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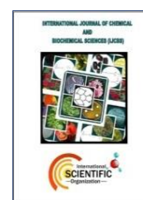

Preliminary Qualitative, Quantitative Screening of different extracts of *Adansonia digitata* L. and Isolation, Characterization of Kaempferol 3-O Rutinoside from *Adansonia digitata* L.

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Abstract

Adansonia digitata L belongs to family *Malvaceae* known as Baobab is found in Southern Africa, Botswana, Namibia, Mozambique, and other tropical India where it is suitable. The baobab fruit known for its nutritional and polyphenolic content. *Adansonia digitata* L use of this plant in alternative system of medicine. That indicates significance of research. On this plant Limited data is available on Phytochemistry. So, this study focuses detailed report of phyto study of plant. In this investigation Preliminary Qualitative, Quantitative screening of Leaf and Fruit extracts of *Adansonia digitata* L for of solvents like Aqueous, Ethanol, Ethyl Acetate, Chloroform and Pet ether was carried. In this study, based on Preliminary screening the flavonoids were extracted from Ethanolic Fruit extract of *Adansonia digitata* L. LC/MS analysis was carried out for fruit extract only as it contains maximum amount of Total flavonoid Content and Total Phenolic Content.Ethanolic Fruit extract of *Adansonia digitata* L shows better results as compared to other Extracts especially for Total flavonoid Content and Total Phenolic Content. The presence of kaempferol 3-O-rutinoside was confirmed from LC/MS/MS data for crude fruit extracts and compared with Standard Sample. The isolated kaempferol 3-O-rutinoside structure was identified and confirmed by using UV, LC-MS, IR , ^{1}H NMR, ^{13}C NMR, HPTLC, HPLC spectroscopy.The study summarizes the phytochemistry of plant extract, it also confirmed that the Ethanolic fruit Extract holds maximum secondary metabolites that might be beneficial for cardio protective, Hepatoprotective Action.

Keywords:*Adansonia digitata* L, Kaempferol 3-O-Rutinoside, phytochemistry.

Full length article **Corresponding Author*, e-mail: patilshirish23@gmail.com

1. Introduction

Due to their unparalleled chemical variety, natural products like plant extracts—whether in the form of pure chemicals or standardized extracts—offer countless prospects for the development of novel drugs. Over 80% of people worldwide get their primary healthcare from traditional medicine, according to the World Health Organization (WHO). Asia's use of herbal remedies is a reflection of centuries of environmental interactions between people and their surroundings. Numerous compounds found in plants used in traditional medicine can be used to treat both viral and chronic illnesses. Men turned to ethnopharmacognosy as a result of side effects and microbial resistance to chemically synthesized medications developing. Thousands of phytochemicals derived from plants were discovered to be safe and generally efficient substitutes [1].Baobab extracts have been used traditionally for various medicinal purposes and also reported as anti-oxidant, anti-diabetic & cardiotonic activity by previous researchers. These activities may be due to higher content of vitamin-c, rich in polyphenols & calcium contents also.In previous research polyphenols& vitamin-c were isolated from other plants sources successfully using different methods of isolation and evaluated for in-vitro studies and molecular dockings. It concludes molecular mechanism of actions and binding capacity of bioactive molecules like polypheonols & vitamin-c to the receptor successfully for desired actions of Cardioprotective, Anti-diabetic and Antioxidant activity. Future studies are needed to determine the individual phyto-constituents present in the extracts to identify which group may be eliciting the effects on desired potential activity.The interest to undertake this investigation is due to the fact that no detailed study regarding molecular mechanism of action and receptor binding capacity of active chemical constituents from *Adansonia digitata L* (Bombacaceae) for Cardioprotective, Anti-diabetic and Anti-oxidant activity. Also it is reported that antioxidant property of drugs may be responsible for prevention of cellular damage (cell

membrane) and cure of many chronic disorders such as cardiovascular, cancer and diabetic etc.Many different types of solvents were evaluated for the Soxhlet extraction process, and then a comprehensive identification process involving both qualitative and quantitative analysis was completed. Determining the chemical marker—a component that is present in the plant product and has a known chemical composition—is a crucial step in the quality control of herbal medicine products. These ingredients might or might not have anything to do with their pharmacological action. Although these compounds can be identified and quantified using a variety of analytical techniques, we employed thin layer and column chromatography to isolate and purify Kaemferol 3-O Rutinoside from Adansonia digitata fruit extract. The structure of the further extracted Kaemferol 3-O Rutinoside was determined by means of FTIR, UV, LC-MS, and nuclear magnetic resonance spectroscopy.

2. Materials and Methods

2.1. Chemicals

Chloroform were purchased from Fischer scientific lab,(India),Methanol were purchased from Rankem, (New Delhi), Ethanol, Ethyl acetate, Formic acid, Glacial acetic acid, Toluene,Sodium chloride ,Potassium hydrogen phosphate, Potassium chloride , Dimethyl sulphoxide (DMSO),5-Fluoro uracil were purchased from Research-lab Fine Chem. Industries(Mumbai), Silica (60-120), precoated with silica gel 60 F254 (20 × 20 cm, 0.2 mm thickness)were obtained from Merck Ltd (Mumbai, India). Acetonitrile, Orthophosphoric acid. The remaining chemicals and solvents used were of standard analytical grade and HPLC grade respectively.

2.2. Plant material

Adansonia digitata L plant was collected from rural region of Miraj (MS). Plant was Authenticated from Botanical survey of India, Western Regional center, Pune (MS),No.BS/WRS/100-1/Tech.2020/126 After authentication, the leaves and fruits were crushed into small sizes and kept in a tightly closed containers in dark places until subjected to the extraction process.

2.3. Preparation of extracts

2.3.1. Aqueous extract

Chloroform water I.P. was used to macerate the leaf and fruit powder of Adansonia digitata L.A rotary flash evaporator (Superfit Rotary Vacuuma) was used to concentrate the mixture under reduced pressure after it had been filtered through muslin cloth. The extract was then left on a water bath to retain its crude extract, and the residue was eventually dried in a vacuum desiccator over anhydrous calcium chloride to produce an aqueous extract.It was determined what the aqueous extract's percentage yield was[1].

2.3.2. Alcoholic extract

Adansonia digitata L leaf and Fruit powder was extracted with Ethanol, Pet ether, Ethylacetate, Chloroform(50°C-70°C temperature varies solvent according to Boiling Point)in a continuous hot extraction method using Soxhlet apparatus. The extracts were concentrated in a rotary flash evaporator (Superfit Rotary Vacuuma) and residue was calculated[1].

2.4. Phytochemical Screening

2.4.1. Qualitative test

Test qualitative A phytochemical investigation was performed to find out whether primary and secondary metabolites were present.Utilizing the established protocols established by the Association of Official Analytical Chemistry, the biomolecule included in the plant extract was identified. In this investigation, tests for phytochemicals such as alkaloids, glycosides, tannins, saponins, steroids, flavonoids, and carbohydrates were conducted[2].

2.4.2. Determination of total phenolic content

Calculating the extract's total phenol content (quantitative test) Important plant components with redox characteristics that provide antioxidant activity are phenolic compounds. Aromatic secondary metabolites found in many different parts of the plant kingdom are called phenolic acids.[3] The term "phenolic acids" in general, designates phenols that having one carboxylic acid functionality, However, when talking about plant metabolites, it refers to a distinct group of organic acids. These naturally occurring phenolic acids contain two distinctive carbon frameworks: the hydroxyl cinnamic and hydroxyl benzoic structures. Although the basic skeleton remains the same, the numbers and positions of the hydroxyl groups on the aromatic ring make the difference and establish the variety[4]. Folin-Ciocalteu's method (FC) is a colorimetric method based on the transfer of electrons between reagents and polyphenols. Different solvent extract chloroform, ethanol: water and ethyl acetate used for determination of phenolic content. The reaction mixture was prepared by mixing 1 ml of methanolic solution of all extracts, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved inwater, and 2.5 ml 7.5% NaHCO3. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5ml 10% Folin-Ciocalteu's reagent dissolved in water, and 2.5 ml of 7.5% of NaHCO3. The samples were thereafter incubated in a thermostat at 45ºC for 45ºmin. The same procedure was repeated for the standard solution of gallic acid (Standard) in methanol (10to100µg/ml) and blank then the calibration line was constructed, and absorbance measured λ max at 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained.

2.5. Isolation and characterization of marker compounds

2.5.1. Thin layer chromatography

Development of TLC profile for separation of phyto-constituents present in ethanol enriched extract. The TLC was performed on pre-coated silica plates (20 × 20 cm, 0.2 mmthickness) silica gel 60 F254. The mobile phase was selected Ethyl acetate: Methanol: Glacial acetic acid: Formic acid (25:5:1:1, v/v/v/v) as mobile phase. The sample application was performed with the help of capillary. The linear ascending development was carried out in a solvent system (10mL) of Ethyl acetate: methanol: Glacial acetic acid formic acid (25:5:1:1,v/v/v/v) in a twin trough glass chamber (20 ×10 cm) previously saturated with solvent for 25min at room temperature (25–27°C) and relative humidity (40–45%). After development, TLC plates were dried with the help of hair dryer for 2 minutes. Visualization was done under UV at 254 nm.TheTLC as shown in figure1.HPTLC of the isolated compound A Camag HPTLC with Linomat V automatic sample applicator and TLC Scanner III along with

software Win CATS version 1.4.0 were used for analysis. The most suitable solvent system was found to be Ethyl acetate: methanol: Glacial acetic acid: formic acid (25:5:1:1,v/v/v/v) for quantitative analysis.[6,7]

2.5.2. Column Chromatography

The vertical glass tube is utilized for column chromatography. The silica gel slurry was made from chloroform and transferred to column with vigorous shaking of the column in such a way that the silica gel in the column settles. Fifteen grams ethanol extract adsorbed to silica gel 100-200 mesh size on rotatory evaporator at temperature 400 ^{0}C.The ethanol extract was then to form solid mass. Such solid masses then load on column-settled silica. The first the mixture fraction from the above column load on next column utilizing the silica gel 230-400 mesh size. By using gradient system with chloroform & methanol different ratio of solvent combination.[8,9] For the first 100% chloroform & 5-7% v / v methanol in chloroform, the column was thenrun off, collected all fractions of 50 ml each respectively at a 6-8 ml / min rate of flow. TLC was performed using mobile phase mentioned in table no 5.5.1 on pre-coated silica plate. The TLC fractionno.1,2,3,4,5,6,7,8,9,10,11,12,13,14,15were pooled together & concentrated after observation.

2.5.3. UV-Visible Spectroscopy

Isolated fraction AA1from column chromatography was scanned by Shimadzu UV/Vis spectrophotometer in the range of 200 to 400 nm. To obtain homogenous components, another preparative TLC was carried out with this fraction labeled AA1. [10] The band was obtained when viewed with UV light which were scrapped out, dissolve in methanol and filtered and λmax was found to be 270nm.

