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ANALYTICAL CHARACTERIZATION OF QUERCETIN ISOLATED FROM LEAVES OF Psidium guajava L.

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Abstract: Guava is a significant food crop also as restorative plant observed tropical and subtropical areas. *Psidium guajava linn.* is an individual from Myrtaceae family, which contains 133 genera and excess of 3,800 plant species. Guava is wealthy maximum of phenols, triterpenes, flavonoids, oils, saponins, lectins, vitamin-c and fiber. The leaf of guava is reach source of flavonoids specifically quercetin, kamiferol luteol. The guava is helpful to increase bowel movement is ascribed due to favonoids and fibres. The flavonoids have pharmacological activity like antibacterial, antioxidant, hepatoprotective. In isolation of quercetin separate form from leaves of *Psidium guajava l.* by using methanol and fallowed the different chemical and analytical test like Lead acetic acid derivation test, Sodium Hydroxide test and Shinoda test , thin layer chromatography mobile phase was Ethyl acetate: Formic acid: GAA: Water, and different procedures UV, IR, NMR, HPLC and HPTLC of Quercetin was studied .

Index Terms **- Chromatography, Flavonoids, *Psidium Guajava L*, Quercetin**

1. Introduction The *Psidium guajava l*., from the Myrtaceae family, is regular observe in all through tropical and subtropical zones [1] among others, and these natural exercises have mostly been identified with the phenolicand flavonoid mixes [2]. *Psidium guajava l* is a significant food crop and restorative plant in tropical and subtropical region , the Myrtaceae family, which contains 133 genera and in excess of 3,800 plant species. These days, elective restorative methodologies dependent on the utilization of phenolic mixes in food items as "utilitarian nourishments" and "nutraceuticals" are being created. Actually, the limit of plant-inferred nourishments to lessen the danger of ceaseless ailments has been exhibited [3]. Guava is wealthy in tannins, phenols, triterpenes, ß flavonoids, basic oils, saponins, carotenoids, lectins, nutrients and unsaturated fats. Guava natural product is higher in nutrient of Vitamin-C than citrus organic products (80 mg of Vitamin-C in 100g of foods grown from the ground) apparent measures of Vitamin A too Guava organic products are additionally a decent wellspring of gelatin and reach source of pectin [4]. Queretin exibit various pharmacological effects like Quercetin has several pharmacologic actions; it prohibit the movement of intestine, and decrease capillary permeability in the abdominal cavity [5]. Flavonoid is naturally occouing group of compound contain quercetin which exhibit the various pharmacological activity , chemically it is flavones nucleous containing two benzene ring fused together with heterocyclic pyrone ring [13,14,15].Quercetin is the most generally dispersed and widely examined flavonoid found in different food sources, including natural products, vegetables, nuts, wine, and seeds [6, 7] . Quercetins have wide range of pharmacological and biological application, which used in pharmaceutical, cosmetic and neutraceutical industries [8]. Quercetin is one of the significant bioflavonoids present in excess of twenty plants material and which is known for its calming, antihypertensive, vasodilator impacts, antiobesity, antihypercholesterolemic and antiatherosclerotic studies [9,10]The physical properties of Quercetin is a yellow, crystalline solid with a bitter taste powder, insoluble in water, slightly soluble in alcohol, soluble in glacial acetic acid and aqueous alkaline solutions [11, 12].

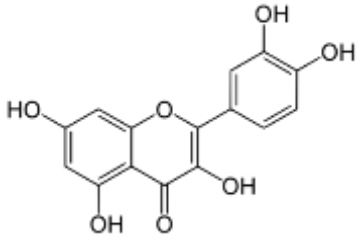


Figure No. 01: IUPAC name 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one

2. MATERIALS AND METHODS

The plant leaves of *Psidium Guajava l* collected it from the farm in Bhor , Pune district, (Maharashtra), India, and it was cleaned. Authenticated by Dr. P.G. Diwakar for Joint Director, Botanical Survey of India, from Botanical Survey of India, Koregaon road, Pune by comparing morphological features. The gerbarium of plant specimen deposited at Botanical survey of India , Pune voucher specimen number BSI/WRC/Tech./2010.

