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Isolation And Identification of Flavonoids from *Madhuca Indica* (Sapotaceae): A Comprehensive Phytochemical Analysis

Mudita Mishra[1*], Seema Tomar[1], Ashutosh Mishra[2]

[1 * ,1] Faculty of Pharmaceutical Sciences, Motherhood University, Roorkee
[2.] Department of Pharmaceutical Science, Acharya Narendra Deo College of Pharmacy Babhnan Gonda, Uttar Pradesh

***Corresponding Author :** Mudita Mishra
*Faculty of Pharmaceutical Sciences, Motherhood University, Roorkee
Email id: muditam8@gmail.com

Abstract :

Madhuca indica is a forest tree that grows in Malaysia and central and northern India. It is frequently seen in Bangladesh and other regions of the Indian subcontinent. These parts are used to treat a wide range of illnesses, including infestations of the helm, low testosterone count, headaches and migraines bloating, infections, rheumatic arthritis, cholera, paralysis, snakebite, debility, tonsillitis, influenza, piles, arthritic pain, and bacterial infections. They are also used as a purifying blood and an antidote to a poisonous substance

In current flavonoids contained in Madhuca indica aerial parts were extracted, identified and characterized. Sequential Soxhlet extraction was subjected to preliminary phytochemical screening and flavonoid quantification. The results showed that maximum yield of the flavonoid content (% w/w) were obtained from ethyl acetate extract. This ethyl acetate extract was subjected to column chromatography for isolation of flavonoid. Characterization of isolated flavonoid was done by UV, IR, 1H NMR and MS. On the basis of chemical and spectral analysis structure were elucidated as quercetin, rutin apigenin and naringenin flavonoid.

Key words
Madhuca indica, isolation, Flavonoids, quercetin chromatography Quercetin

1. Introduction

Polyphenolic compound has been identified as most importent molecule which have ability to modify and influence several cellular pathways Due to their capacity to affect a number of cellular processes "The most common group of polyphenolic compounds in the human diet and those found in plants in large quantities" are called flavonoids (Figueira et al., 2017). Despite the fact that more than 4000 flavonoids have been found, many fruits and vegetables seem to contain significant amounts of certain flavonoids. Flavonoids are divided into a number of subgroups, including flavone, flavanone, flavonol, isoflavonoid, anthocyanidin, and chalcones, based on the variations in functional groups and their relative locations of the 15-carbon skeleton (aglycons) (Nijveldt et al., 2001). Though in smaller amounts, flavonols—the original bioflavonoids, like quercetin rutin apigeninare likewise widely distributed (Miean & Mohamed 2001). A subclass of polyphenols, flavonoids are a family of phytochemicals that have several health benefits, including anti-inflammatory and anti-cancer properties (Karak et al., 2019). They are also among the most powerful and prevalent antioxidants in our diet. Madhuca indica, popularly referred to as "Mahua," is a tropical plant that is commonly found in India's central and northern plains and woodlands. It is a member of the Sapotaceae family, grows quickly to a height of 20 m, and has evergreen or semievergreen leaf. It also does well in arid climates (Quamar et al 2021).

Madhuca indica is a forest tree that grows in Malaysia and central and northern India. It is frequently seen in Bangladesh and other regions of the Indian subcontinent. Madhuca indica is an Indian plant with great therapeutic potential that is not being completely used since people are unaware of it. Mahua trees are quite potent pharmacologically and can be used to cure a wide range of illnesses (Srinivasa., 2022). Different parts of the tree, including complete young plants, leaves, stems, bark, roots, fruits, flowers, and seeds are employed in Bangladesh's traditional medical system. The goods made from trees are either sold in local and regional markets or consumed by the communities themselves. Trees with numerous uses, including decorative, medicinal, spiritual, and cultural. *Madhuca indica* a tree that is worshipped by tribal has versatile uses **(Lakshmiprasanna & Aparna., 2020).**

Entire plant and almost all plant parts are used to treat a wide range of illnesses, including, low testosterone count, headaches and migraines bloating, infections, rheumatic arthritis, cholera, paralysis, snakebite, debility, tonsillitis, influenza, piles, arthritic pain, and bacterial infections (Khan et al., 2011). They are also used as a purifying blood and an antidote to a poisonous substance. From the bark of this tree, two proto basic glycosides, called madhucosides A and

B, were discovered. These two substances significantly inhibited the production of hypochlorous acid by neutrophils and the release of superoxide from polymorph nuclear cells (Sengar & Singh., 2009).
The aim of the study was to isolate the flavonoids using Flash Chromatography and was characterized through their spectral analysis like IR, 1H, 13C NMR and MS.

2. Materials and Methods

2.1 Chemicals and standard drugs
All the chemicals and solvents used were of analytical grade, Silica gel (G) 60 F and 0.25 readymade aluminium sheets (Merck, Germany),

2.2 Plant material and preparation of extract
Air-dried *Madhuca indica* (Sapotaceae) Bark were procured from local areas of Haridwar in the month of March and identified by Dr. Lal Babu Chaudhahary, senior principal scientist and Professor of A CSIR, division of CSIR Department of Plant diversity, systematics, and herbarium division, National Botanical Research Institute, Lucknow, India and the accession number of *Madhuca indica* was 106870. Fresh plant material was properly washed with running water to remove dust and then allowed to air dry in the shade at room temperature. The dried plant material was further processed with a grinder into a coarse powder before the extraction procedure started. The powdered crude material (1 kg) was defatted with petroleum ether and then extracted successively with chloroform, ethyl acetate, and methanol, using Soxhlet extractor followed by cold maceration (7 days) with 50 % methanol. The extracts were concentrated using rotary vacuum evaporator to yield MIEA extract (MIEA yield: 3.9 % w/w), The extract was subsequently subjected to phytochemical screening and quantitative estimation

2.3 Thin layer chromatography and Phytochemical screening
The prepared extract was screened for the presence of different phytochemicals employing thin layer chromatographic (TLC) techniques (Wagner and Bladt, 1996). Thin layer plates precoated with silica gel G (Merck, 0.25 mm thickness) were used. Development was carried out by trying different solvent systems and finally was finalized After development of chromatogram in the solvents, the plates were dried and sprayed with FeCl3 reagents for the detection of flavonoids. Visualization was carried out under visible and UV light (l: 366 nm). In preliminary phytochemical screening presence of flavonoid also spotted (Tivari et al., 2011).

