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Investigation of phytochemicals and Spectral data of aqueous, ethanolic, and methanolic extracts of Smilax china

Apada Reddy Gangadasu[1*], Rakesh Jat[2], and P. Selvam[3]
[1*]*Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan – 333001*
[2]*Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan – 333001*
[3]*Intrensic Herbals Laboratory., Rajapalayam - 626117*

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ABSTRACT
Secondary metabolites termed as phytochemicals are produced by plants as a kind of defence against pathogenic microbial invasion and environmental stress. These phytochemicals have been demonstrated to offer therapeutic benefits in the treatment of human ailments as well as pharmacological effects. It is common knowledge that the active components in medicinal plants combine to lessen both the primary and secondary symptoms of a range of illnesses. Popularly known as "Jin Gang Teng" or "Ba Qia," Smilax china (SC) is a common ingredient in traditional Chinese medicine (TCM). It is used to treat diuretic and rheumatoid arthritis problems, detoxify the body, and treat tumours, inflammatory diseases, lumbago, and gout. In the current investigation, the DPPH assays, in vitro anti-inflammatory activity, and anti-diabetic activity against alpha-amylase were used to evaluate the ability of the aqueous extract and ethanolic extract (SC-A & SC-ET) in scavenging free radicals. Additionally, SC looked into the lethality of brine shrimp and antibacterial efficacy against S. aureus and P. aeruginosa. Aqueous and ethanolic extracts of Smilax china were investigated for their in-vitro antioxidant, antibacterial, anti-diabetic, and anti-inflammatory properties. Due to the presence of physiologically active compounds in the herbal preparation, the data given provide scientific proof for the antioxidant and other therapeutic efficacy of the SC A, SC ME, & SC ET preparation.

Keywords: Smilax china; phytochemical assays; Rutin, Quercetin, Smilax China Rhizome, Spectral Data

I. INTRODUCTION

People have been looking for medications in nature to treat their diseases since ancient times. The usage of medicinal plants began instinctively, just as it does with animals [1]. Because there was insufficient information at the time, neither about the causes of the ailments nor about which plant and how it could be used as a cure, everything was based on experience. As the reasons for the use of various medicinal plants for the treatment of specific ailments were identified over time, the use of medicinal plants shifted away from an empiric framework or towards one based on explicatory facts. Plants have been the source of treatment and prophylaxis until the invention of iatrochemistry in the 16th century [2]. The ability of pharmacists and physicians to respond to the challenges that have arisen with the spread of professional services in the facilitation of man's life has increased as knowledge of the development of ideas related to the use of medicinal plants as well as the evolution of awareness has increased [3]

Smilax china

The perennial climbing deciduous shrub Smilax china L. (Liliaceae) is widely found in Southern China and Southeast Asian countries. S. china leaves are utilized as a detoxicant in traditional Chinese medicine [4]. Smilax china L. derived compound extract has various pharmacological properties, including anti-inflammatory, anti-cancer, and antioxidant properties [5]. The main active components of Smilax china L. are stilbene, flavonoids, polyphenols, and steroidal saponins [6, 7]. Polyphenol compounds like resveratrol are also found in Smilax china L. [8]. Free radical scavenging and antioxidant enzyme promoting activities were observed in the extracts of Smilax china L. root [9, 10]. The alcoholic extract of Smilax china protects the induction of lipid peroxidation, induced by $FeSO_4$. This may be due to chelation of iron, conversion of Fe^{2+} to Fe^{3+}, increased level of reduced glutathione, or by scavenging hydroxyl, superoxide radicals, and other oxygen molecules responsible for lipid

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peroxidation [11, 12]. In this study, the ability of the aqueous extract & Ethanolic extract (SC-A & SC-ET) in scavenging free radicals was assessed by using DPPH assays, in vitro anti-inflammatory activity, and ant diabetic activity against alpha-amylase. SC also investigated for antibacterial activity against S. aureus and P. aeruginosa and Brine shrimp lethality test.

Method of preparation of sample

10gm of SC is heated with 100ml water and solvent separately for 5 hours under reflux condenser in a water bath, cool, and filter. The filtrate is evaporated under a vacuum to get SC-A, SC-Me, and SC-ET extract.

II. RESULTS

PHYTOCHEMICAL SCREENING

The results of different solvent extracts of Smilax china's phytochemical analysis is presented in Table 1.