2.5.4. FTIR spectroscopy

FTIR has proven to be a valuable tool for the characterization and identification of functional groups present in AA1 isolated fraction from given extract. In Attenuated Total Reflectance (ATIR) model, IR (α-ATR Bruker Germany spectrometer) was used to collect the sample IR spectra. The spectra were collected in 24 scans within the range of 4000–600cm−1,with a resolution of 4cm−1for each sample using opus software.[11,12]

NMR Spectroscopy of the isolated compound only fraction AA1 was additionally elucidated by 1HNMR by using solvent D6 + CDCL3 MIX. The analysis was done at the BRUKER instrument of 400MHz.

2.5.5. LC-MS Spectrometry

About 10mg of sample was dissolved in 1ml of DMSO and diluted to 10mL with methanol. Filtered and injected volume of 10uL.[13] Mobile Phase-A: 0.1% Formic acid in water Mobile Phase-B: Acetonitrile Diluent: Methanol and Dimethyl sulphoxide with methanol was used. Column: Accu core C18, 50 x 4.6, 5µ Particle size from Thermo Scientific and Processing Software:Mass Lynx V4 was used.LCMake:Waters,USAModel:1525µ Binary Pump. MS Make: Waters, USA Model: Xevo G2-XS QT Capillary Voltage: 3.0KV Collision Energy:20V,Ramp Collision Energy: 30-90V Source Temp: 150˚C Desolvation Temp: 450 ºC,Conegas:50L/Hr Desolvation Gas Flow:800L/Hr were used.HPLC of isolated compound Using a Systronics PU-2080 Plus intelligent detector and a C18 column (250 x 4.6 mm, 5 µm) for isolation, the gallic acid content of the extract was estimated using flash chromatographic fraction no. FA004 at 270 nm. The tests reported in the literature were used to determine the choice of mobile phase. Gallic acid was shown to have affinity for the stationary phase because the acidified water prevented it from ionising.5. The elution process used ACN, or 0.2% acetic acid (60:40), as the mobile phase. The injection loop and mobile phase flow rates were adjusted to 20µL and 1.0 ml/min, respectively. For the purpose of quantitatively determining the composition of the specified fraction, FA004 was diluted with mobile phase at various concentrations between 0.01 and 0.5 mg/ml.

3. Result

3.1. Preliminary Phytochemical screening

Characteristics, Physical properties and Percentage Yield of Different extracts of *Adansonia digitata* L.was observed as shown in table below (Table 1).Preliminary Qualitative screening of Multiple extracts of *Adansonia digitata* L was carried by using Different Reagents. Ethanolic Fruit extract of Adansonia digitata L (EFAD) shows better results as compared to other Extracts.

3.2. Quantitative phytochemical screening of Adansonia digitata L leaf and fruit extracts

3.2.1. Estimation of Total Phenolic Content

The total phenolic content was determined using Folin-Ciocalteau method. Phenolic content was calculated from the regression equation of the standard plot($y=0.004x+0.0039,R^2=0.9967$)and is expressed as Gallic acid equivalents. The total Phenolic content present in *EFAD*is 42.93mgGAE/g.

3.2.2. Estimation of total flavonoid content

The total flavonoid content was determined using aluminium chloride method. Flavonoid content was calculated from the regression equation of the standard plot ($y=0.0074x+0.0205,R^2=0.9951$)and is expressed as quercetin equivalents.[14,15] The total flavonoid content present in *EFAD* are29.56mgQUE/g. In this investigation Preliminary Quantitative screening of Multiple extracts of *Adansonia digitata* was carried for Phenolic and flavonoid content. Ethanolic Fruit extract of *Adansonia digitata* L(EFAD) shows better results as compared to other Extracts especially for Total flavanoid Content(TFC) and Total Phenolic Content(TPC).The *Ethanolic Fruit extract of Adansonia digitata* L*(EFAD)* fruit part of this plant was selected for isolation of active compounds namely isolated kaemferol 3-o rutinoside from extract as rich content for flavnoid and phenolic content. Characterization of Isolated kaemferol 3-o rutinoside and standard sample of kaemferol 3-o rutinoside done by using HPLC, NMR, TLC, IR method for estimation and quantification of kaemferol 3-o rutinoside and compared with standard.

Table1: Characteristics, physical properties and percentage yield of different extracts of *Adansonia digitata* L is observed

Sr. No.	Solvents	% Extraction yield	Color	Consistency
1	ALAD	09.12	Green	Semi solid
2	AFAD	10.35	Brownish Green	Semi solid
3	EALAD	08.50	Green	Semi solid
4	EAFAD	05.17	Brownish Green	Solid
5	CLAD	07.93	Brownish Green	Solid
6	CFAD	13.21	Brown	Solid
7	PELAD	08.14	Green	Solid
8	PEFAD	11.78	Brownish Green	Solid
9	ELAD	13.30	Green	Solid
10	EFAD	12.70	Brownish Black	Solid

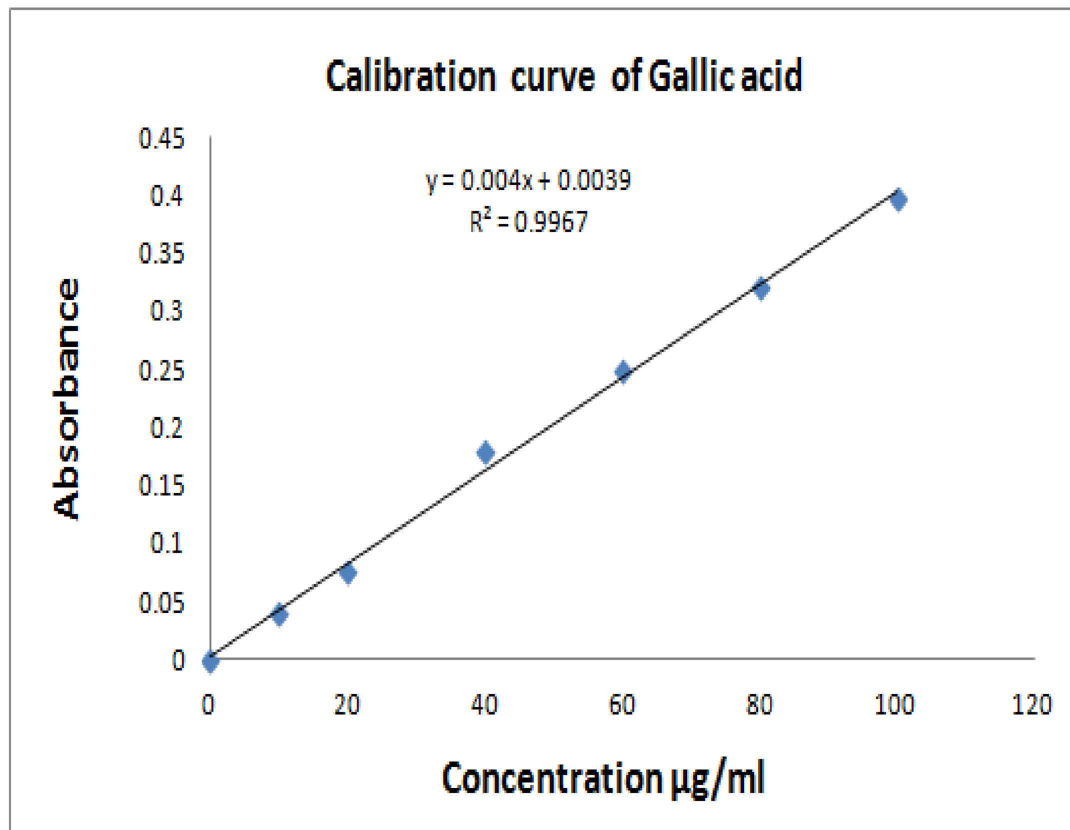

Figure 1: Standard curve for quantification of total phenolic content

Table 2: Qualitative Phytochemical Screening of *Adansonia digitata* L Leaf and Fruit Extracts

Phytochemical Constituents detected in *Adansonia digitata* L Leaf and Fruit Extracts											
Sr.No.	**Test**	**AFAD**	**ALAD**	**EFAD**	**ELAD**	**CFAD**	**CLAD**	**EAFAD**	**EALAD**	**PEFAD**	**PELAD**
		Observation& Inference									
1.	Tests for Alkaloids:										
	a. Mayer's	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	b. Dragendroff's	(-)	(+)	(+)	(+)	(-)	(+)	(+)	(+)	(-)	(+)
	c. Wagner's	(-)	(+)	(+)	(+)	(-)	(+)	(+)	(+)	(-)	(+)
	d. Hager's	(-)	(-)	(+)	(+)	(-)	(+)	(+)	(+)	(-)	(+)
2.	Tests for glycosides:										
	a.Libermann-Burchard's	(-)	(-)	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(+)
	b. Legal's	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(+)
	c. Modified Borntrager's	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(+)
3.	Tests for Flavonoids:										
	a. Ferric chloride	(-)	(+)	(+)	(-)	(-)	(-)	(+)	(+)	(+)	(-)
	b. Shinoda	(-)	(-)	(+)	(+)	(-)	(-)	(+)	(+)	(+)	(-)
	c. Zinc – HCL reduction	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(-)	(-)
4.	Test for saponins:										
	Foam	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
5.	Test for the phenolic compounds and tannins:										
	a. Ferricchloride	(+)	(-)	(+)	(+)	(-)	(+)	(-)	(+)	(+)	(+)
	b. Lead acetate	(+)	(+)	(+)	(-)	(+)	(+)	(+)	(+)	(-)	(+)
6.	Test for Amino Acid										
	a. Ninhydrin	(+)	(-)	(+)	(+)	(+)	(-)	(+)	(-)	(+)	(+)
	b. Tyrosine	(-)	(-)	(+)	(+)	(-)	(+)	(+)	(-)	(+)	(-)
	c. Tryptophane	(+)	(+)	(+)	(-)	(-)	(+)	(+)	(-)	(-)	(-)
	d. cysteine	(+)	(+)	(+)	(+)	(-)	(+)	(+)	(-)	(-)	(+)
7.	Test for steroid										
	Salkowski	(+)	(+)	(+)	(-)	(+)	(+)	(+)	(-)	(+)	(+)
8.	Test for Proteins										
	a. Millions	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(+)	(+)	(+)
	b. Biuret	(-)	(-)	(-)	(+)	(+)	(-)	(+)	(+)	(-)	(+)

Table 3: Phytochemical Constituents detected in Adansonia digitata L Leaf and Fruit Extracts (Glycosides, Tannins & Phenols