2.4. Determination of total flavonoids (TF)
Flavonoid content was determined by the aluminium chloride colorimetric method **(**Chang et al., 2002**)** Briefly, to 1 ml of test solution (1 mg/ml), 1.5 ml of 95 % alcohol, 0.1 ml of 10 % aluminum chloride hexahydrate (AlCl3.6H2O), 0.1 ml of 1 M sodium acetate (CH3COONa) and 2.3 ml of distilled water was added. After incubation at room temperature for 40 min, absorbance of the reaction mixture was measured at 435 nm against corresponding blank, prepared in the same manner without adding AlCl3. quercetin was used as a reference standard and results were expressed as mg of rutin equivalents (RE)/g of extract. All determinations were performed in triplicate.

2.5 Isolation using column chromatography
Silica gel (60–120) was put into the column. A gradient method using toluene and ethyl acetate as solvent was used to elute the column. A rota evaporator was used to concentrate each 50 ml portion before it was allowed to crystallise. There were 400 fractions in all that were collected. Groups were formed from the fractions with the same Rf values. Using the repeated recrystallization process, the isolated chemical was purified.

2.6 Compound characterization
Physical, chemical, and spectral investigations were used to identify the substances. Using a melting point device, the melting points were measured in open-glass capillaries. Using TMS (tetramethyl silane) as the internal standard, 1HNMR and 13CNMR spectra were acquired on a Brunauer 400 MHz spectrometer. Mass spectra were captured using the Bruker Daltonics ESIesquire 3000 instrument. Thermo Scientific's Nicolet 380 FTIR spectrometer was used to acquire IR spectra from KBr pellets, while the Shimadzu UV-1700 (double-beam) spectrophotometer was used to capture UV spectra.

3. Results and discussion

3.1 Phytochemical screening
The ethyl acetate extract were screened for the presence of different phytochemicals employing thin layer chromatographic (TLC) techniques. Thin layer plates precoated with silica gel G (Merck, 0.25 mm thickness) were used. Development was carried out with different solvent systems such as toluene : chloroform: methanol: water (5:3:1, v/v/v),

chloroform: methanol: water (7:3 v/v/), and toluene: ethyl acetate (8:2, v/v). After development of chromatogram in the solvent, the plates were dried and sprayed with FeCl3 reagents for the detection of flavonoids. Visualization was carried out under visible and UV light (l: 366 nm). The quantitative estimation of flavonoid in MIEA extract were carried out also.

3.2 Determination of flavonoid

The content of flavonoids (mg/g) was determined by the regression equation of the calibration curve (y = 0.008x - 0.004 R^2 = 0.993) and was expressed in quercetin equivalents (RE) and was found to be 1.250

3.3. column chromatographic isolation of flavonoid

The ethyl acetate extract of dried stem bark of *Madhuca indica* were chromatographed on silica gel column and thus isolated and purified four flavonoids; Quercetin, apigenin and naringenin rutin and quercetin was achieved.

Fractions 13–38 of the mobile phase (toluene: ethyl acetate 90:10) were used to isolate Compound A (Quercetin). 8% FeCl3 soln was used to detect the compound's presence. From fractions 47–74, compound B (Rutin) was separated using the mobile phase (toluene: ethyl acetate 90:15). After the crystals were extracted from the mobile phase, acetone and methanol were used to recrystallize the particles for purification. The compound's presence was ascertained by misting an 8% FeCl3 solution onto a TLC plate. Fractions 75–118 of the mobile phase (toluene: ethyl acetate 80:20) were used to isolate compound C (Apigenin). After the crystals were extracted from the mobile phase, acetone and methanol were used to recrystallize the particles for purification. After increasing polarity, Pooled Fractions 211-260 were rechromatographed over a silica gel subcolumn using eluents (toluene: ethyl acetate 80:20). Fractions 6-27 of the subcolumn were used to isolate Compound D (Naringenin) using a 60:40 toluene:ethyl acetate mobile phase. The chemical was purified by wiping it with cold methanol.

3.4. Identification and spectral analysis of isolated flavonoid

COMPOUND A

Compound a was isolated as Yellow crystals, 40 mg (0.5% w/w yield) Rf 0.74 (ethylacetate: toluene 30:70);m.p. 315-317°C. Compound gave positive glycoside test; UV λmax (methanol): 240 and 440 nm; ^{1}H-NMR (400MHz, DMSO) showed signals at δ 6.14 (d, *J* = 2.0 Hz, 1H), 6.37 (d, *J* = 2.1 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 1H), 7.50 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.64 (d, *J* = 2.2 Hz, 1H), 9.33 (d, *J* = 26.7 Hz, 2H), 9.58 (s, 1H), 10.77 (s, 1H), 12.46 (s, 1H) (Figure 4.3). IR (KBr, cm^{-1}) spectrum showed broad peak at 3153 (OH), 1864 (C–H Aromatic), 1710 (C=O), 1623 (C=C Aromatic), 1293 (C-O-C) (Figure 3.2). ESI-MS m/z 302.04 [M+H], 303 [M+2] (Figure 3.3). So the mass of the compound was 302. Thus, on the basis of above physical, chemical and spectral data compound A was characterized as Quercetin (Figure 3.4) having molecular formula $C_{15}H_{10}O_7$