Table 1: Phytochemical investigation of rhizome extracts of Smilax china

Plant constituents test / reagents used	SC A	SC Me	SC ET
1 Carbohydrates test			
a) Molisch's Reagent Testing	-	-	-
b) Fehling's testing	-	-	-
c) Benedict's testing	-	-	-
d) Barfoad's testing	-	-	-
2. Protein & Amino acids Test			
a) Biuret Test	-	+	+
b) Millon's testing	-	+	+
c) Xanthoprotein testing	-	+	+
d) Ninhydrine testing	-	+	+
3. Test for Phytosterols			
a) Salkowski's Test	+	-	+
b) Liebermann – Burchard Reaction Test	+	-	+
4. Saponin Test			
a) Foam testing	-	+	-
5. Glycosides Test			
a) Legal's Testing	-	-	+
b) Killer killani Testing	-	-	+
c) Borntrager's Testing	-	-	+
6. Phenolic Compounds Assessing Test			
a) Ferric Chloride Solution Test	-	-	+

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7. Flavonoid Assessing Test a) Shinoda's Testing	-	-	+
8. Alkaloids Assessing Test a) Mayer's Reagent Testing b) Wagner's Reagent Testing c) Dragendorff's Reagent Testing d) Hager's Reagent Testing	 - - - -	 - - - -	 + + + +

"+" indicates chemical constituents are present "- "indicates chemical constituents are absence

PREPARATIVE THIN-LAYER CHROMATOGRAPHY (TLC)

Results of Preparative Thin Layer Chromatography (T.L.C) investigation of diverse solvent excerpts and isolated compounds were shown in Table 2.

Table 2: Results showing TLC of different Smilax china extracts

Rutin	Quercetin	SC A	SC ET	SC ME
				0.92(Blue)
			0.88(Blue)	0.88(Blue)
0	0.14(Blue) 0.07(Blue)		0.07(Blue)	0.07(Blue
0.04(Blue)				

TEST FOR FLAVONOIDS:

Compound 1 (SC Me 1)

Clearly indicate flavonoids, Quercetin.

Sample dissolved in sodium hydroxide form yellow colour and add dilute HCL colour disappear.

HPLC ANALYSIS

The retention time of pure quercetin standard (RT 4.68) was determined to be RT 4.06, whereas the component isolated from Smilax china methanolic extract was found to be RT 4.06. These findings support the presence of quercetin in Smilax china rhizomes under comparable conditions.

UV ANALYSIS

The UV analysis results of Smilax china Crude extract, SC ME I and SC ME II is presented in Table 5.

Table 5: UV-spectrophotometric analysis of SC ME and compounds

S. No.	Name of the sample (chloroform extract)	Λ max (nm)
1.	Crude extract	257
2.	SC ME I	262
3.	SC ME II	272

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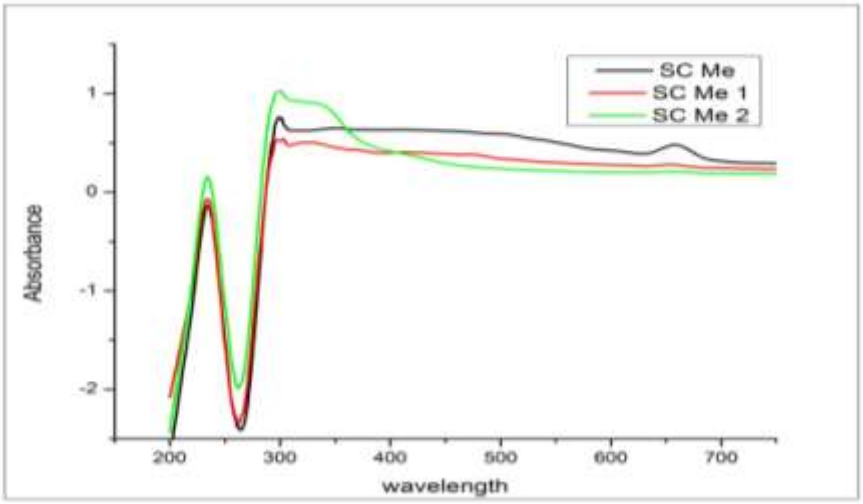

Figure 1: The graph showing the UV analysis results of SC ME and compounds

FT-IR ANALYSIS

The UV analysis results of Smilax china SC Me, SC ME I and SC ME II is presented in Figures 2, 3 & 4.