Sr.No.	Test	AFAD	ALAD	EFAD	ELAD	CFAD	CLAD	EAFAD	EALAD	PEFAD	PELAD
		Observation & Inference									
A)	Test for Cardiac Glycosides										
1	Legal	(-)	(+)	(+)	(-)	(-)	(-)	(+)	(-)	(+)	(-)
2	Keller-Killiani	(+)	(-)	(+)	(+)	(-)	(+)	(-)	(+)	(+)	(+)
3	Raymond's	(+)	(+)	(+)	(-)	(-)	(+)	(-)	(+)	(-)	(-)
4	Baljet	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(+)
B)	Test For Anthraquinone Glycosides										
1	Brontrager's	(+)	(-)	(+)	(-)	(-)	(+)	(+)	(+)	(+)	(-)
C)	Test for Saponin Glycosides										
1	Foam Test	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(-)	(-)
D)	Tests For Tannins and Phenolic Compounds										
1	5%Fecl3solution	(-)	(+)	(+)	(+)	(-)	(-)	(+)	(-)	(-)	(-)
2	Lead acetate sol.	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(-)	(+)	(-)
3	Bromine water	(-)	(+)	(+)	(-)	(-)	(-)	(+)	(+)	(-)	(+)
4	Acetic acid sol.	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(-)	(-)	(-)
5	Dil.Iodine sol.	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(-)	(+)
6	Dil.HNO_3 sol.	(+)	(-)	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(-)
7	Dil.$KMnO_4$ sol.	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(+)

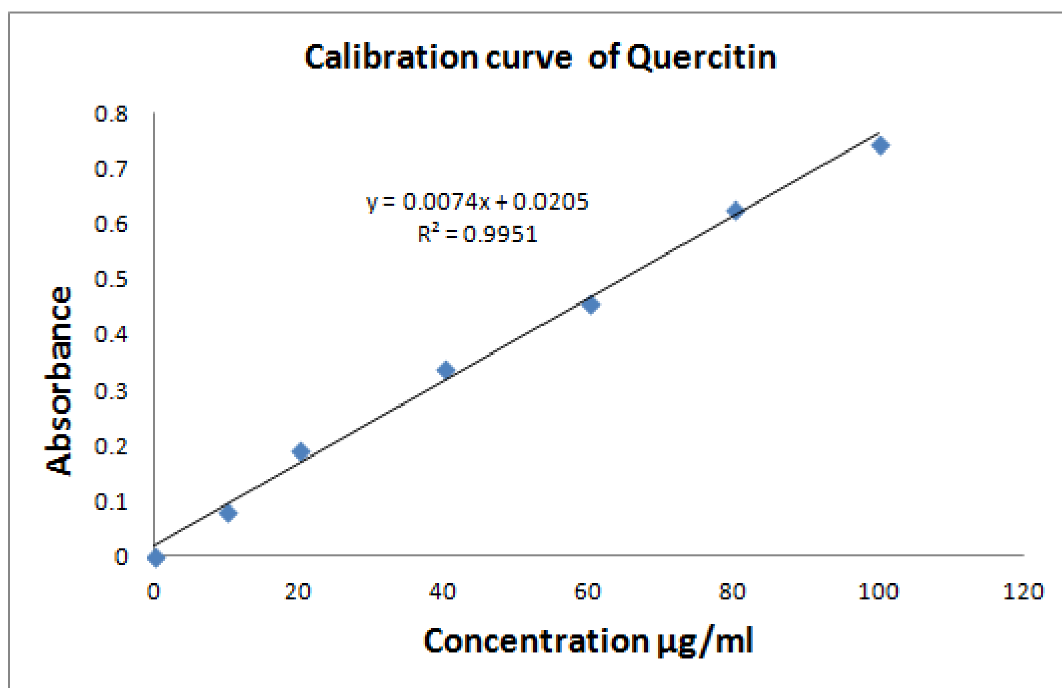

Figure 2: Standard curve for quantification of total flavonoid content

Table 4: Total Flavonoid Content and Alkaloid content *Adansonia digitata* Leaf and Fruit Extracts

Sr. No.	Extract Name	Total Flavonoid content	Total Alkaloid content
1.	ALAD	08.02 mg QUE/g	09.00 mg GAE/g
2.	AFAD	09.16 mg QUE/g	11.93mg GAE/g
3.	PEFAD	15.01 mg QUE/g	18.38mg GAE/g
4.	EALAD	17.06 mg QUE/g	21.32 mg GAE/g
5.	CFAD	19.47 mg QUE/g	24.72mg GAE/g
6.	CLAD	19.56 mg QUE/g	31.02mg GAE/g
7.	PELAD	20.56 mg QUE/g	31.42mg GAE/g
8.	ELAD	21.92 mg QUE/g	30.13mg GAE/g
9.	EAFAD	23.29 mg QUE/g	29.78mg GAE/g
10.	**EFAD**	**29.56 mg QUE/g**	**42.93 mg GAE/g**

Table 5: Observation Table

Constituent Name	Mobile Phase	Ratio	Standard	Standard RfValue	Rf Value	Isolation Status
Isolated sample from Extract	Ethyl acetate: Methanol: Glacial acetic acid: Formic acid	25:5:1:1 v/v/v/v	Kaempferol 3-Rutinoside	**1.085**	**1.041**	**Present**

Table 6: HPTLC of Ehanolic extract at 254nm

Compound	Rt(min)	Area%	Quantity(mg)
Isolated Sample Kaemferol 3-O Rutinoside	8.422	96.42	100