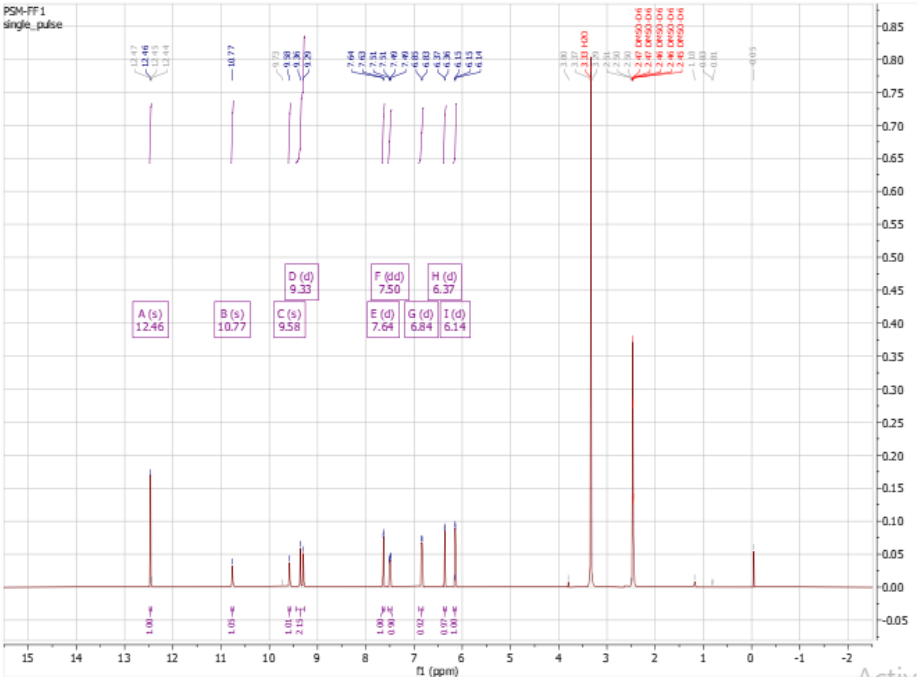


Figure.3.1: ^{1}H-NMR of Quercetin.

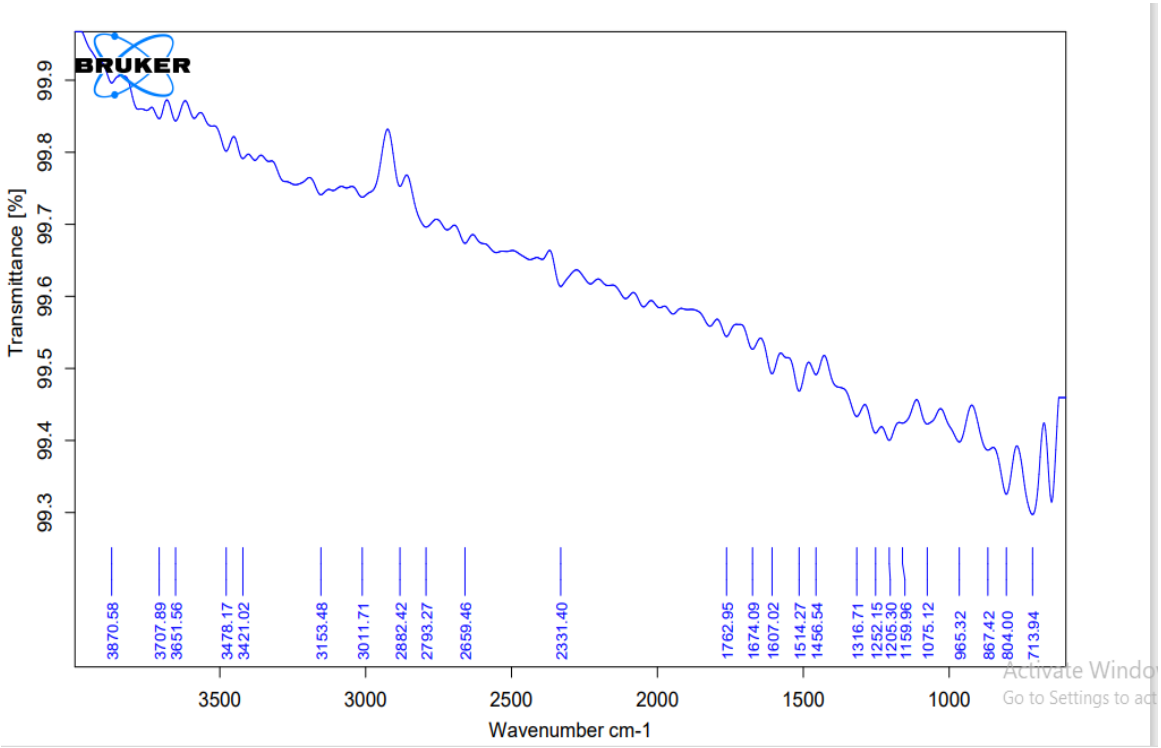
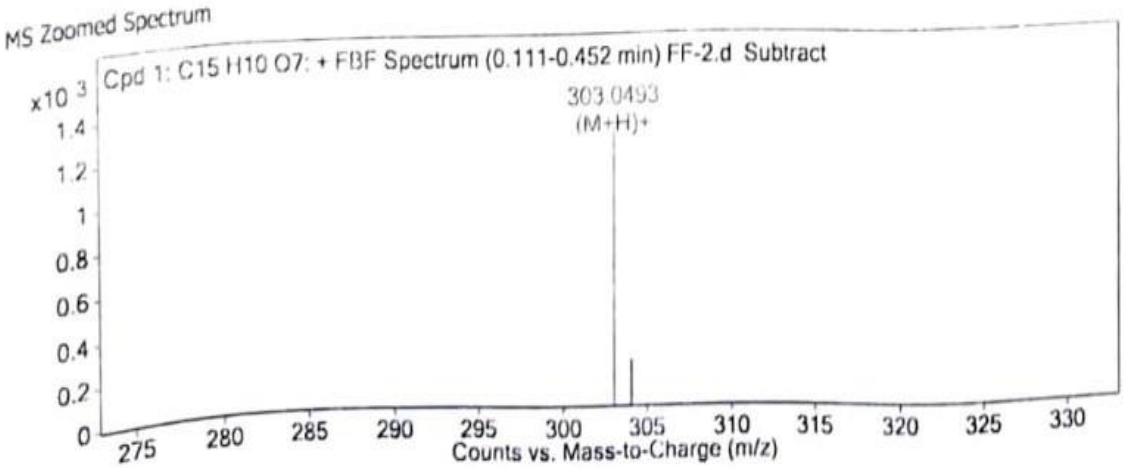


Figure 3.2: IR Spectra of Quercetin.



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
303.0493	1	1296.93	C15H11O7	(M+H)+
304.0528	1	160.61	C15H11O7	(M+H)+

Figure 3.3: Mass Spectra of Quercetin.