Figure 2: The graph showing the FT-IR analysis results of SC

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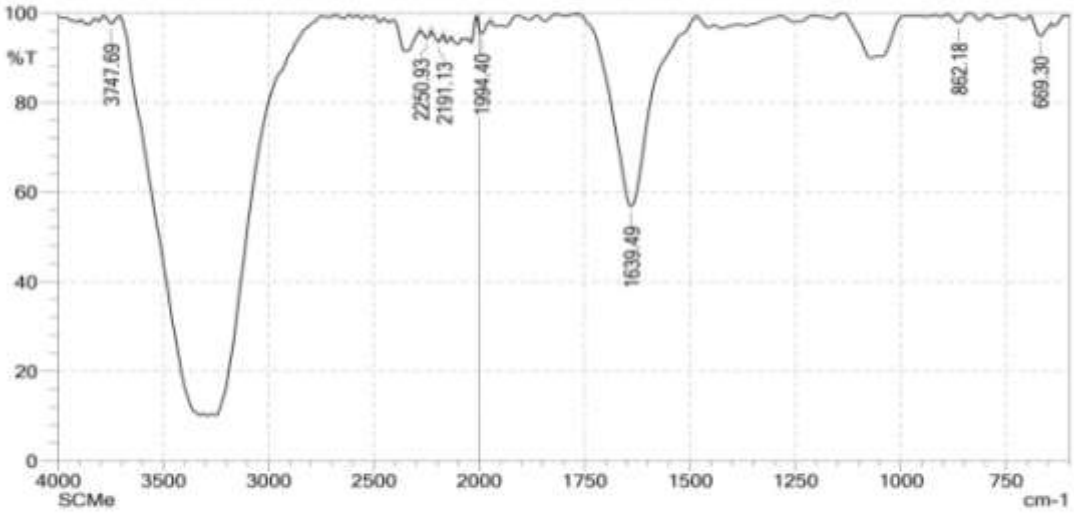

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	669.30	94.78	3.69	692.44	642.30	178.042	97.482	
2	862.18	97.79	2.03	887.26	837.11	58.893	49.754	
3	1639.49	56.78	42.72	1766.80	1485.19	4515.834	4367.671	
4	1994.40	95.30	3.42	2013.68	1973.18	119.942	67.179	
5	2191.13	93.43	1.94	2227.78	2169.92	317.766	56.091	
6	2250.93	94.23	1.73	2279.86	2227.78	252.837	42.807	
7	3747.69	97.53	1.69	3780.48	3707.18	115.488	58.560	

ME

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Figure 3: The graph showing the FT-IR analysis results of SC ME – I

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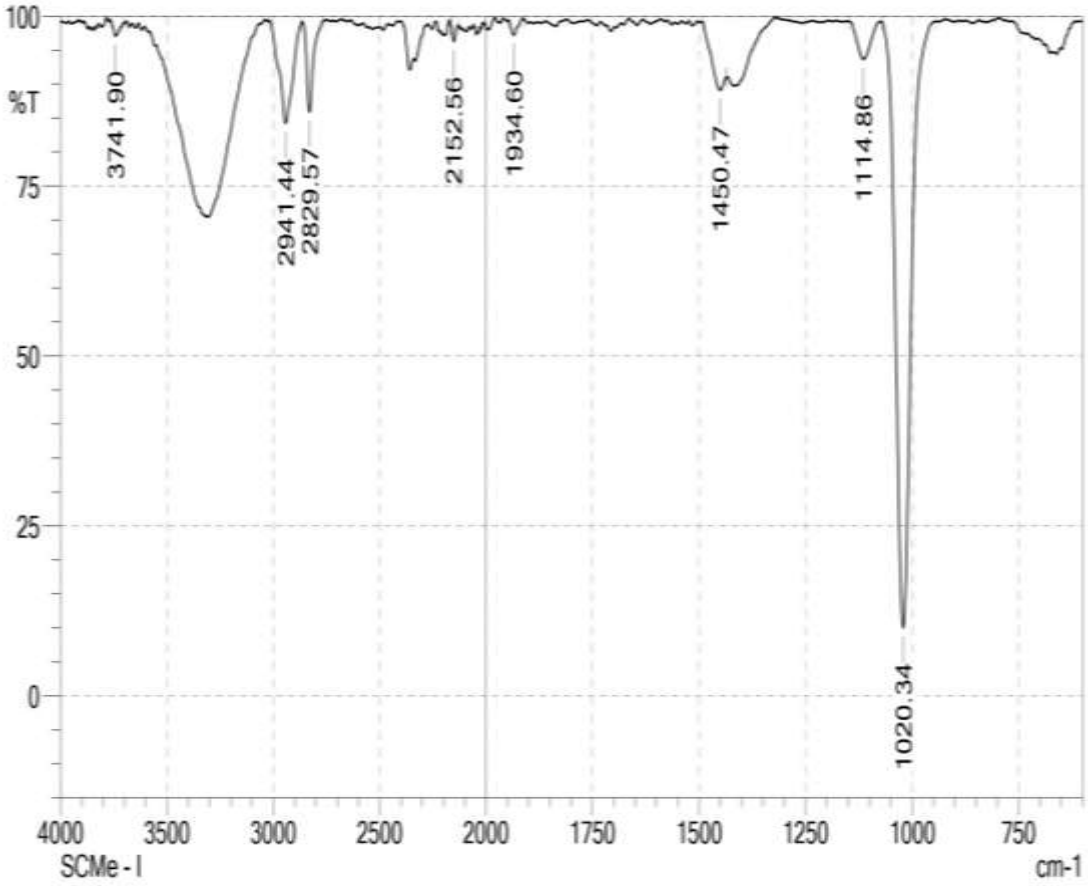