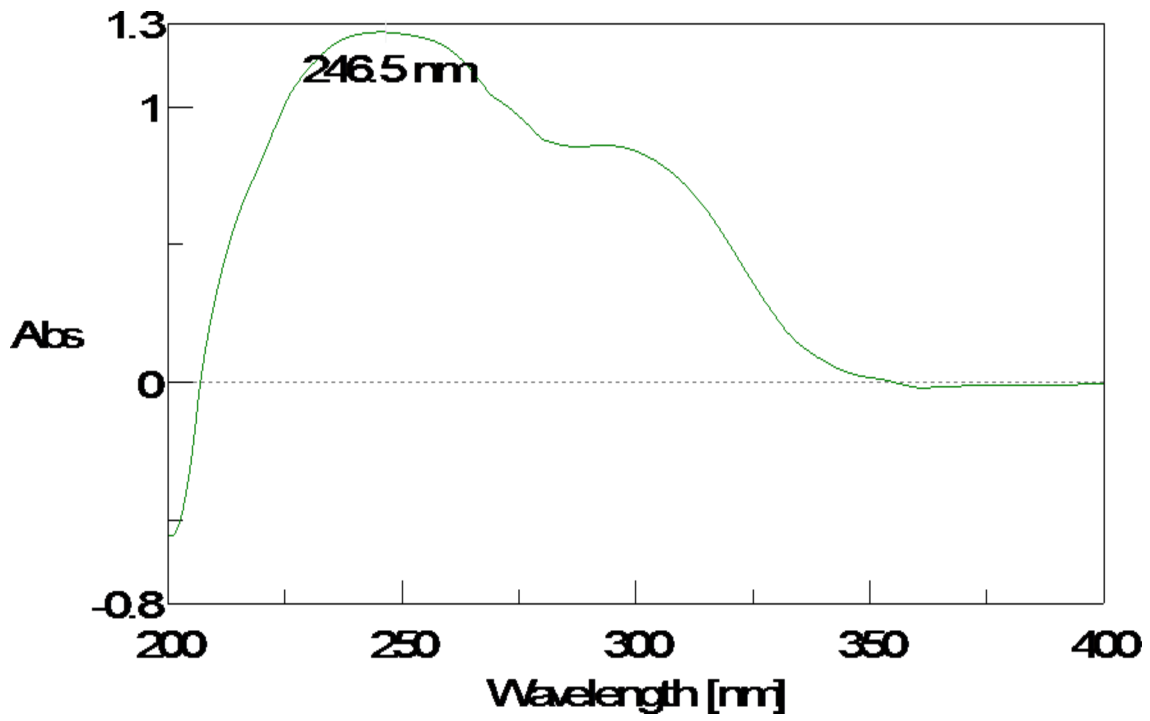

Figure 3: UV spectra for isolated Kaemferol 3-O Rutinoside

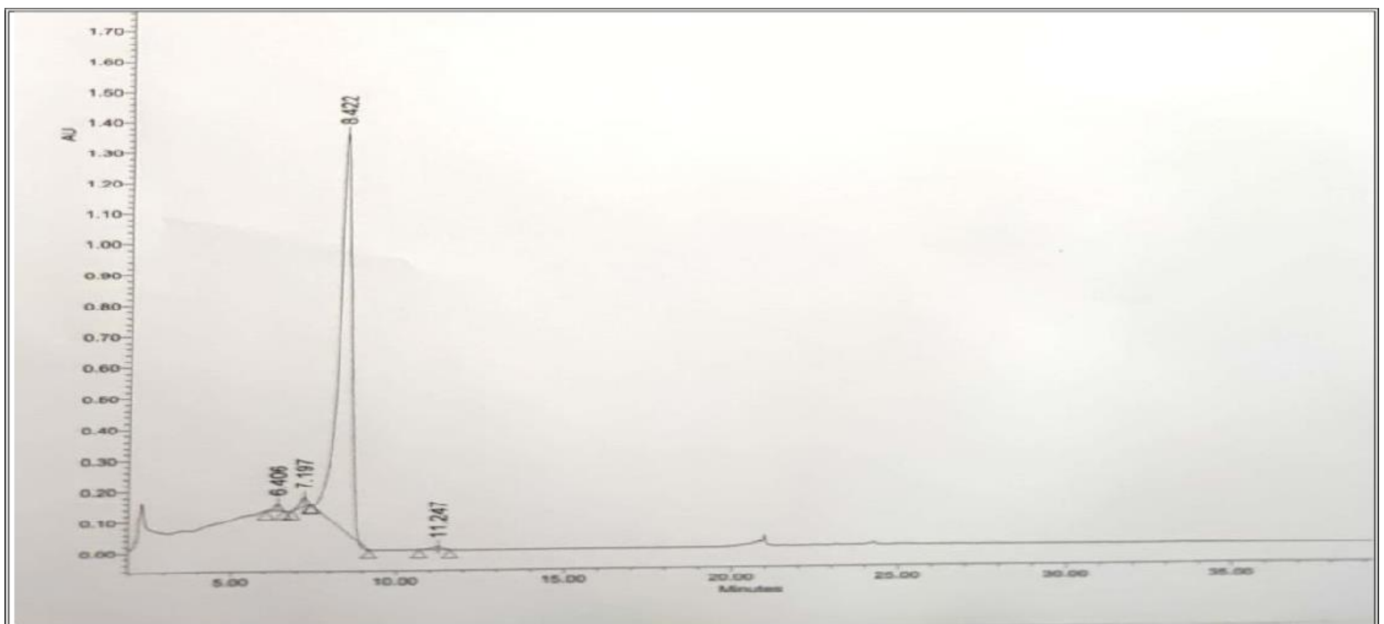

Figure 4: HPLC Chromatogram of isolated kaemferol 3-O rutinoside sample

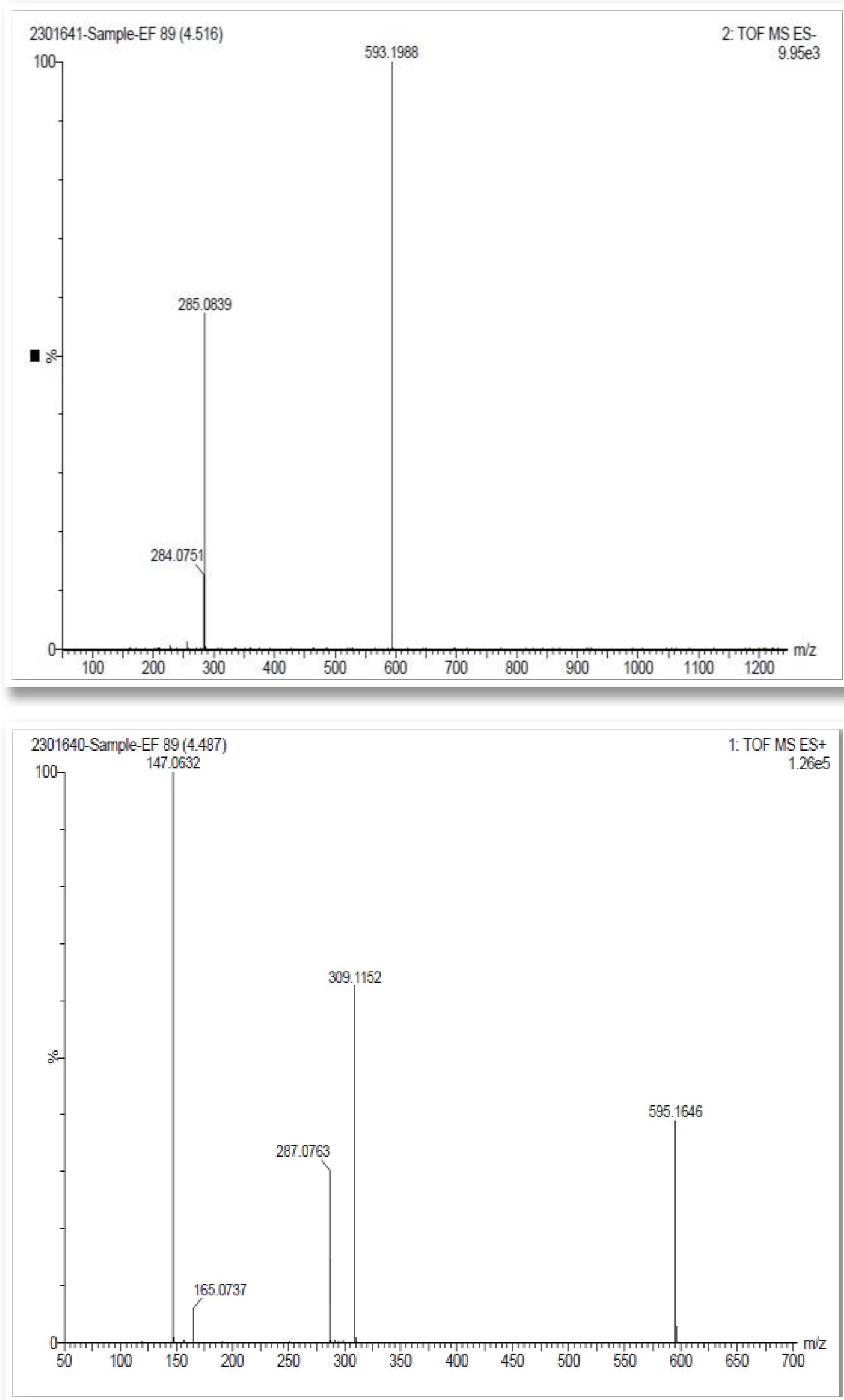

Figure 5: Mass data of isolated sample of kaemferol 3-O Rutinoside

Table 7: Mass Spectroscopy Result of isolated Sample Kaemferol 3-O Rutinoside

Sr. No	Name	RT	Area (µV*sec)	Height(uv)	Base Peak(m/z)
Negative Mode (Kaemferol3-O Rutinoside-Isolated Sample from Extract)					
1	Sample	4.491	16185899	828540	593.1988
Positive Mode (Kaemferol3-O Rutinoside-Isolated Sample from Extract)					
2	Sample	4.487	1600921	98310	595.1646

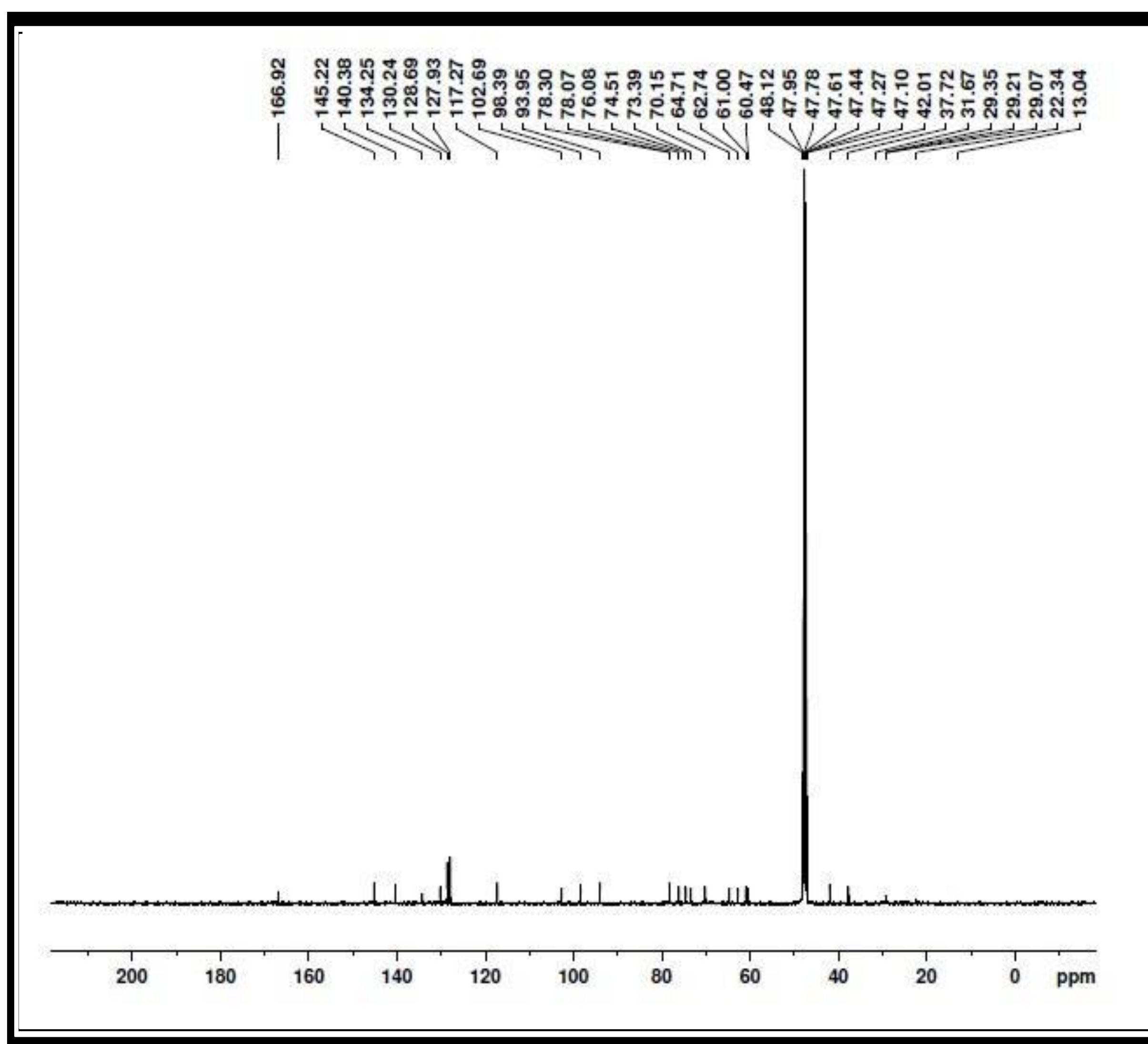

Figure 6.^{13}CNMR of isolated Sample Kaemferol 3-O Rutinoside

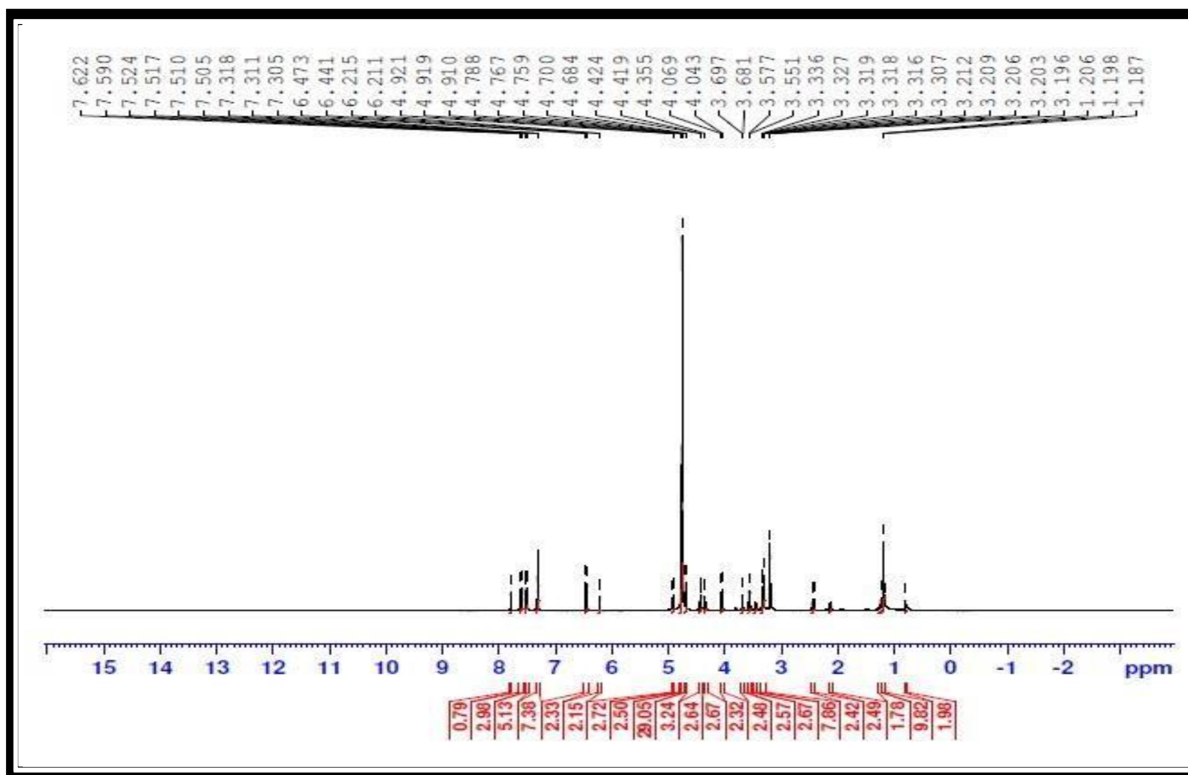

Figure 7:^{1}H NMR Data of isolated Sample Kaemferol 3-O Rutinoside

Table 8: ^{13}CNMRdata(500MHzDMSO-D6) δppm

Position no	Interpreted value 13CNMR
1	93.95
3	140.38
4	102.69
5	37.72
6	78.30
7	62.74
8	64.71
9	42.01
10	62.74
1’	98.39
2’	74.51
3’	76.08
4’	70.15
5’	76.08
6’	64.71
1’’	134.25
2”	130.24
3”	128.69
4”	134.25
5”	128.69
6”	130.24
CO	166.92
α	145.22
β	117.27