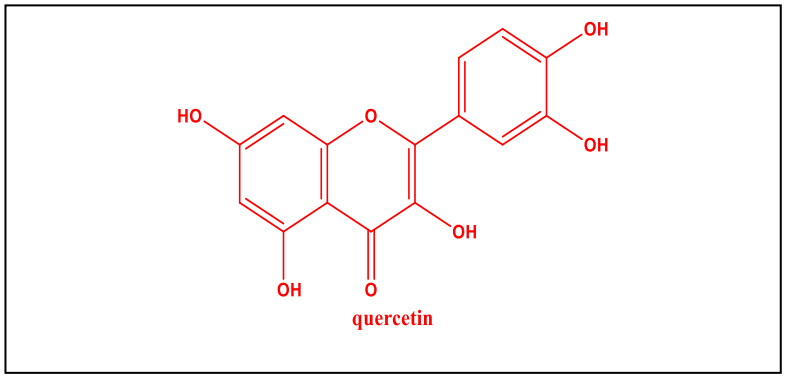


Figure 3.4 Structure of Quercetin.

COMPOUND B

Compound B was isolated as Light yellow crystals, 15 mg (0.5% w/w yield) Rf 0.34 (Dichloromethane: methanol 4:1); m.p. 315-317°C. Compound gave positive Shinoda test; UV λmax (methanol): 258 nm; ^{1}H-NMR (400MHz, DMSO), showed signals at δ 0.95 (d, J = 6.2 Hz, 3H), 3.03 (t, J = 9.4 Hz, 2H), 3.18 (t, J = 6.8 Hz, 3H), 3.19 – 3.33 (m, 3H), 3.66 (d, J = 10.5 Hz, 1H), 4.34 (s, 1H), 4.39 (s, 1H), 4.52 (s, 1H), 5.03 – 5.13 (m, 2H), 5.25 – 5.34 (m, 2H), 6.15 (d, J = 2.0 Hz, 1H), 6.35 (d, J = 2.0 Hz, 1H), 6.80 (d, J = 8.1 Hz, 1H), 7.49 (s, 1H), 9.18 (s, 1H), 9.66 (s, 1H), 10.82 (s, 1H), 12.57 (s, 1H) (Figure 3.5). IR (KBr, cm^{-1}) spectrum showed broad peak at 3450 (OH), 3020 (C–H Aromatic), 1715 (C=O), 1653 (C=C Aromatic), 1300 (C-O-C) (Figure 3.6). ESI-MS m/z 610 [M+H], 611 [M+2] (Figure 3.7). So the mass of the compound was 610. Thus, on the basis of above physical, chemical and spectral data compound B was characterized as Rutin (Figure 3.8) having molecular formula $C_{27}H_{30}O_{16}$.

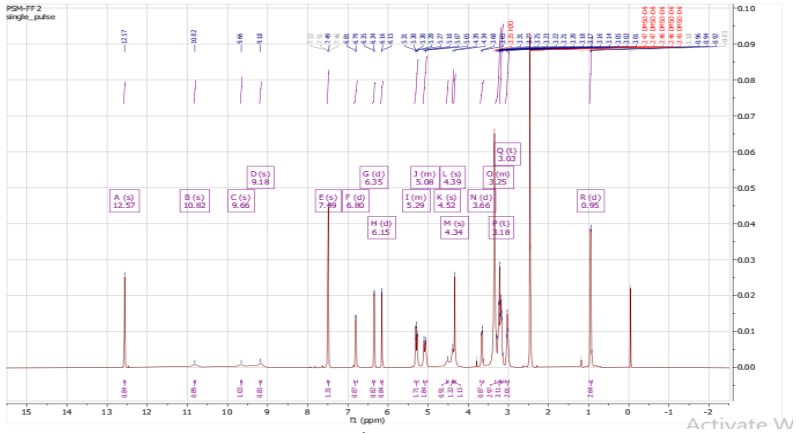


Figure 3.5 ^{1}H-NMR of Rutin

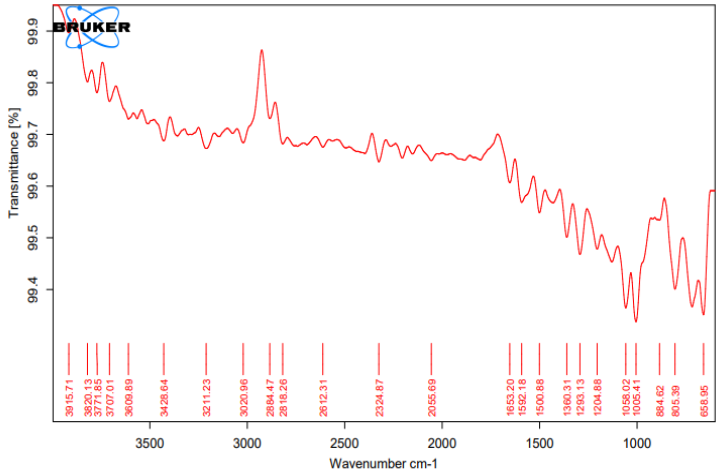


Figure 3.6: IR Spectra of Rutin.