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	1020.34	10.00	89.22	1070.49	945.12	3598.811	3501.370	
2	1114.86	93.67	5.56	1147.65	1070.49	252.113	192.075	
3	1450.47	89.11	4.20	1490.97	1435.04	411.098	138.824	
4	1934.60	97.20	2.30	1948.10	1913.39	53.222	36.976	
5	2152.56	96.24	2.39	2164.13	2129.41	83.735	37.116	
6	2829.57	85.89	13.40	2866.22	2760.14	531.609	459.156	
7	2941.44	84.23	15.04	3020.53	2866.22	1153.407	1041.780	
8	3741.90	97.05	2.26	3766.98	3703.33	116.807	69.381	

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Figure 4: The graph showing the FT-IR analysis results of SC ME – II

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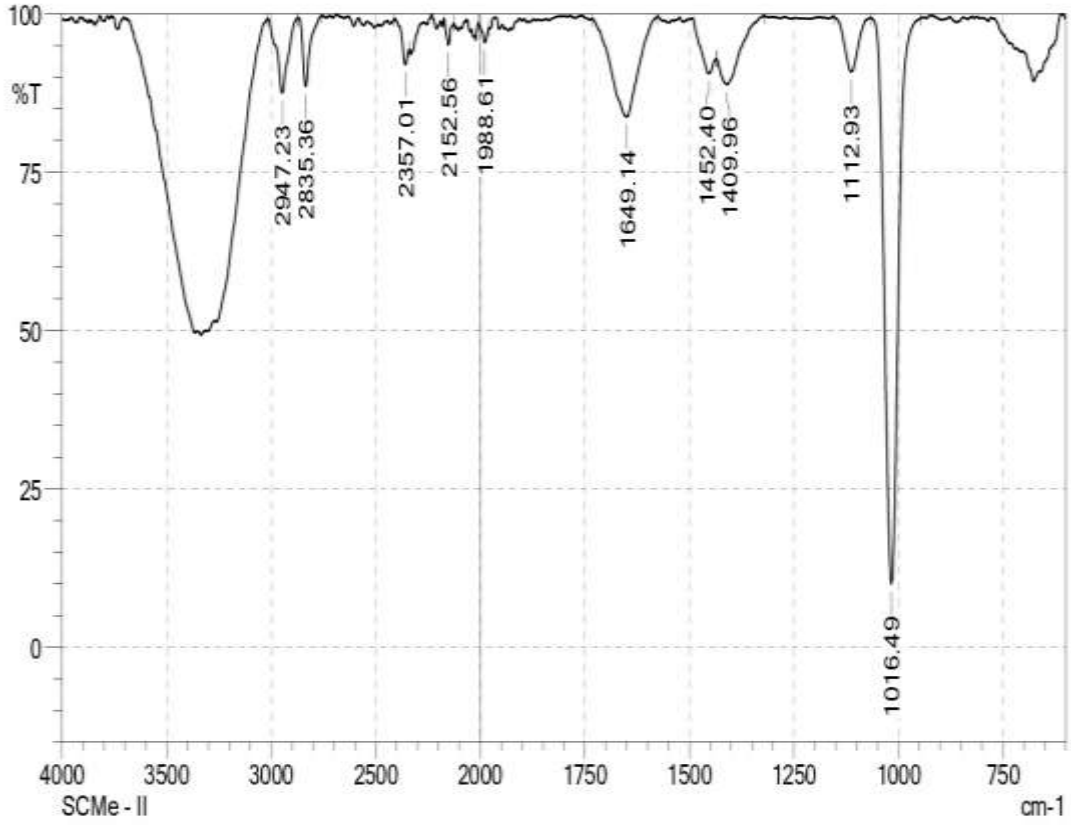