Table 9: Interpretation of ^{13}C NMR Data of isolated Sample Kaemferol 3-O Rutinoside

Sr.No	NMR Peakc-13	Interpretation
1	130and134ppm	Aromatic carbon
2	166ppm	Carboxylic Carbon.
3	140to145ppm	C=C Unsaturated Carbon.
4	102.15ppm	C-O of aromatic ring
5	78.30ppm	-CH of aromatic

Table 10: Interpretation of ^{1}H NMR Data of isolated Sample Kaemferol 3-O Rutinoside

Sr.No	NMRPeak	Probable assignments
1	4.8ppm	Alkene Proton
2	6.2ppm	Hydroxyl Proton
3	6.4to6.5ppm	Hydroxyl Proton
4	7.3ppm	Aromatic Proton
5	7.5ppm	Aromatic Proton
6	7.6and7.8ppm	Aromatic Proton

Table 11: IR Interpretation of Isolated Sample of Kaemferol 3-O Rutinoside

Srno.	Wave no.(cm^{-1})	Functional group	Compound class
1	3365.17	O-H Stretching	Alcohol
2	2924.52	C-H Str	Alkane
3	1637.27	C=CStr	Alkene
4	1523.49	C-H Bending	Aromatic Comp
5	1452.14	C-H Bending	Alkane
6	1332.57	O-H Bending	Alcohol
6	1257	C-O str	Ester
7	1177	C-O Str	Alcohol

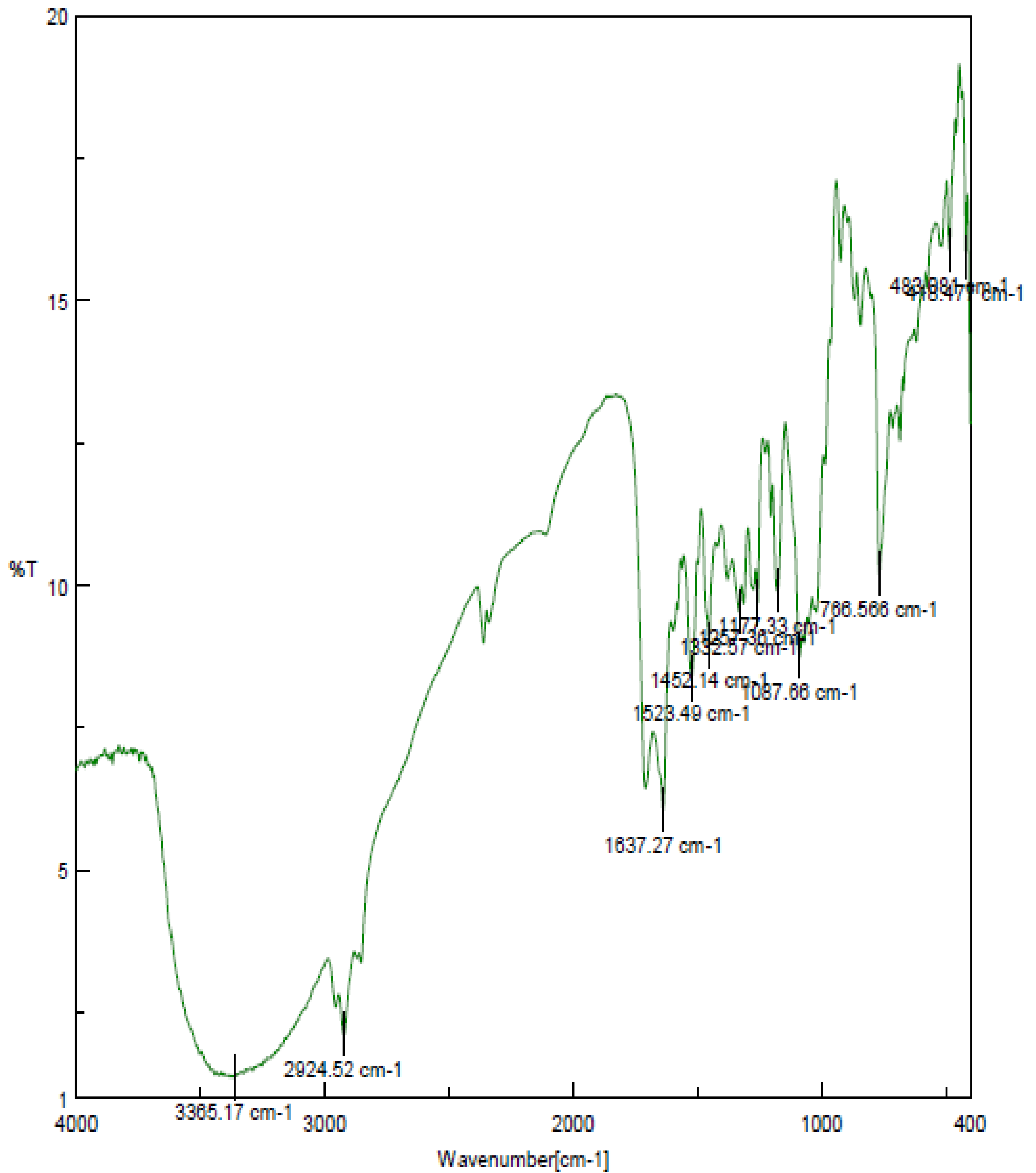

Figure 8: IR Spectra of Isolated Sample of Kaemferol 3-O Rutinoside

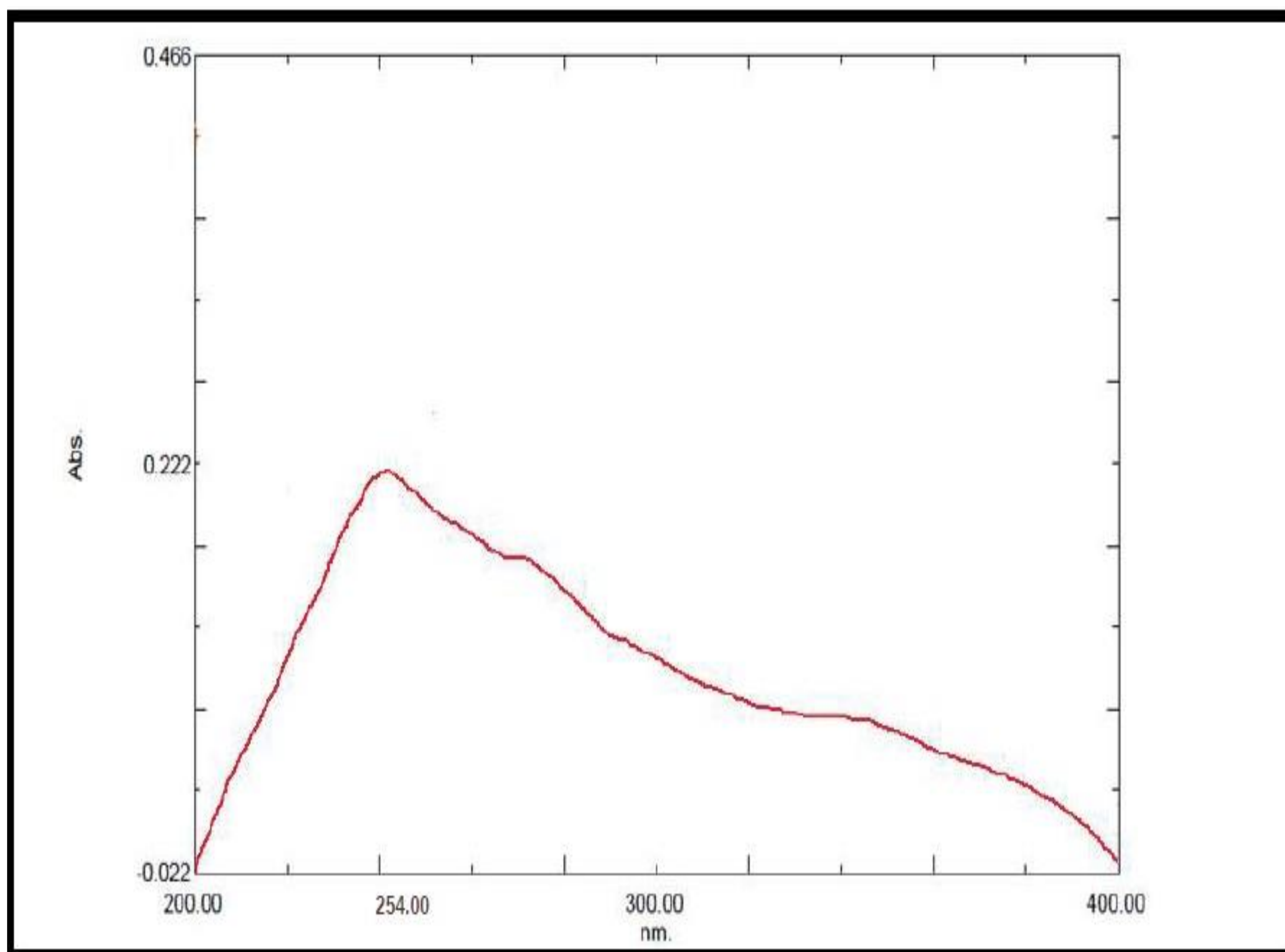

Figure 9: UV spectra for Standard Sample Kaemferol 3-O Rutinoside

Table 12: Mass spectroscopy of Standard Sample of Kaemferol 3-o Rutinoside

Sr.no	Name	RT	Area(µV*sec)	Height(uv)	BasePeak(m/z)
Negative Mode (Kaemferol3-O Rutinoside-Standard)					
1	StdSample	4.491	1350720	88212	593.1988
Positive Mode (Kaemferol3-O Rutinoside-Standard)					
2	Std Sample	4.481	1420921	700310	593.2886