2.1 Reference Material:

Quercetin-3-β-d-glucoside and quercetin-3-β-d-galactosid were supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

2.2 Chromatographic requirements

Prepared Precoated thin layer chromatography (TLC) plates wit (silica gel 60F-254) with the adsorbent layer thickness of 0.25 mm (E-Merck), silica gel (Merck) and kieselgel 60,

2.3 Phytochemical screening:

2.3.1 Extraction and isolation: The dried powder material of leaves *Psidium guava l* (250gm) sample is extracted in 80% methanol(250 ml) in soxhlet for 24 hrs. The extract was concentrated and reconcentrated in petroleum ether (40°- 60°C) for 12 hrs (fraction-I), concentrate and mix separetly in ethyl ether for 12 hrs (fraction-II) and ethyl acetate (fraction-III) in succession. Each of the steps was repeated three times to ensure complete extraction in each case. Fraction I was rejected since it was rich in fatty substances whereas fraction II was analysed for the free flavonoids in each of the samples. Fraction III of each of the test samples was hydrolysed by refluxing with 7% H2SO4 (10 ml/gm residue) for 5 hours. The mixture was filtered and the filtrate extracted with ethyl acetate in a separating funnel. The ethyl acetate layer was washed with distilled water till neutrality and dried in vaccum. The residues were taken up in small volumes of ethanol separately and then subjected to various tests for quercetin [16]

2.3.2 CONFIRMATORY TEST for test of flavonoid - Quercetin) [17, 18, 19]

a) Lead acetate test: Few drops of 10 percent lead acetate are added to the extract. Development

of yellow coloured precipitate confirms the presence of flavonoids.

b) Sodium Hydroxide test: To the extract-increasing amount of Sodium Hydroxide was added gives yellow colour, which disappeared after addition of acid.

c) Shinoda test (Magnesium Hydrochloride reduction test): To the test solution add few fragments of Magnesium turning and add cone. Hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

2.3.4 ANALYTICAL CHARACTERIZATION OF QUERCETIN:-

A. THIN LAYER CHROMATOGRAPHY - For Quercetin[18, 20]:

a. Steps involved in performing TLC:

I. **Preparation of TLC plate:** Arranged the slurry of adsorbent media (silica gel-G) in distilled water and poured the slurry on the TLC glass plates to get a slender layer.

II. **Activation of TLC plate:** Heating in oven for 30 min. at 105^{O}C activated TLC plate.

III. **Sample application:** Dunking the capillary tube into the solution for be analyzed and applied the example by slim contacted to the meager layer plate at a point around 2 cm from the base and air dried the sample spot.

IV. **Chamber saturation:** The glass chamber for TLC should be saturated with mobile phase. Mobile phase poured into the chamber and capped with lid. Allowed saturating about 30 min.

V. **Chromatogram development:** After the saturation of chamber and spotting of samples on plate, it was kept in chamber. The solvent level in the bottom of the chamber must not be above the spot that was applied to the plate, as the spotted material will dissolve in the pool of solvent instead of undergoing chromatography. Allowed the solvent to run around 10-15 cm on the silica plate.

VI. **Visualization:** Plates were removed and were examined under UV cabinate and suitable visualizing agent (Vanillin-sulphuric acid, Methanolic ferric chloride solution) after that R_f was calculated by following formula in given table no.1

Table No. 1: Formula for determination of retention factor

R_f =	Distance Traveled By Solute From Origin Line
	Distance Traveled By Solvent From Origin Line

b. Solvent system for thin layer chromatography for Flavonoid (Quercetin):

The giving table no 02.mention the TLC use the solvent system for identification of quercetin contain the mobile phase contain Ethyl acetate, Formic acid, Glacial acetic acid (GAA) and Water [20].

Table No. 2: Development system for T.L.C. of Flavonoid (Quercetin)

Stationary phase	Silica gel-G
Mobile phase	Ethyl acetate: Formic acid: GAA: Water (100:11:11: 26)
Chamber saturation	30 min.
Visualization	Solution of Anisaldehyde – Sulphuric acid

B. UV of Quercetin [27]: - 0.1g of Quercetin is dissolved in the 100ml ethanol (1000µg/ml).0.1ml of that solution is removed and diluted 100ml (10µg/ml).And then λ max was observed.

