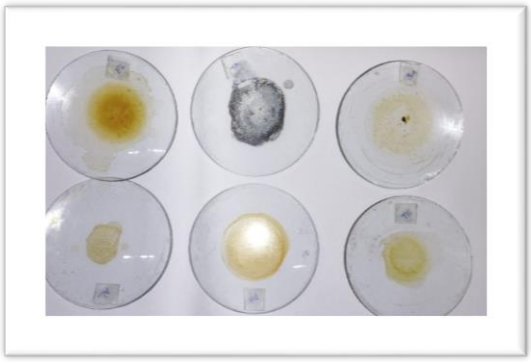

TABLE 3.2: Quantitative analysis of ethanolic extract of *rosa centifolia*

S.NO	PHYTOCHEMICAL COMPOUNDS	YIELD (mg/g)
1	FLAVONOIDS	0.031
2	TANNINS	0.025
3	SAPONINS	0.007
4	ALKALOIDS	0.004
5	PHENOL	0.015
6	TERPENOIDS	0.005

3.3 QUANTITATIVE ANALYSIS OF TOTAL FLAVONOID CONTENT IN ETHANOLIC EXTRACT

The standard solution was analysed for linearity. A linear response was identified (r^2=0.9452), and the equation was y= 0.0045x+0.223. The [BMIM] Cl extract using NaCl as the separation salt had the highest flavonoid content (61.75 mcg/g).

The equation of the slope was relates our result to the reference article. So, we can use this equation to calculate the total flavonoid content in the ethanolic extract.

Graph 3.1: Standard calibration curve for total flavonoid content for standard quercetin with ethanol extract.

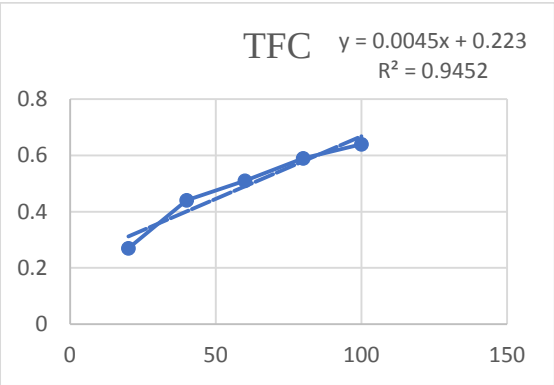

TABLE 3.3: Total flavonoid content of ethanolic extract of roots of *rosa centifolia*

Total flavonoid contents in ethanol *Rosa centifolia*	61.75 mcg/g

3.4 ISOLATION OF QUERCETIN BY COLUMN CHROMATOGRAPHY OVER PREPARATIVE TLC

Elution of the column first with n- hexane, increasing amount of ethyl acetate in n-hexane and finally with methanol yielded a number of fractions. The preparations of solvent systems used to obtain quercetin (36 mg/50 g) were ethyl acetate-methanol (10:90) from fraction 22. The compounds were detected on TLC plates by spraying with LibermannBurchard reagent and heated at 100□ for 10 minutes.

FIGURE: 3.3 Column Chromatography

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FIGURE: 3.4 Fraction photos

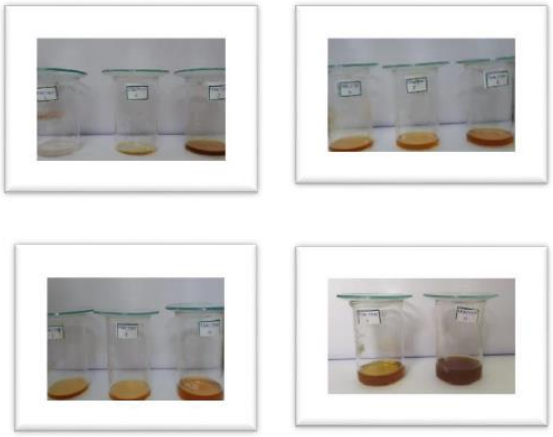

FIGURE: 3.5 ReChromatography

FIGURE: 3.6 Fraction photos

FIGURE: 3.8 Quercetin from ethanolic extract

FIGURE: 3.7 Preparative TLC

CHARACTERIZATION OF ISOLATED COMPONENT OF QUERCETIN

3.5 UV VISIBLE OF SPECTROSCOPY

Quercetin melting point 314-315□, MV: 302.238 g/mol which corresponds to the molecular formulae $C_{15}H_{10}O_7$.The UV λ_{max} value of compound 2-(3, 4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one was 255.10nm and 371.50 nm.