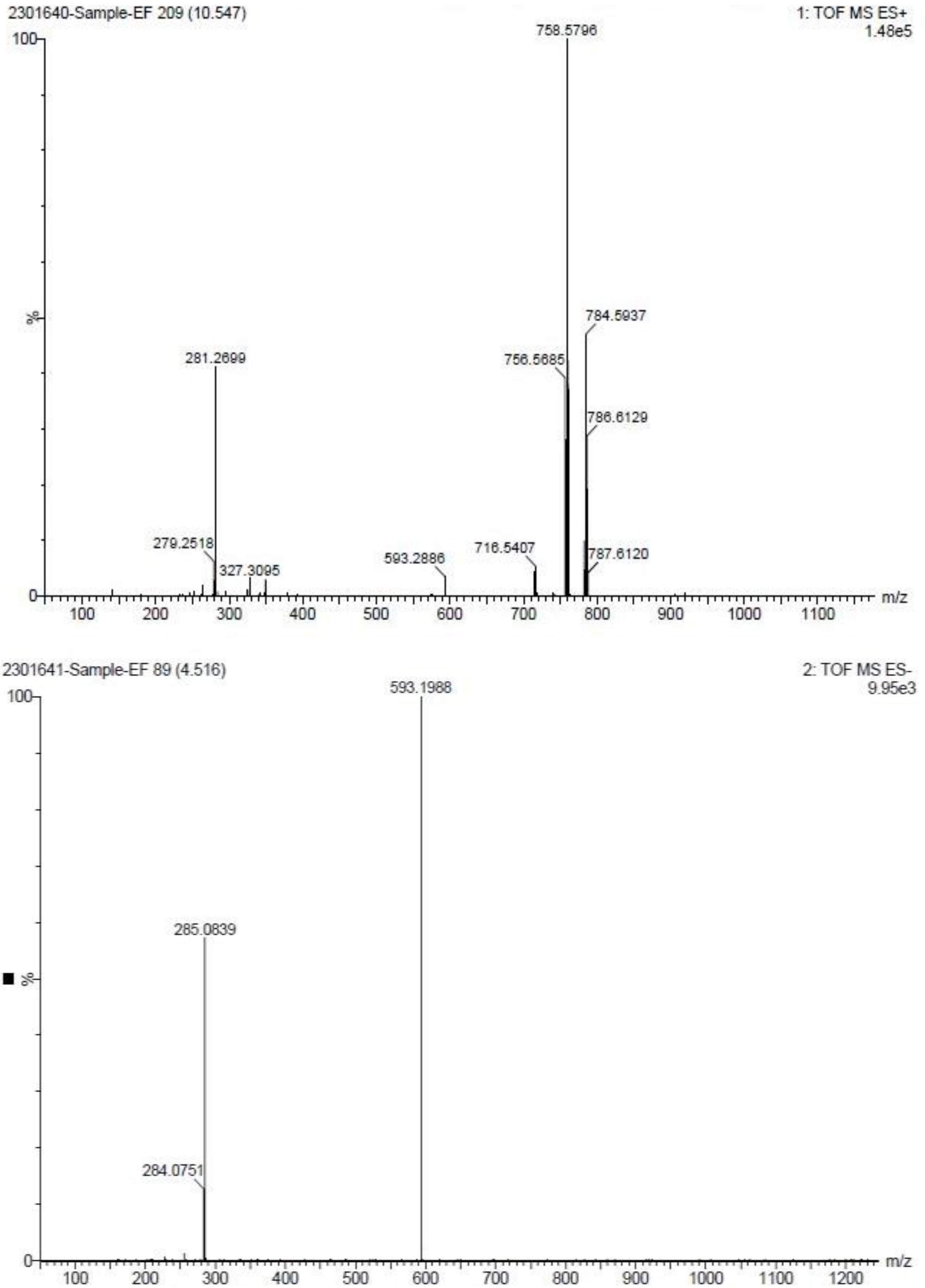

Figure 10: Mass Spectroscopy of Standard Sample of Kaemferol 3-O Rutinoside

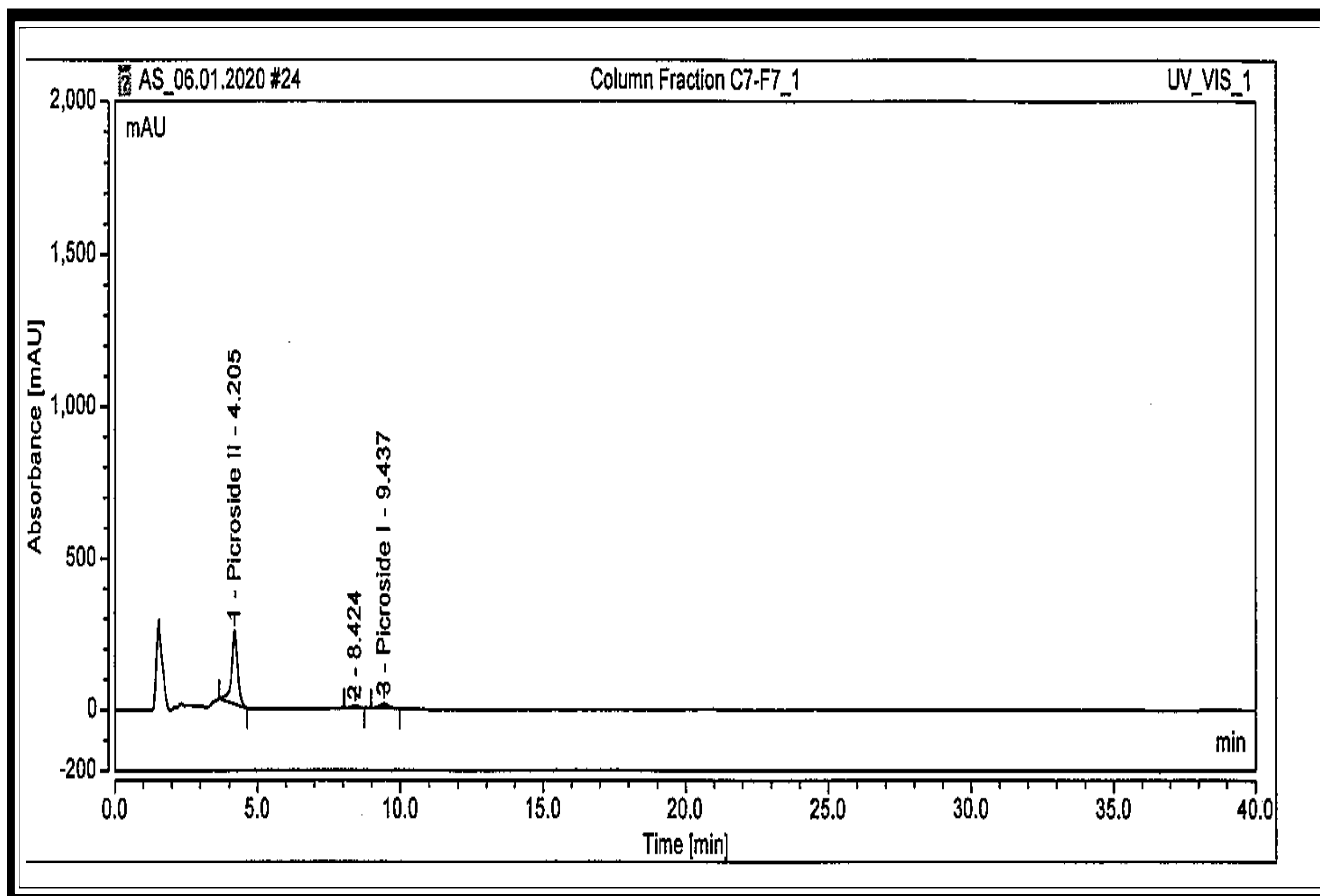

Figure 11: HPLC Chromatogram of Standard sample Kaemferol 3-O Rutinoside

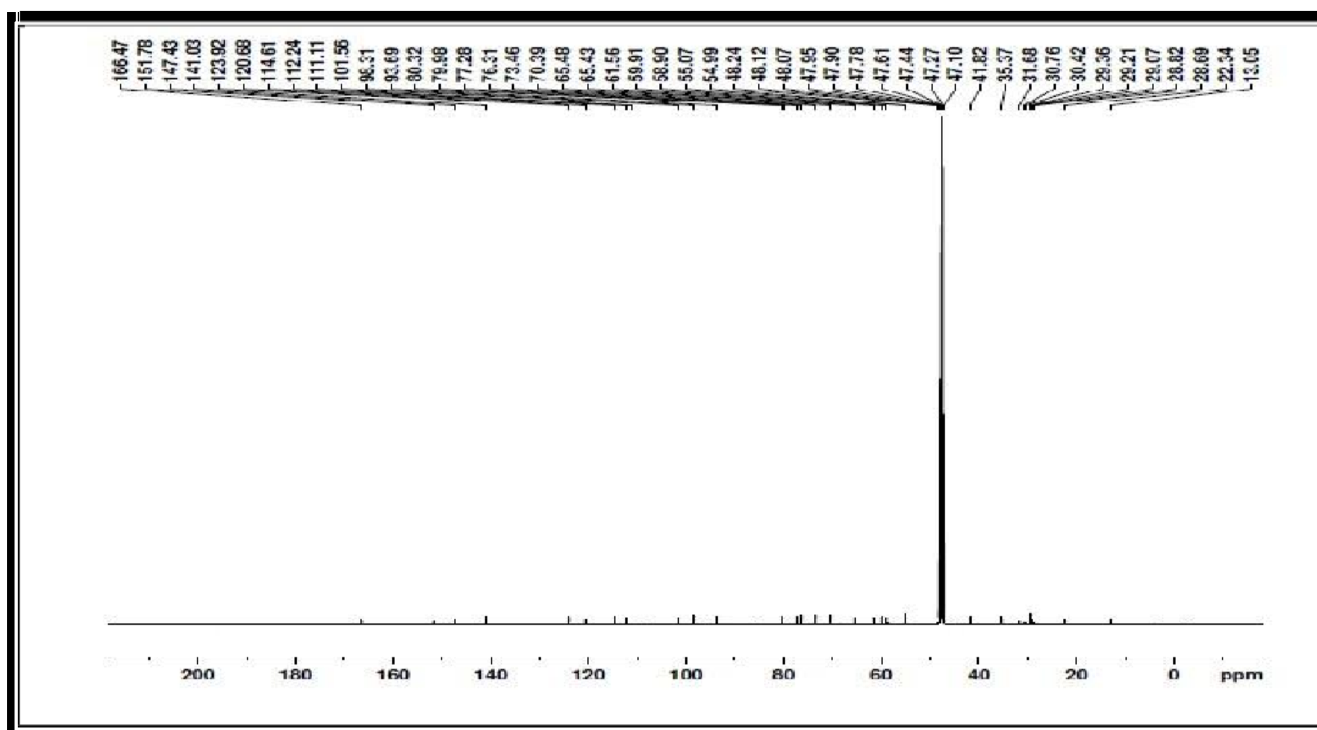

Figure 12:^{13}C NMR Spectroscopy of the Standard sample of Kaemferol 3-O Rutinoside