C. IR of Quercetin [25 26]: - The IR spectrum of Quercetin is mixed with the Dried KBr disc has been determined on Perkin-Elmer. Infrared Spectrophotometer and the structural assignment have been correlated for the characteristic band is listed.

D. NMR of Quercetin[22]:- The NMR spectroscopy used for interrelation and help for identification of compound and its molecular structure .The NMR spectroscopy for quercetin generally solution of extract and isolated Quercetin from leaves of *Psidium guavaja linn* in DMSO solution using procure from thermosil Scientific .

Data Collected on: Varian-NMR-mercury 300.

Fid file: Proton, Carbon.

Pulse sequence: Proton (s2 pul), Carbon (s2 pul).

Solvent: DMSO.D_6

E. HPLC [21]: The HPLC analysis of Quercetin perform the column condition 4×125 mm Hypersil ODS with mobile phase 0.5% Orthophosphoric acid in water and Methanol , flow rate 1ml/min column wash 20 min 100% and 18 min 100% . The column temperature is 25 ^{0} C. The sample injected volume 10µl.

F. HPTLC[23, 24]:

a) Stationary phase: The 05 x 10 cm aluminum-backed HPTLC plate coated with 250 µm layers of Silica gel G 60 F254 (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai) was prewashed with methanol and activated at 110ºC for 10 min was used as stationary phase.

b) Mobile phase: Toluene: Ethyl Acetate: Formic Acid (5:5:0.3 % v/v) was used as mobile phase.

c) Development: The plate was developed in an ascending manner with a solvent system consisting of Toluene: Ethyl Acetate: Formic Acid (5:5:0.3 % v/v) in a development chamber presaturated (20 min) with the mobile phase. Developing distance was 1 cm from lower edge of the plate. The length of each chromatogram run was 8 cm. The developed plates were air dried.

I. **Densitometer scans:** Plate was scanned at 400 nm using scanner-3 (CAMAG) operated in reflectance–absorbance mode and controlled by Win CATS software (Version 1.4.3). The slit dimensions were 5 x 0.45 mm and the scanning speed was 20 mm/s. The source of radiation used was deuterium lamp emitting continuous UV spectra between 200-400 nm**.**

II. **Documentation:** The profile obtained was video documented at 254 nm and visible light after development of the plate.

3. RESULT AND DISCUSSION:

The phytochemical extraction and isolation of quercetin from plant *Psidium guava l* dried residues in vaccum. They were taken up in small volumes of ethanol separately and then subjected to various tests for quercetin.

- The Percentage yield calculated was found to be of Quercetin is 160 mg

3.1 Physical Characteristics:

a) **Colour** :Yellow
b) **Odour** :Characteristic
c) **Taste** :Tasteless
d) **Melting Point** :31^{0}C Dissociates
e) **Solubility** : Soluable in Ethanol, Methanol And Alkaline solvent

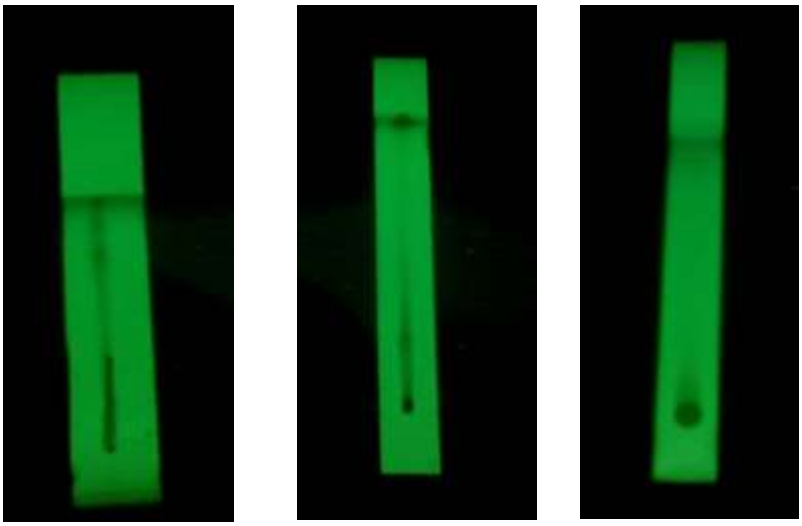
3.2 Chemical Test:

Table No 03: Chemical confirmatory test for isolated Quercetin from leaves of *Psidium guava L*.