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	1016.49	10.00	89.40	1066.64	945.12	3252.735	3180.408	
2	1112.93	90.83	8.67	1155.36	1066.64	353.457	309.110	
3	1409.96	88.85	5.54	1435.04	1321.24	652.004	232.644	
4	1452.40	90.58	4.29	1490.97	1435.04	362.859	143.912	
5	1649.14	83.76	15.75	1732.08	1575.84	1197.375	1118.652	
6	1988.61	95.48	2.57	2009.83	1978.97	86.577	31.187	
7	2152.56	95.10	3.82	2173.78	2125.56	136.107	82.001	
8	2357.01	92.03	3.57	2409.09	2339.65	295.315	75.392	
9	2835.36	88.56	10.94	2873.94	2756.28	466.463	415.846	
10	2947.23	87.53	11.81	3014.74	2873.94	783.968	690.503	

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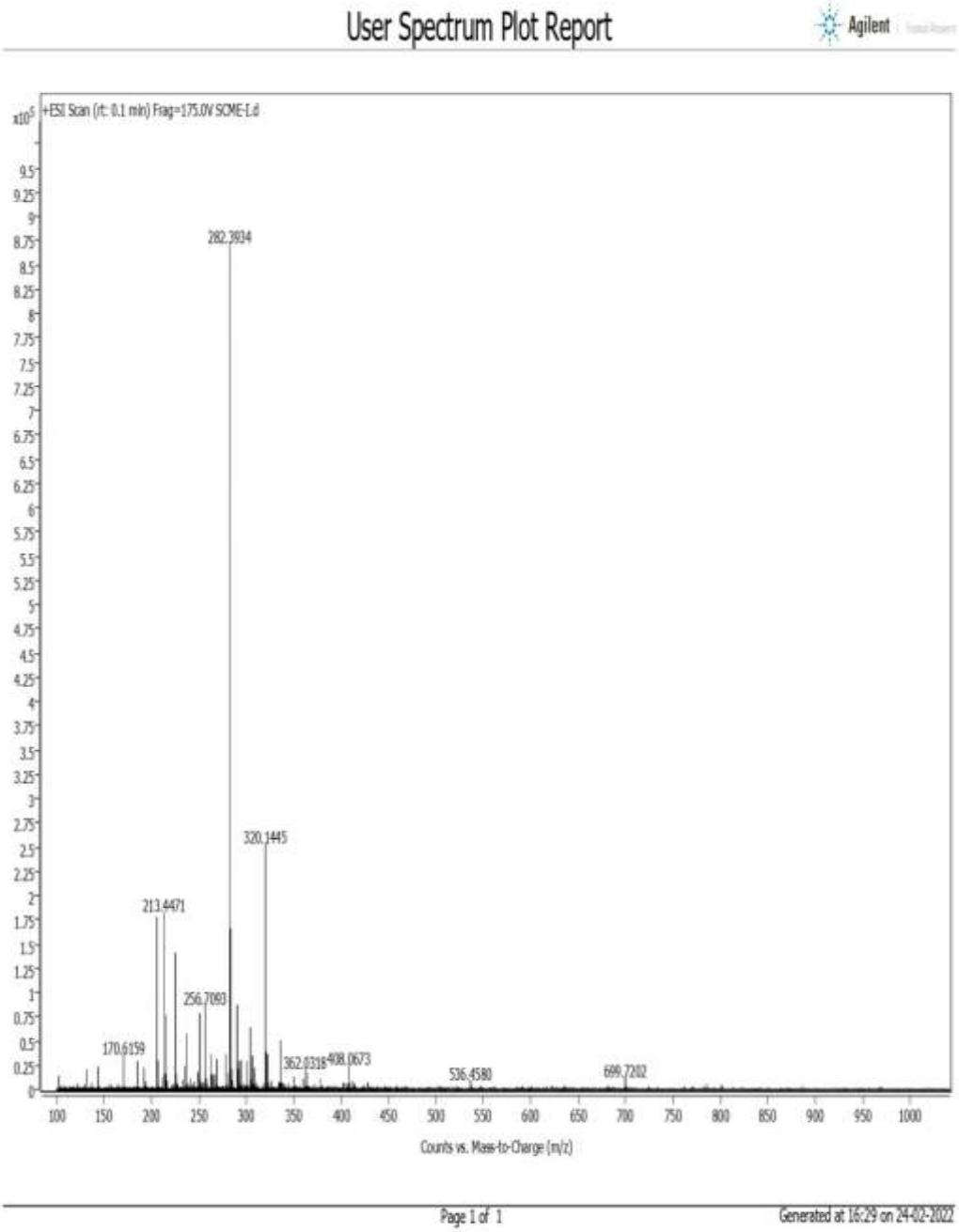
L.C-M.S ASSAY