Table 13: Interpretation of ^{13}C NMR Data of Standard sample of Kaemferol 3-O Rutinoside

Position no	Interpretedvalue13CNMR
1	93.69
3	141.03
4	101.56
5	35.37
6	80.32
7	59.91
8	65.48
9	41.82
10	61.56
1’	98.31
2’	73.46
3’	79.98
4’	70.39
5’	78.08
6’	61.56
1’’	122.91
2”	114.18
3”	147.43
4”	151.78
5”	114.61
6”	123.92
CO	166.47
α	55.07
COCH3	-

Table 14: Interpretation of ^{13}C NMR Data of Standard sample of Kaemferol3-O Rutinoside

Sr.No	NMR Peakc-13	Interpretation
1	47.10 to47.97	Methoxy Carbon
2	130 and134ppm	Aromatic carbon
3	166ppm	C=O Carbon
4	141ppm	Unsaturated carbon C=C
5	101.56	C-O of aromatic ring
6	77.28ppm	-CH of aromatic

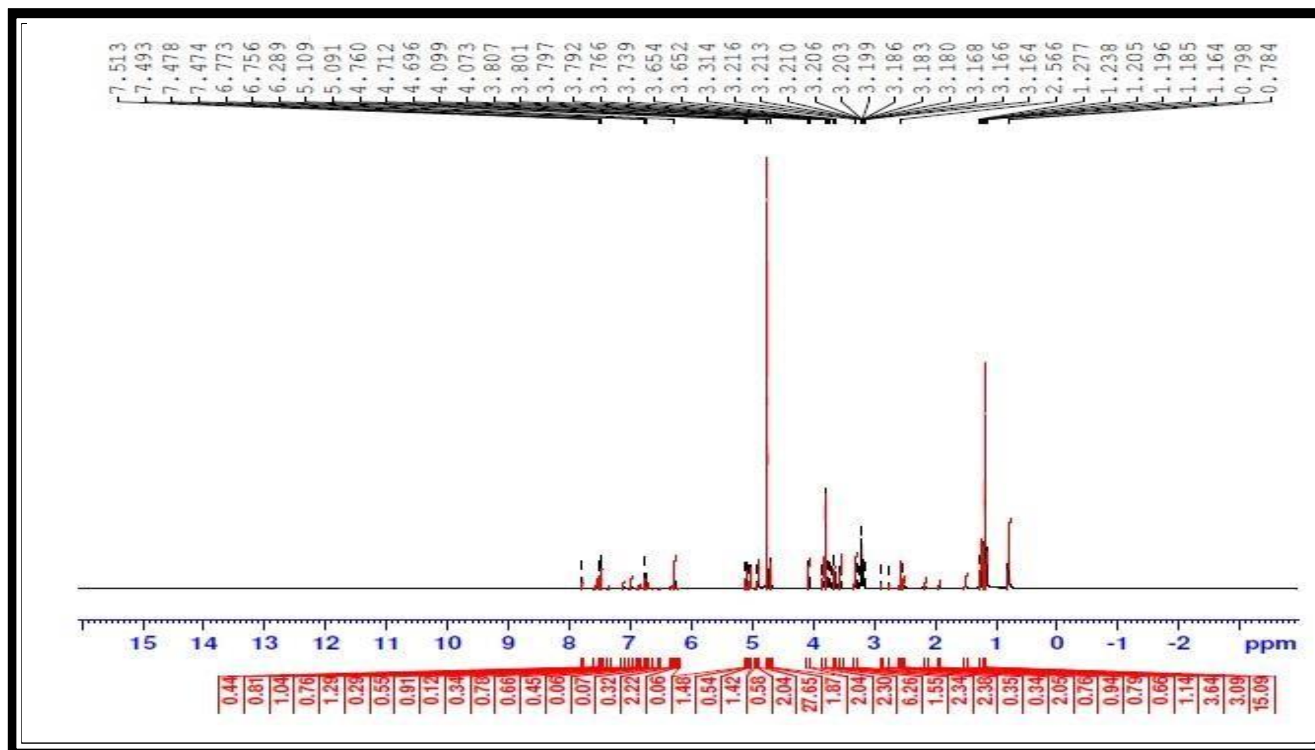

Figure 13: ^{1}H NMR Spectroscopy of standard sample of Kaemferol 3-O Rutinoside

Table 15: Interpretation of ^{1}H NMR Data of standard sample Kaemferol 3-O Rutinoside

Sr.No	NMR Peak	Probable assignments
1	3.71ppm	Methoxy Proton.
2	4.7ppm	Alkene Proton
3	6.3ppm	Hydroxyl Proton
4	6.7to6.8ppm	Hydroxyl Proton
5	7ppm	Aromatic Proton
6	7.5ppm	Aromatic Proton
7	7.8ppm	Aromatic Proton

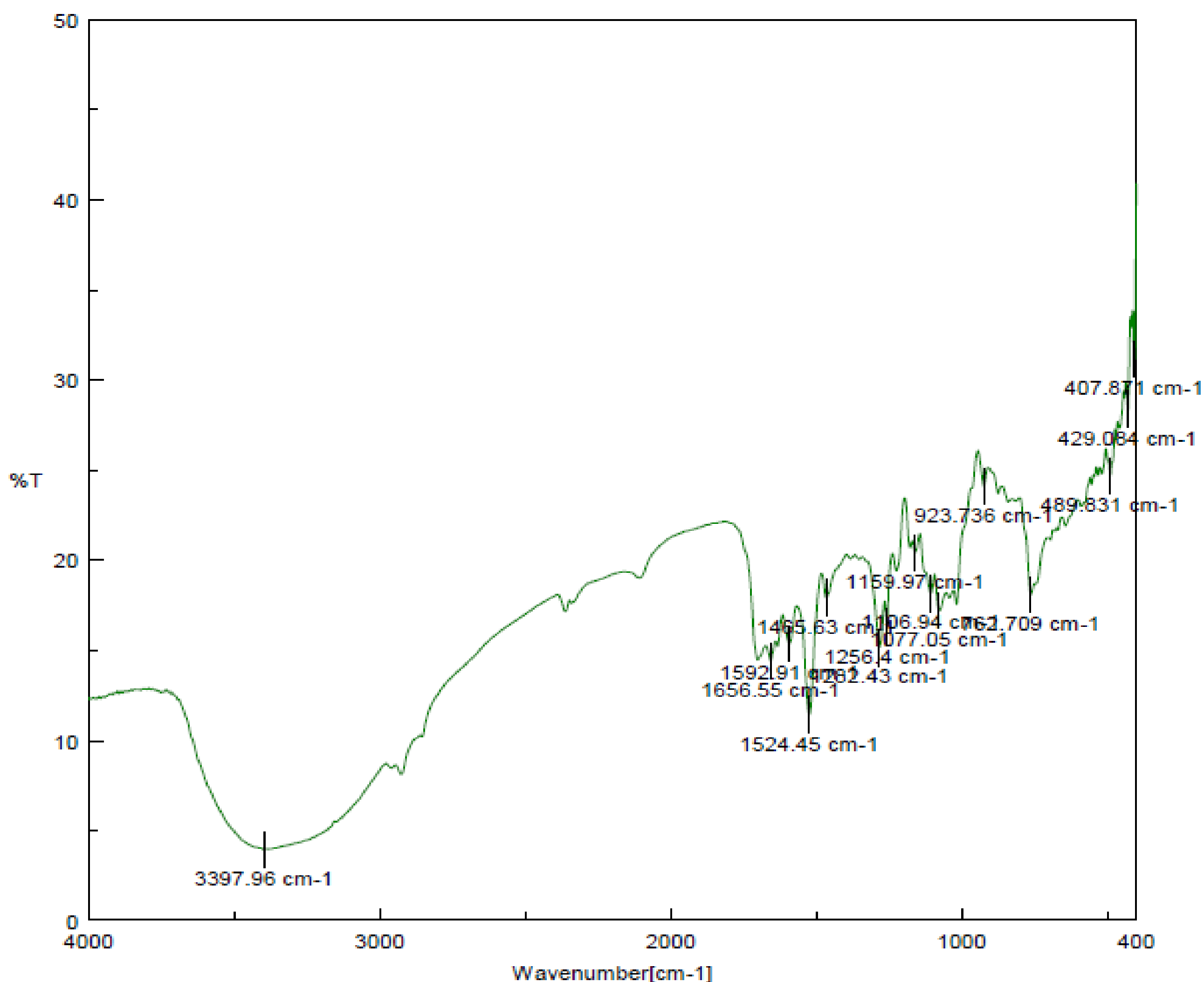

Figure 14: IR Spectra of standard sample Kaemferol 3-O Rutinoside

Table 16: Interpretation of IR spectra of standard sample Kaemferol 3-O Rutinoside

Sr.No	Wave no.(cm^{-1})	Functional group	Compound class
1	3397.96	O-H Str	Alcohol
2	1656.55	C=O Str	Carbonyl
3	15.92.91	C=C Str	Aromatic Ring
4	1524.45	C-H Bend	Aromatic Ring
5	1456	C-H Bending	Methyl Group(CH3)
6	1282	O-H Bending	Alcohol
7	1256.4	C-O Str	Alkyl Aryl Ether
8	1159.97	C-O Stre	Ester
9	1106.94	C-O Stre	Aliphatic Ether
10	1077	C-O Stre	Alcohol

3.3. Isolation and Characterization of isolated Kaemferol 3-O Rutinoside by spectroscopic Method

3.3.1. TLC development for isolated Kaemferol 3-O Rutinoside

TLC was performed of silica gel glass slide. Ethyl acetate: Methanol: Glacial acetic acid: Formic acid 25:5:1:1 v/v/v/v respectively was used.

3.3.2. HPTLC

HPTLC was performed on pre-coated silica plates 20x20 (0.20 mm) silica gel 60 F254. The mobile phase Ethyl acetate: Methanol: Glacial acetic acid: Formic acid gives in ratio25:5:1:1 v/v/v/v respectively was used Visualization was done under UV at 254 nm, and The extract and standard sample was observed one major spot at (Rf 0.50)on TLC.