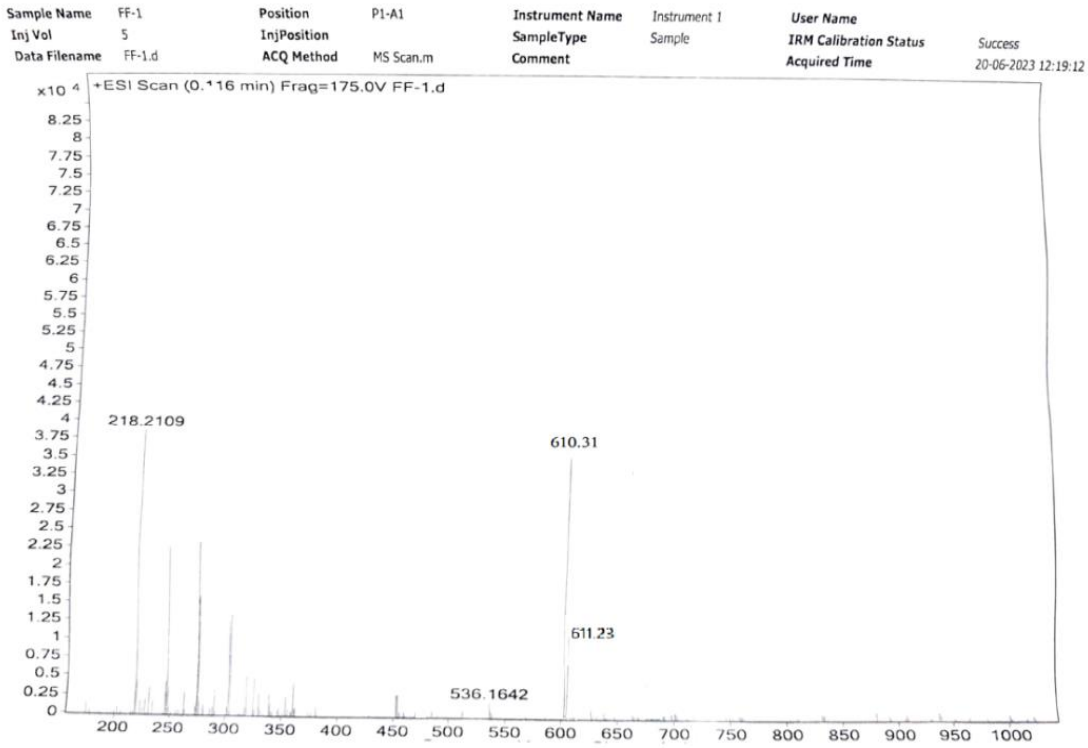


Figure 3.7: Mass Spectra of Rutin.

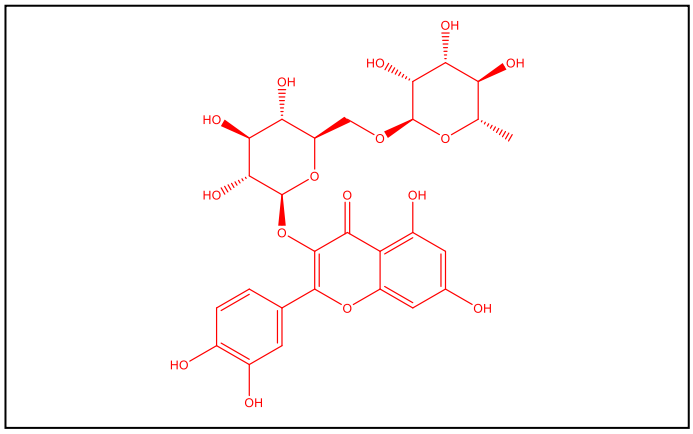


Figure 3.8: Structure of Rutin

Compound C

Compound C was isolated as yellow crystalline solid, 30 mg (0.5% w/w yield) Rf 0.34 (Dichloromethane: methanol 4:1); m.p. 345-355°C. Compound gave positive Shinoda test; UV λmax (methanol): 268 and 332 nm; ^{1}H-NMR (400MHz, DMSO), δ 6.15 (d, *J* = 2.1 Hz, 1H), 6.44 (d, *J* = 2.1 Hz, 1H), 6.76 (s, 1H), 6.84 – 6.92 (m, 2H), 7.85 – 7.93 (m, 2H), 10.34 (s, 1H), 10.81 (s, 1H), 12.93 (s, 1H) (Figure 3.9). IR (KBr, cm^{-1}): 3452 (OH), 3150 (C–H Aromatic), 1715 (C=O), 1653 (C=C Aromatic), 1353 (C-O-C) (Figure 3.10). ESI-MS m/z 271.06 [M+H], 272.06 [M+2] (Figure 3.11). So, the mass of the compound was 271. Thus, on the basis of above physical, chemical and spectral data compound C was characterized as Apigenin (Figure 3.12) having molecular formula $C_{15}H_{10}O_5$