Sr. No.	Test	Observation	Inference
1	Lead acetate test	Yellow colour precipitate	Flavonoid
2	Sodium Hydroxide test	Yellow colour, which disappeared after addition of acid.	Flavonoid
3	Shinoda test (Magnesium Hydrochloride reduction test)	Pink scarlet	Flavonoid

The chemical test show positive response of isolated compound presence of flavonoid confirmed. The leaves of Psidium guava show maximum number of phenolic and flavonoid constituent, which gives the maximum pharmacological activity. The Tabel no 03 shows positive test for lead acetate, sodium hydroxide test and shinoda test gives positive result of isolated compound, so we confirm the isolated compound is quercetin.

3.4 THIN LAYER CHROMATOGRAPHY – For extract and isolated Quercetin



A B C

Figure No 02:- TLC of *Psidium guava L* Extract (A) and Isolated Compound Quercetin (B) and standard Quercetin (C)

The figure no 02 indicate the different spot were observed, the spot are simultaneously *Psidium guavaja l* extract , isolated compound and reference substance for confirmation. The result shows the positive response observes under UV light identified the similar spot of isolated compound and reference substance confirmed the isolated compound is quercetin.

3.5. UV Spectra of Quercetin:

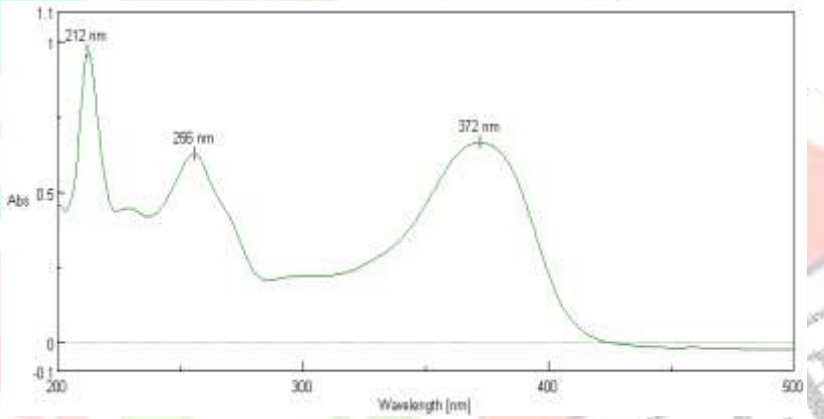


Figure No 03: U.V. Spectra of reference Quercetin.

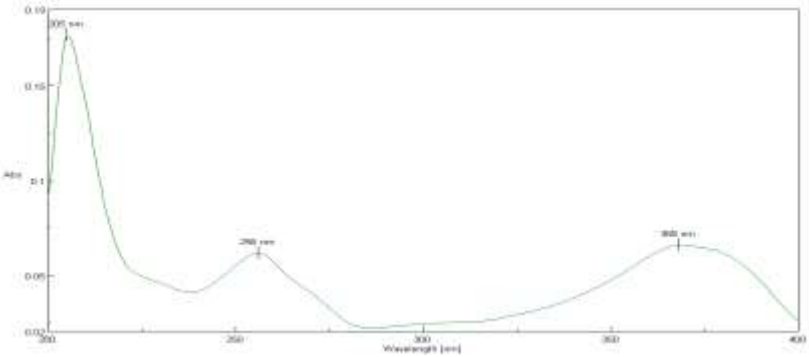


Figure No 04: U.V. Spectra of Isolated Quercetin from *Psidium guava L*

Table No 04: ƛ max was observed of U.V Spectrum of Isolated Compound (Quercetin).