The result of our UV wavelengths are similar to the reference article mentioned in the above. So, we confirmed the drug Quercetin is present in our ethanolic extract.

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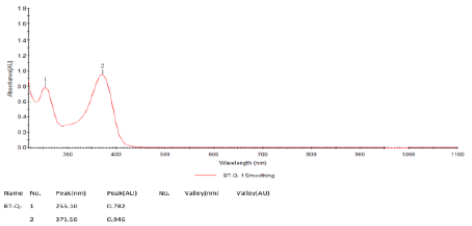
GRAPH: 3.2 UV- Visible Spectroscopy

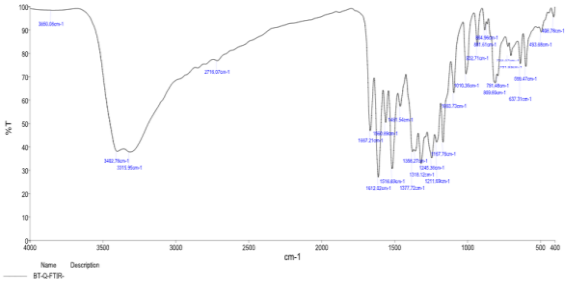


3.6 FT-IR OF ISOLATED FLAVONOID OF ETHANOLIC EXTRACT OF ROSA CENTIFOLIA ROOTS

In the IR spectrum of isolated compound very broad peak at 3402.78cm^{-1} (O-H stretching vibration of phenol), 1667.21cm^{-1} (C=O Aryl Ketonic stretch), 1612.02cm^{-1} (C---C aromatic ring stretch), 1516.69cm^{-1} (C=O aromatic stretch), 1461.54cm^{-1} (C=C aromatic stretch), 1377.72cm^{-1} (O-H bending of phenol), 1318.12cm^{-1} (C-H bond in aromatic hydro carbon), 1245.36cm^{-1} (C-O stretch of aryl ether), 1211.69cm^{-1} (C-O stretch of phenol), 1167.76cm^{-1} (C-CO-C stretch and bending in ketone), 932.71 cm^{-1},809.60 cm^{-1}, 701.93 cm^{-1}, 637.31 cm^{-1} (C-H bending of aromatic hydrocarbon).

The result of our FTIR wavenumbers are similar to the reference article mentioned in the above. So, we confirmed the drug Quercetin was present in our ethanolic extract.

Graph: 3.3 The FT-IR isolated flavonoid of ethanolic extract of rosa centifolia roots.

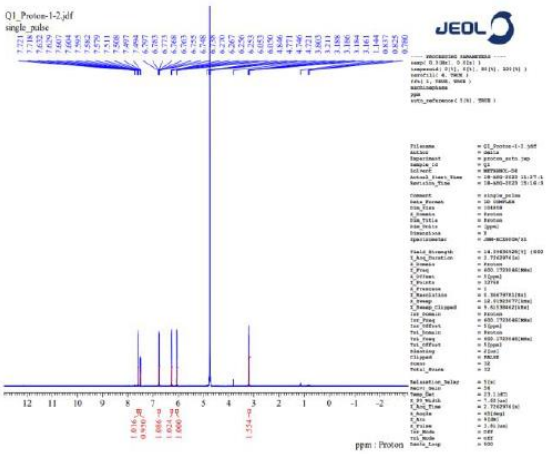


3.7 H1-NMR OF ISOLATED FLAVONOID OF ETHANOLIC EXTRACT OF ROSA CENTIFOLIA ROOTS

In its ^{1}H-NMR spectra shows, bands between δ 6 - 6.7 shows aromaticity, δ 7- 7.6 shows the presence of phenolic OH group, δ 3.2- 3.5 shows the presence of -CH_2-, -CH and an instance peak at δ 4.771 shows the presence of OH group.