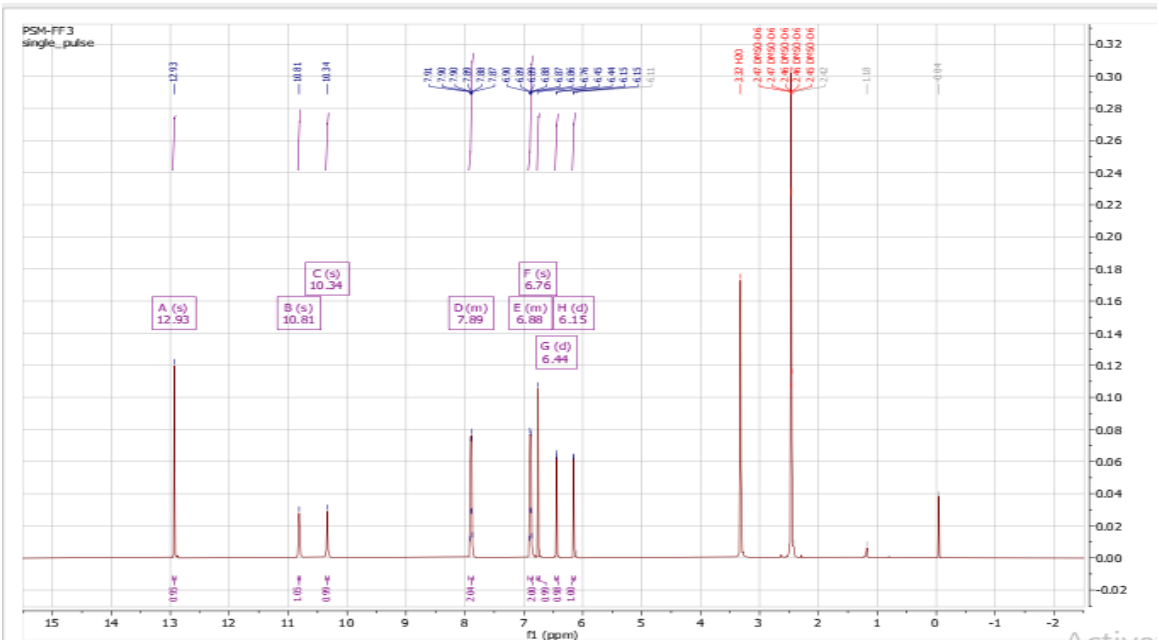


Figure 2.9: ^{1}H-NMR of Apigenin

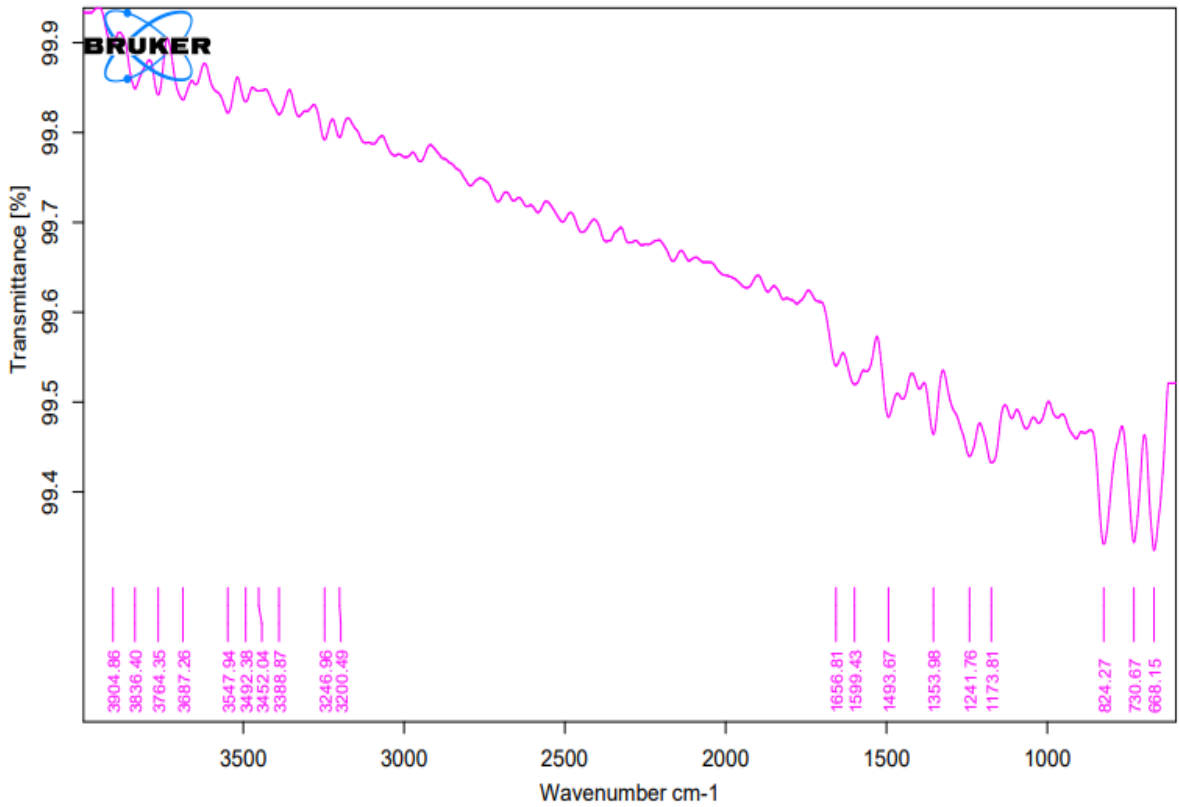
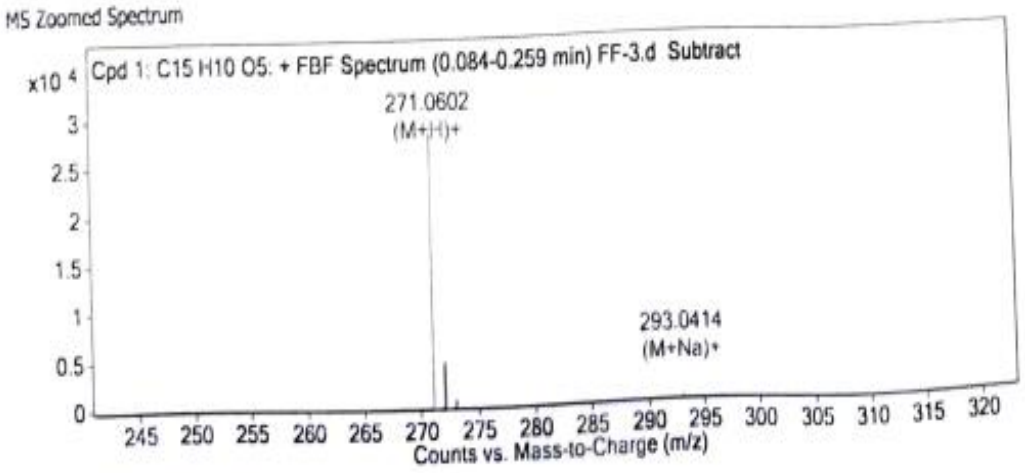


Figure 3.10: IR Spectra of Apigenin



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
271.0602	1	28458.89	C15H11O5	(M+H)+
272.0632	1	4418.44	C15H11O5	(M+H)+
273.0653	1	573.92	C15H11O5	(M+H)+
293.0414	1	321.59	C15H10NaO5	(M+Na)+
294.0447	1	21.45	C15H10NaO5	(M+Na)+

Figure 3.11: Mass Spectra of Apigenin.