Sr. No	Standard	Isolated
	Absorbance nm	Absorbance nm
1	212	205
2	256	256
3	372	368

The table no 04 shows the UV spectroscopy study of isolated compound from leaves of *Psidium guavaja l* , which is compare with the reference compound similar ƛ max was observe , so we confirm the isolated compound is Quercetin.

3.6. IR of Quercetin:

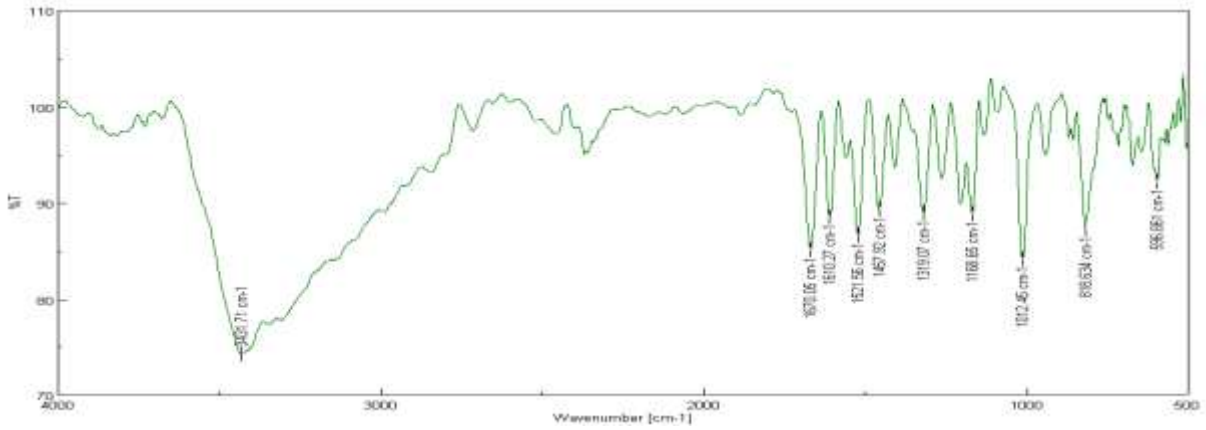


Figure No. 05. I.R. Spectra of Reference Quercetin.

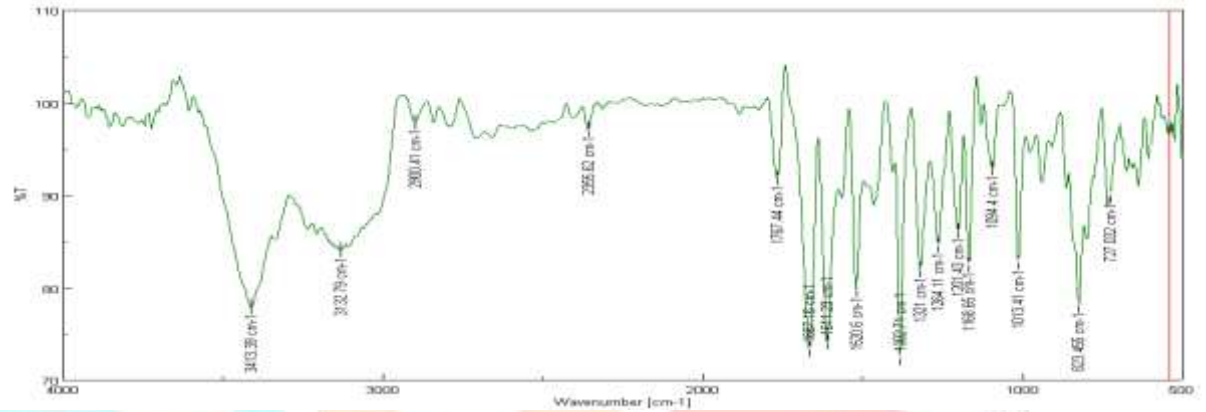


Figure No.06: I.R. Spectra of Isolated Quercetin.