Liquid Chromatography Mass Spectroscopy (L.C-M.S) investigation has been performed by using API-2000.

Figures 5: The graphical representation portraying L.C-M.S outcomes of SC-I



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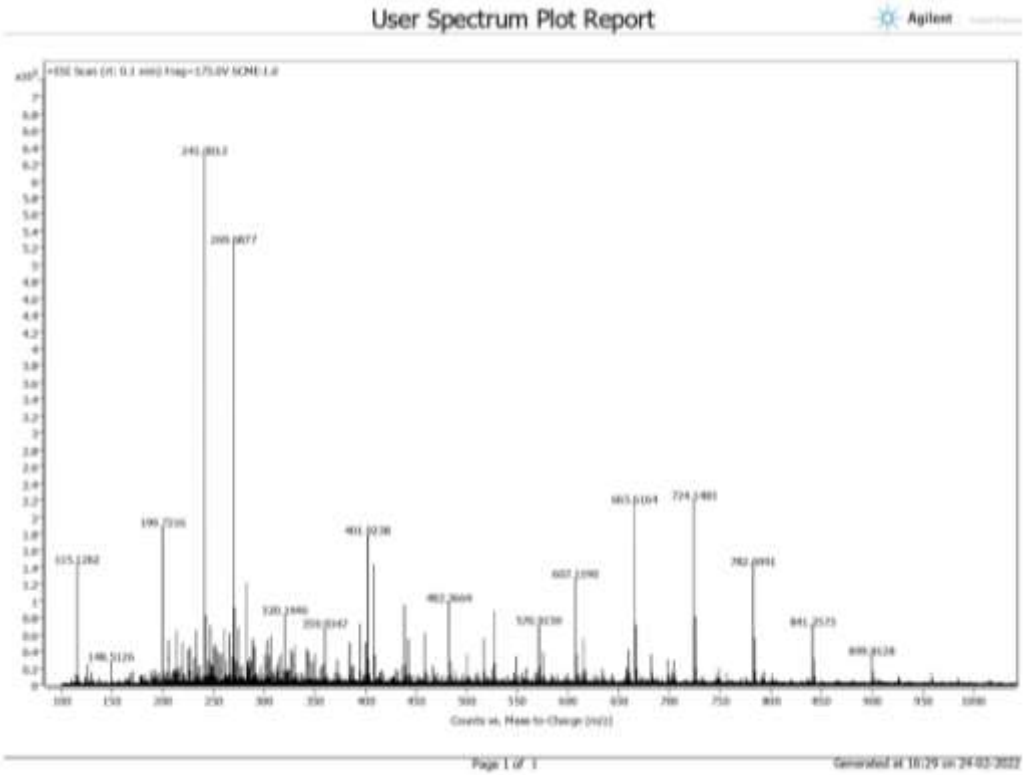