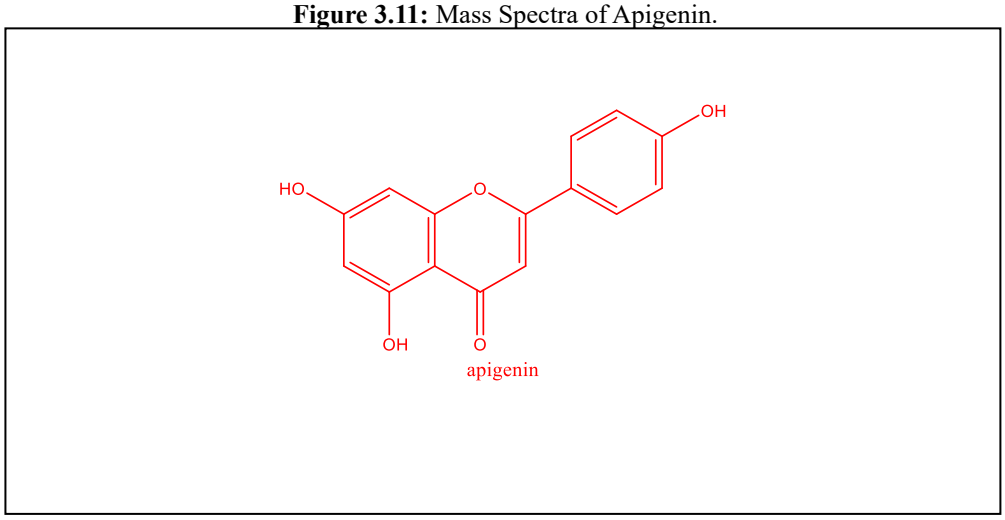


Figure 3.12: Structure of Apigenin

Compound D

Compound D was isolated as off white crystalline solid, 50 mg (0.5% w/w yield) Rf 0.34 (Dichloromethane: methanol 4:1); m.p. 250-251°C. Compound gave positive shinoda test UV λmax (methanol): 324 nm; ^{1}H-NMR (400MHz, DMSO), δ ppm: δ 1.08 – 1.22 (m, 3H), 2.68 (ddd, *J* = 17.3, 9.6, 3.1 Hz, 1H), 3.15 (td, *J* = 9.9, 5.1 Hz, 2H), 3.22 – 3.44 (m, 2H), 3.40 (s, 3H), 3.63 (s, 2H), 3.57 – 3.71 (m, 1H), 4.48 (d, *J* = 5.8 Hz, 1H), 4.56 (t, *J* = 4.6 Hz, 1H), 4.65 (d, *J* = 4.3 Hz, 1H), 4.71 (d, *J* = 4.6 Hz, 1H), 5.02 – 5.07 (m, 1H), 5.11 (t, *J* = 7.6 Hz, 2H), 5.30 (d, *J* = 5.0 Hz, 1H), 5.46 (td, *J* = 13.6, 2.9 Hz, 1H), 6.06 (dt, *J* = 12.4, 2.4 Hz, 2H), 6.72 – 6.79 (m, 2H), 7.29 (dd, *J* = 8.6, 3.3 Hz, 2H), 9.60 (d, *J* = 1.9 Hz, 1H), 12.02 (d, *J* = 2.6 Hz, 1H) (Figure 3.13). IR (KBr, cm^{-1}): 3249 (OH), 3164 (C–H Aromatic), 1766 (C=O), 1621 (C=C Aromatic), 1350 (C-O-C) (Figure 3.14).ESI-MS m/z 272 [M+H], 273 [M+2] (Figure 3.15). So, the mass of the compound was 272. Thus, on the basis of above physical, chemical and spectral data compound A was characterized as Naringenin (Figure 4.16) having molecular formula $C_{15}H_{12}O_5$.