Sr. No.	Frequency(cm^{-1})	Assignment
1	3413.39-3132.79	OH- Bonded
2	2900.41-2355.62	-CH Strech
3	1767.44	-C=O
4	1667.16	-C=C-
5	1611.23	-C=O Aromatic
6	1520.6	-C=C- Aromatic
7	1382.71-1264.11	-C-O-C
8	1201.43	-C=O Strech
9	1168.65-1094.4-1013.41	-C-O-C
10	727.032-823.455	-C-H-bending

Table No. 05: Frequency & Assignment of Quercetin.

The IR spectrum of Quercetin is highly specific for each chemical structure with a small structure difference resulting in significant spectral changes. The table no 05 shows the spectra of isolated compound of same compound are compared with standard compound. FTIR Spectrum is characteristic of entire molecule and it helps structural information by referring to generalized chart of characteristic group Frequencies**.**

3.7. NMR of Quercetin:-

Data Collected on: Varian-NMR-mercury 300.

Fid file: Proton, Carbon.

Pulse sequence: Proton (s2 pul), Carbon (s2 pul).

Solvent: $DMSO.D_6$

The figure no 07 and 08 shows the Proton NMR range from 01 to 13ppm of isolated quercetin , the identified chemical shift interpreted the structure of quercetin according to their number of Hydrogen molecule.

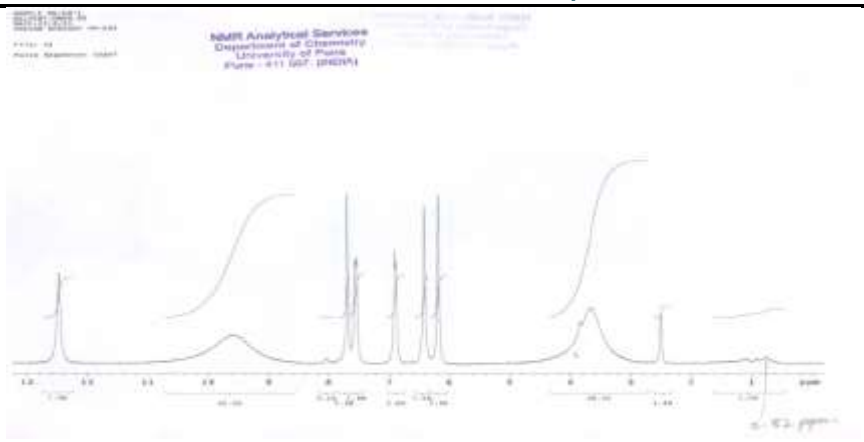


Figure No 07: NMR of Reference Quercetin ranges from 01 to 13

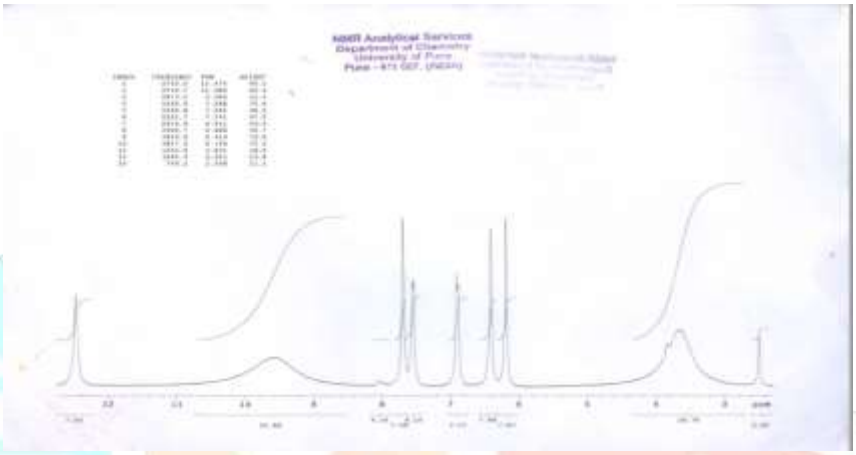


Figure No 08 : NMR of Isolated Quercetin from *Psidium guava l.* ranges from 01 to 13

Table No 06: Characterization Proton N.M.R.