3.4. Isolation of Kaemferol 3-O Rutinoside from Extract by column chromatography

For isolation of compound 15 gm of Ethanolic Fruit extract of Adansonia digitata L*(EFAD)*was load on column packed with silica gel 100-200 mesh size. First 100%chloroform and then, 3-4% v / v methanol in chloroform was run off in the column. Collected the all fractions of 100 ml each respectively. After observing the TLC fractionno.1-2,3-4,5-6,7-9,10-12,13-14,15-17,18-21,22-23,24-25,26-28,40werepooledtogether& concentrated. Fraction no- shows the targeted compound. The dried weight of that fraction was 100 mg, 200mg, 100mg, 100mg resp. column fraction subjected to thenext column chromatography. Visualization was done under UV at 254 nm, and The Kaemferol 3-O Rutinoside was observed one major spot at (Rf 0.43 $\pm$0.03) on TLC as illustrated n fig.4The UV spectra for isolated Kaemferol 3-O Rutinoside from extract was carried out by UV spectroscopy. The extract shows strong absorption band at around 246.5nm, of wavelength. UV spectra of extract is shown in Figure 3.

3.4.1. Analytical HPLC

Analytical HPLC was performed to check the purity of Kaemferol 3-O Rutinoside-Sample shownin: fig.4
Kaemferol 3-o Rutinoside were successfully isolated and purified with prep-RP-HPLC using binary gradient Orthophosphoric acid and acetonitrile. The gradient elution program gavebetter peak shape and required resolution with a small time for running. The fraction collect or was set as time based from time 8.42 min for isolated Kaemferol 3-O Rutinoside-Sample. HPLC evaluated the purity of fractions andfound to be highly pure and identified by retention time with reference standard. The purity of isolated kaemferol 3-o rutinoside sample is 96.42%.

3.5. ^{1}HNMR of isolated Kaemferol 3-O Rutinoside-Sample

The isolated Kaemferol 3-O Rutinoside-Sample^{1}H NMR spectrum revealed 3 kinds of proton signals namely, hydroxyl protons, aromatic protons of the analyte and a solvent proton signal.The^{1}HNMR spectrum showed hydroxyl protons resonating at δ 6.2 and in between 6.4 to 6.5 ppm two singlet also 7.3 to 7.5 ppm for aromatic proton and two singlet at 7.6 ppm for aromatic proton and other proton occurs at 7.8ppm corresponding to aromatic ring.

3.5.1. Infrared spectroscopy

IR spectroscopic data of Isolated Kaemferol 3-O Rutinoside-Sample exhibited broad absorption peak at around 3365.17 cm^{-1}was allocate alcohol's O-H stretching vibration. C-HB ending vibration of Aromatic Compound were observed at 1523.49 cm^{-1}. The absorption peaks positioned at 2924.52cm^{-1}and1452.14cm^{-1}are allocated alkane C-H stretching and C-HB ending respectively. C=C stretching vibrations of alkene were observed at1637.27cm^{-1}.C-O stretching vibrations of ester were observed at 1257cm^{-1}. O-H Bending & C-O stretching vibrations of Alcohol were observed at 1332.57 c m^{-1} and 1177 cm^{-1}which confirmed all the possible functional groups in isolated compound.

3.6. Characterization of Standard Sample of Kaemferol 3-O Rutinoside-by spectroscopic methods

3.6.1. UV spectra for Standard Sample Kaemferol 3-O Rutinoside

The UV spectra for Standard Sample Kaemferol 3-O Rutinoside from extract was carried out by UV spectroscopy. The extract shows strong absorption band at around 254 nm of wavelength. UV spectra of extract is shown in Figure 09.

3.6.2. Analytical HPLC

Analytical HPLC was performed to check the purity of Standard Sample of Kaemferol 3-O Rutinosideshownin fig11.Standard sample Kaemferol 3-O Rutinoside were successfully isolated and purified with prep-RP-HPLC using binary gradient Orthophosphoric acid and acetonitrile. The gradient elution program gave better peak shape and required resolution with a small time for running. The fraction collector was set as time based from time 4.205 min for Standard sample Kaemferol 3-ORutinoside. HPLC evaluated the purity of fractions and found to be highly pure and identified by retention time with reference standard.The purity of Standard sample Kaemferol 3-ORutinosideis 89%.

3.7. NMR Analysis

3.7.1. ^{13}C NMR spectroscopy of standard sample Kaemferol 3 -ORutinoside

The standard sample Kaemferol 3-O Rutinoside^{1}H NMR spectrum showed hydroxyl protons resonating at δ 6.3 and in between 6.7 to 6.8 ppm two singlet also 7 to 7.5 ppm for aromatic proton and another proton occurs at 7.8 ppm corresponding to aromatic ring. Alkene Proton appeared at 4.7 ppm as singlet. Methoxy Proton appeared at 3.71 ppm as singlet.

3.7.2. Infrared spectroscopy

IR spectroscopic data of standard sample Kaemferol 3-O Rutinoside exhibited broad absorption peak at around 3397.96 cm^{-1}was allocated to alcohol's O-H stretching vibration. C=O stretching vibration of Carbonyl were observed at 1656.55 cm^{-1}. C=C stretching and C-H Bending vibrations of aromatic Ring were observed at 15.92.91 cm^{-1} and 1524.45 cm$^{-1.}$ The absorption peaks positioned at 1456cm^{-1} area located Methyl Group of C-H Bending vibration.O-H Bending and C-O stretching vibrations of Alcohol were observed at 1282 cm^{-1}and 1077 cm^{-1}respectively. C-O stretching vibration of Alkyl Aryl Ether was observed at 1256.4 cm^{-1}. C-O stretching vibration of ester was observed at 1159.97 cm^{-1}. The absorption peak positioned at 1106.94 cm^{-1}are allocated to Aliphatic Ether C-

O stretching. This confirmed all the possible functional groups in isolated compound.

4. Conclusions

In this investigation Preliminary Qualitative, Quantitative screening of Multiple extracts *of Adansonia digitata* was carried. Ethanolic Fruit extract of *Adansonia digitata* L(EFAD) shows better results as compared to other Extracts especially for Total flavonoid Content(TFC) and Total Phenolic Content(TPC). This study aimed to isolate flavanol glycosides namely Kaemferol 3-O Rutinoside-Sample from the Ethanolic Fruit extract of Adansonia digitata L *(EFAD)*. The isolated compound were find out by utilizing HPLC,^{13}C NMR and ^{1}H NMR spectra, Mass and IR spectroscopy. By comparing spectroscopic results both isolated and standard sample, it is conclude successfully isolate flavanol glycosides namely Kaemferol 3-O Rutinoside-Sample from the Ethanolic Fruit extract of Adansonia digitata L*(EFAD)*.Further studies are warranted to decipher the probable mechanism by which absolute ethanol extract of Kaemferol 3-O Rutinoside exert Pharmacological effect.

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Conflict of Interest

The authors of this research article have no conflicts of interest to disclose.

Abbreviations

HPLC: High Performance Liquid Chromatography
HPTLC: High Performance Thin Layer Chromatography
FTIR: Fourier Transform Infrared Spectroscopy
LCMS-Liquid Chromatography Mass Spectroscopy
NMR-Nuclear Magnetic Resonance
AFAD- Aqueous Fruit extract of *Adansonia digitata* L
ALAD- Aqueous Leaf extract of *Adansonia digitata* L
EFAD-Ethanolic Fruit extract of *Adansonia digitata* L
ELAD Ethanolic Leaf extract of *Adansonia digitata* L
CFAD- Chloroform Fruit extract of *Adansonia digitata* L
CLAD-Ethanolic Leaf extract of *Adansonia digitata* L
EAFAD-Ethyl Acetate Fruit extract of *Adansonia digitata* L
EALAD-Ethyl Acetate Leaf extract of *Adansonia digitata* L
PEFAD -Pet Ether Fruit extract of *Adansonia digitata* L
PELAD-Pet Ether Leaf extract of *Adansonia digitata* L

References

[1] B. Zygmunt, J. Namieśnik. (2003). Preparation of samples of plant material for chromatographic analysis. Journal of chromatographic science. 41(3): 109-116.
[2] P. Ahad, Mohi-ud-in,R. Hassan. (2023). Pharmacognostical standardization, Phytochemical analysis and anti-oxidant and antidepressant potency of Iris Germanica L. Journal of Population Therapeutics and clinical Pharmacology. 30.17
[3] K. Herrmann, C.W. Nagel. (1989). Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. Critical reviews in food science & nutrition. 28(4): 315-347.
[4] R.J. Robbins. (2003). Phenolic acids in foods: an overview of analytical methodology. Journal of agricultural and food chemistry. 51(10): 2866-2887.
[5] J.C. Sánchez-Rangel, J. Benavides, J.B. Heredia, L. Cisneros-Zevallos, D.A. Jacobo-Velázquez. (2013). The Folin–Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. Analytical Methods. 5(21): 5990-5999.
[6] H.T. Wolterbeek and P. Bode. Strategies in sampling and sample handling in the context of large-scale plant biomonitoring surveys of trace element air pollution. Sci. Total Environ. 176: 33–43 (1995).
[7] H. Emons, J.D. Schladot, and M.J. Schwuger. Environmental specimen banking in Germany—present state and further challenges. Chemosphere 34: 1875 (1997).
[8] J. Namiesnik and B. Zygmunt. Role of reference materials in environmental analysis. Sci. Total Environ. 228: 243–57 (1999).
[9] S. Bowadt and S.B. Hawthorne. Supercritical fluid extraction in environmental analysis. J. Chromatogr. 706: 549–70 (1995).
[10] A. Kot and J. Namiesnik, The role of speciation in analytical chemistry. Trends Anal. Chem. 19: 69–79 (2000).
[11] A.M. Kipopoulou, E. Manoli, and C. Samara. Bioconcentration of polycyclic aromatic hydrocarbons in vegetables grown in an industrial area. Environ. Pollut. 106: 369–80 (1999).
[12] L. Holoubek, P. Korinek, Z. Seda, E. Schneiderova, L. Holoubkova, A. Pacl, J. Triska, P. Cudlin, and J. Caslavsky. The use of mosses and pine needles to detect persistent organic pollutants at local and region scales. Environ. Pollut. 109: 283–92 (2000).
[13] M. Howsam, K.C. Jones, and P. Ineson. PAHs associated with the leaves of three deciduous tree species—concentrations and profiles. Environ. Pollut. 108: 413–24 (2000).
[14] S.K. Poole, T.A. Dean, J.W. Oudsema, and C.F. Poole. Sample preparation for chromatographic separations: an overview. Anal. Chim. Acta 236: 3–42 (1990).
[15] W.R. Majors and G. Slack. “Sample Preparation”. In Practical HPLC Method Development. L.R. Snyder, J.J. Kirkland, and J.L. Glajch, eds. John Wiley & Sons, New York, NY, 1997, pp. 100–73.