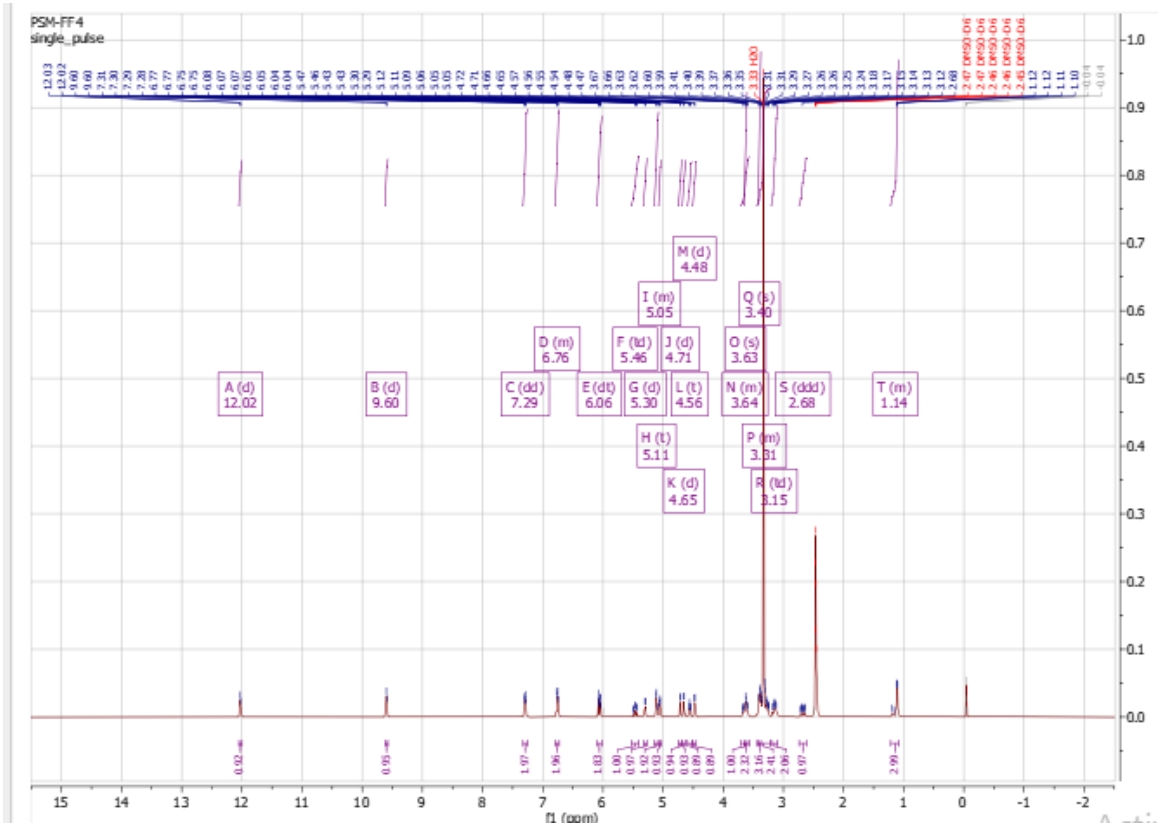


Figure 3.13: ^{1}H-NMR of Naringenin

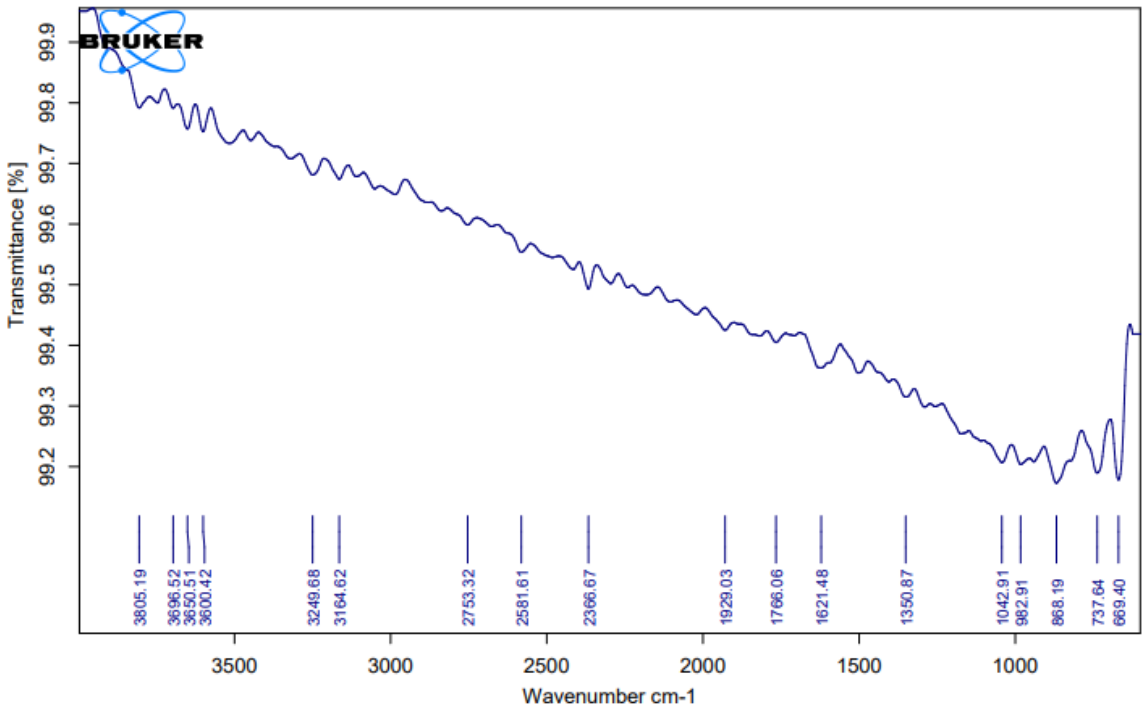
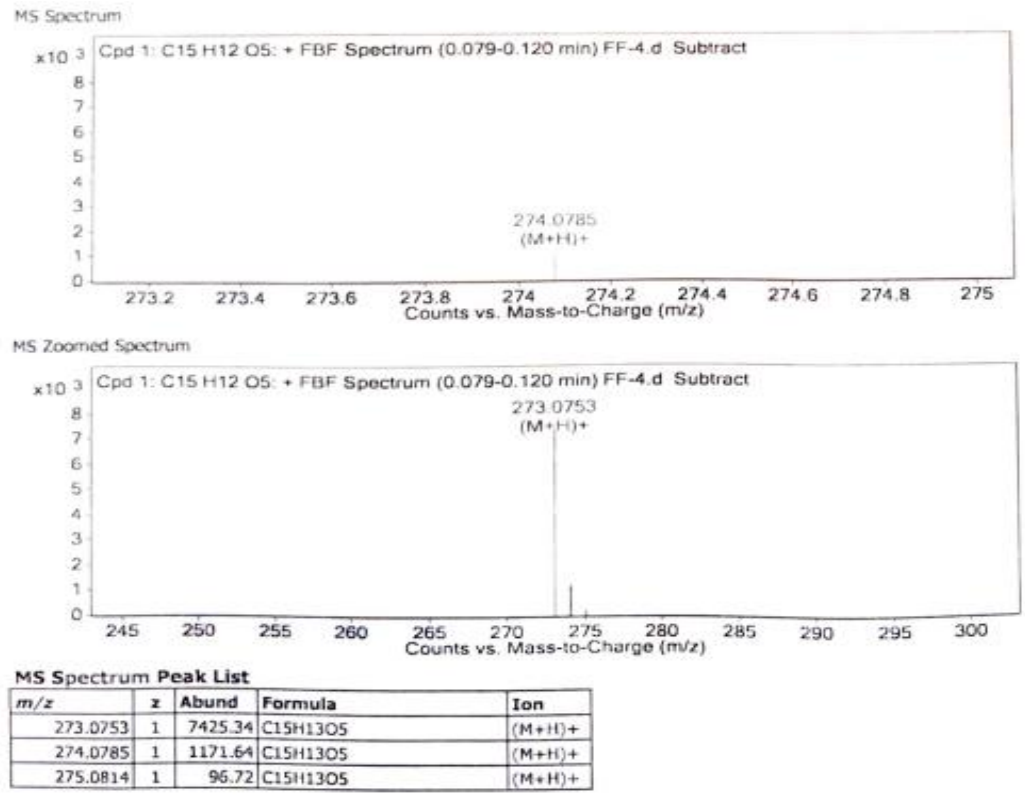


Figure 3.14: IR Spectra of Naringenin



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
273.0753	1	7425.34	C15H13O5	(M+H)+
274.0785	1	1171.64	C15H13O5	(M+H)+
275.0814	1	96.72	C15H13O5	(M+H)+

Figure 3.15: Mass Spectra of Naringenin

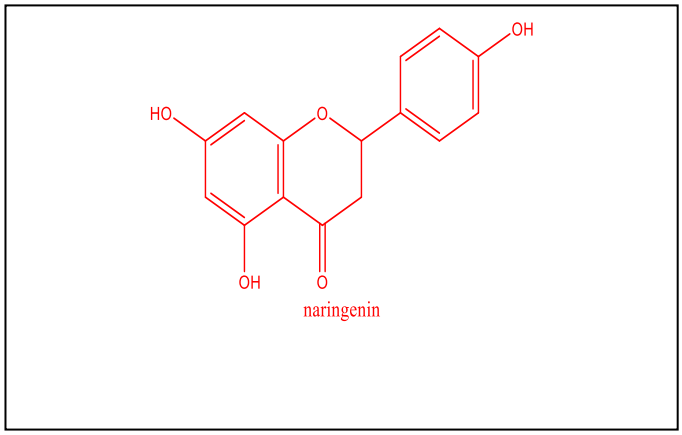


Figure 3.16: Structure of Naringenin

Concluding the aforementioned studies, it was analysed that the studied plant contained flavonoids. The phytochemical screening of isolated compound suggested it to be a flavonoidal molecule. TLC with standard was also performed and it showed same Rf value. The melting point of isolated compounds was same as that of above reported compounds

Conflicts of interest
None to declare

4. Funding
None

5. Acknowledgement

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7. References

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