Sr. no	ppm	Chemical Shift	Group
1	12.475	Aromatic	H-4
2	12.480	Aromatic	H-3
3	9.580	Aromatic	H-4
4	~7.541-7.698	Aromatic	H-2, H-6, H-5
5	~6.190-6.911	Aromatic	H-6,H-5
6	~2.500-3.841	Aromatic	H-2

As we conclude that the figures no 07,08, 09and 10 carbon and proton NMR shows in table no 07 interpretation of the isolated compound is confirmed as Quercetin due to structural relation between reference and isolated have similar value we got after NMR spectroscopy. In this three spectrum observe which are proton signals, aromatic protons and hydroxy protons of the analyte and a solvent proton signal.

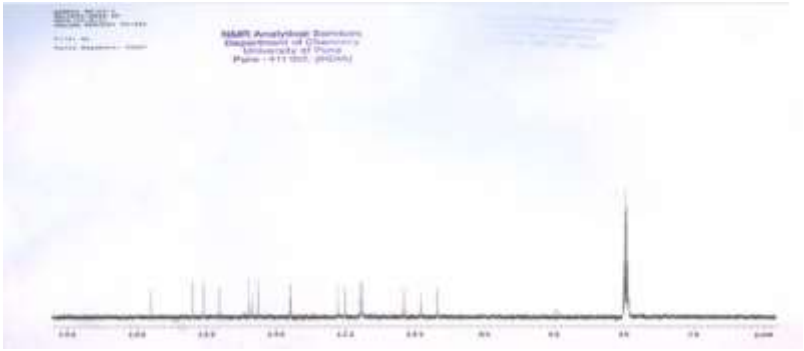


Figure No 09: Carbon NMR – range from 20 to 200 ppm

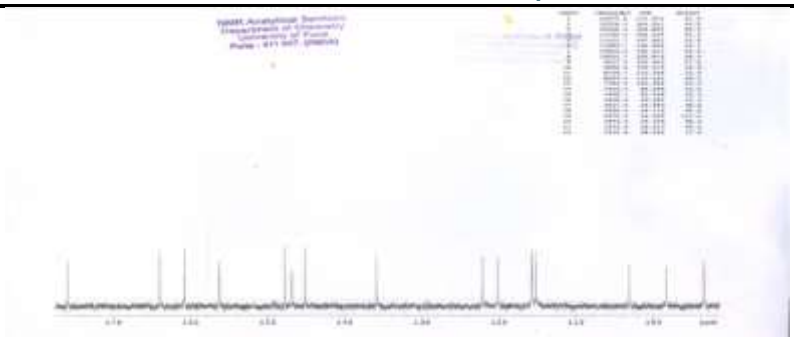


Figure No 10 : Carbon NMR – range from 100 to 200 ppm.

Table No 07: Characterization of Quercetin by Carbon N.M.R.

Sr. No.	C-ppm	Chemical shift	Sr. No	C-ppm	Chemical shift
1	175.953	C-4	7	115.232	C-2
2	~164.032-160.825	C-5	8	103.156	C-4
3	156.294	C-8	9	~93.528-98.349	C-6
4	~145.171-147.82	C-4	10	40.264	C-49
5	135.873	C-3	11	~39.159-39.944	C-9
6	~120.170-122.163	C-6	12	~38.602-38.873	C-8

3.8 HPLC: The HPLC analysis of Quercetin perform the column condition 4×125 mm Hypersil ODS with mobile phase 0.5% Orthophosphoric acid in water and Methanol , flow rate 1ml/min column wash 20 min 100% and 18 min 100% . The column temperature is 25 ^{0} C. The sample injected volume 10µl.

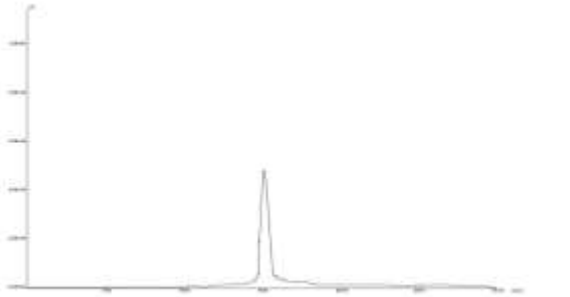


Figure No 11: HPLC of Standard Compound.