The result of our H1-NMR bands are similar to the reference article mentioned in the above. So, we confirmed the drug Quercetin was present in our ethanolic extract.

Graph: 3.4 H1-NMR isolated flavonoid ethanolic extract of rosa centifolia roots.

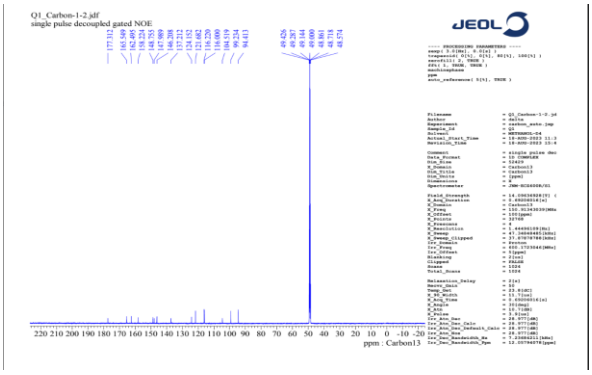


3.8 ^{13}C-NMR OF ISOLATED FLAVONOID OF ETHANOLIC EXTRACT OF ROSA CENTIFOLIA ROOTS

In the ^{13}C NMR spectra of 2-(3,4-dihydroxy-phenyl)-3,5,7-trihydroxy-4H-chromen-4-one showed 148.7(C-1), 137.2(C-2), 177.3(C-3), 162.4(C-4), 99.2(C-5), 165.5(C-6), 94.4(C-7), 158.2(C-8), 104.5(C-9), 124.1(C-10), 116.2(C-1'), 146.2(C-2'), 147.9(C-3'), 116.0(C-4'), 121.6(C-5'). The structure was confirmed by comparison with literature data.

The result of our C13-NMR carbon values are similar to the reference artricle mentioned in the above. So, we confirmed the drug Quercetin is present in our ethanolic extract.

Graph: 3.5 C13-NMR isolated flavonoid ethanolic extract of rosa centifolia roots.



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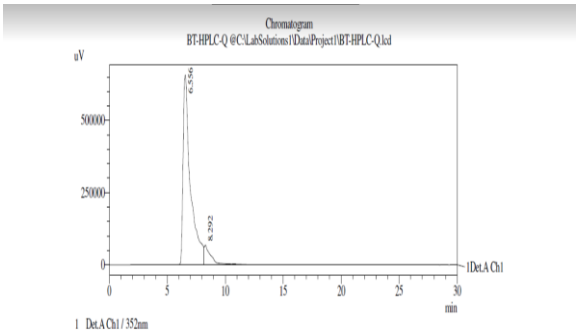
3.9 HPLC OF ISOLATED FLAVONOID OF ETHANOLIC EXTRACT OF ROSA CENTIFOLIA ROOTS:

The retention time of isolated compound from the ethanolic extract of sample was about 6.556 was shown by HPLC peak.

The result of our HPLC peak value is similar to the reference article mentioned in the above. So, we confirmed the drug Quercetin is present in our ethanolic extract.

Graph: 3.6 HPLC of isolated flavonoid of ethanolic extract of rosa centifolia roots



Peak Table

Detector A Ch1352 nm

Peak #	Ret. Time	Area	Height	Area%	Height%
1	6.556	29121681	656753	91.276	90.097
2	8.292	2709827	67964	8.724	9.903
Total		31831508	724717	100.000	100.000

IV.CONCLUSION

Quercetin is a flavonoid compound commonly found in various fruits, vegetables, and plants, including Rosa centifolia. The isolation of quercetin from plant sources usually involves ethanolic extraction and purification processes. From the present work, the roots of Rosa centifolia are typically collected and cleaned to remove any dirt or impurities. After ethanolic extraction, the mixture is usually filtered to remove any solid particles or plant debris. Various purification techniques can be employed to isolate quercetin from other compounds present in the ethanolic extract. These techniques may include column chromatography (CC) and preparative thin-layer chromatography (TLC). Once the compound is isolated, it can be characterized using spectroscopic techniques such as UV-Visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), and nuclear magnetic resonance spectroscopy (NMR). The isolated quercetin can be quantified using analytical techniques like high-performance liquid chromatography (HPLC).

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