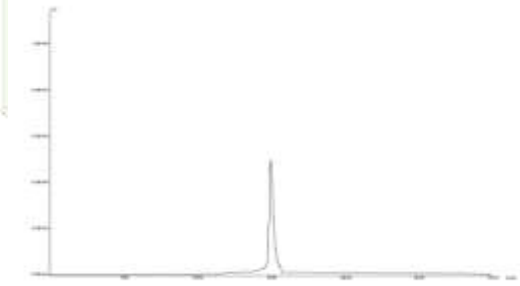


Figure No. 12: HPLC of isolated Quercetin from *Psidium guava l.*

Both peaks observe in figure no 11 and 12 shows similarly in HPLC analysis, so we conclude, as isolated compound is Quercetin.

3.9. HPTLC:

Tabel No. 8: Development System for HPTLC

1.	**Stationary phase**	Silica gel G 60 F254
2.	**Mobile phase**	Toluene: Ethyl Acetate: Formic Acid (5:5:0.3 % v/v)
3.	**Development**	Development chamber pre-saturated (20 min) with the mobile phase
4.	**Source of radiation**	deuterium lamp 200-400 nm
5.	**Detection**	A. at 254 nm B. Visible Light

A B

Figure No.13:Visualization of Developed Plate A. at 254 nm B. Visible Light.

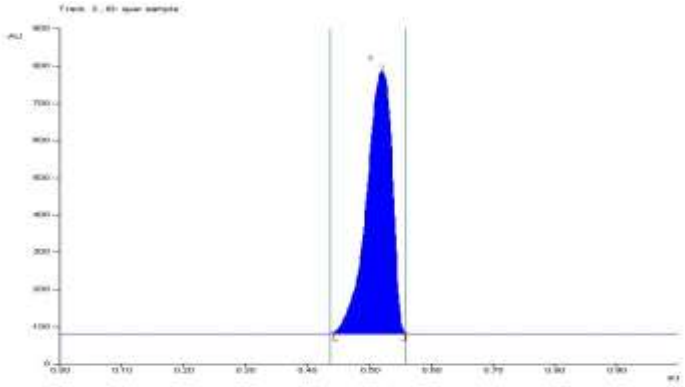


Figure No.14: HPTLC of Quercetin.

The in the figure no 13 and 14 shows the following observation ,

- Quercetin - 400 nm
- Rf - 0.52

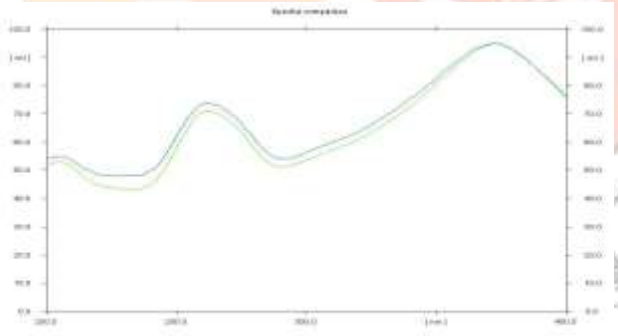


Figure No. 34: Spectra Comparison HPTLC of Quercetin.

- **Blue** –Spectra of RS Quercetin
- **Green** – Spectra of isolated Quercetin from extract

Both the compound run parallel, the blue spectra show the graph of reference substance and green spectra shows the isolated compound of quercetin, which is identified above HPTLC method.

Conclusion:

The present study demonstrated that the Methanolic extract of the aerial part of *Psidium Guajava* Linn and to isolate the flavoinod Quercetin. The Psidium guava has been found most important rich chemical constituent of flavonoid called as Quercetin. The chemical test TLC confirm the flavonoid and the TLC clear identified the bands of flavonoid constituent in extract as well as confirm the quercetin from isolated TLC , the Analytical specification helpful for confirmation of number of Hydrogen , carbon and other oxygen molecule present with the help of IR and NMR spectroscopy. The HPLC and HPTLC method helpful to identified the pure Quercetin molecule from plant *Psidium guavaja l.* so we conclude that according to literature survey *Psidium guava linn* has been found rich source of flavonoid espicialy quercetin. And the presence study confirmed the quercetin.

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