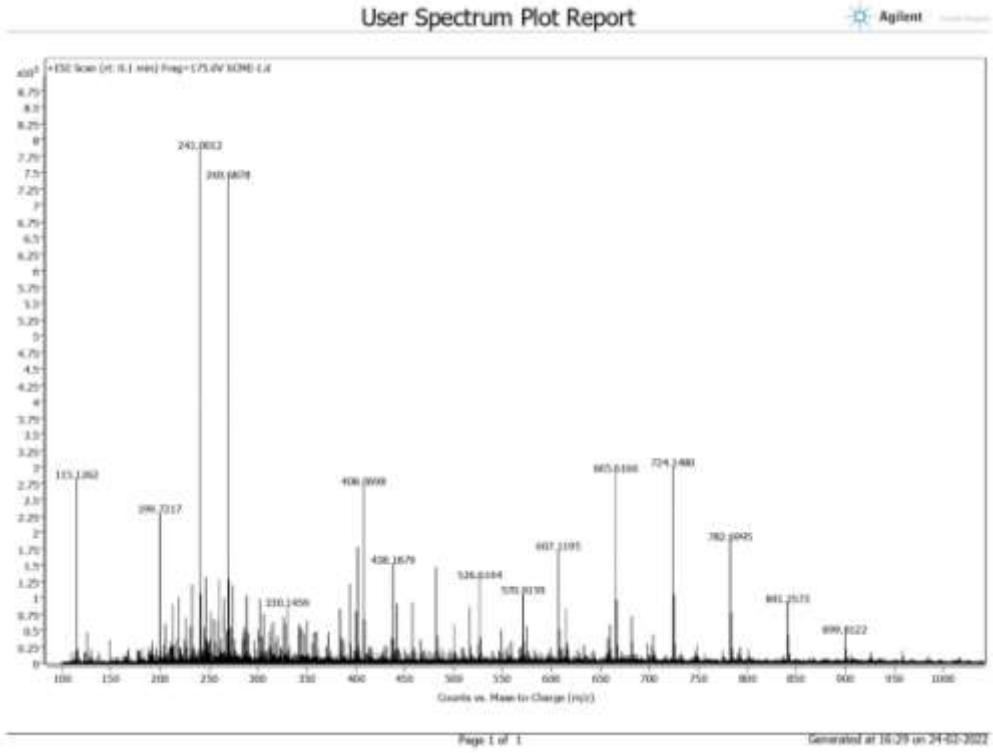

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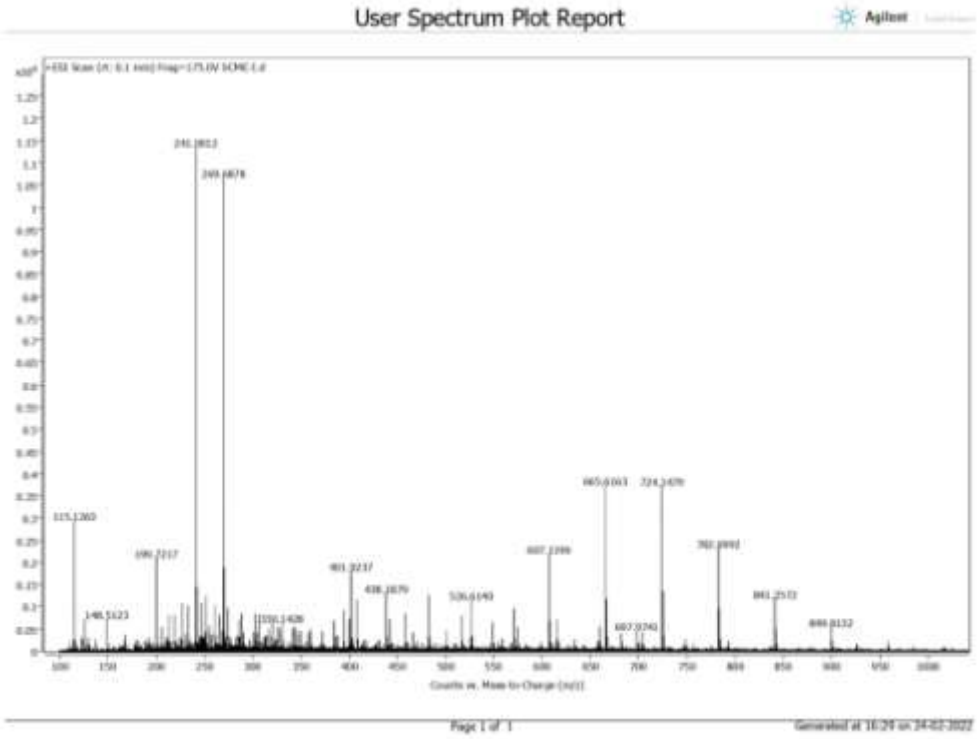

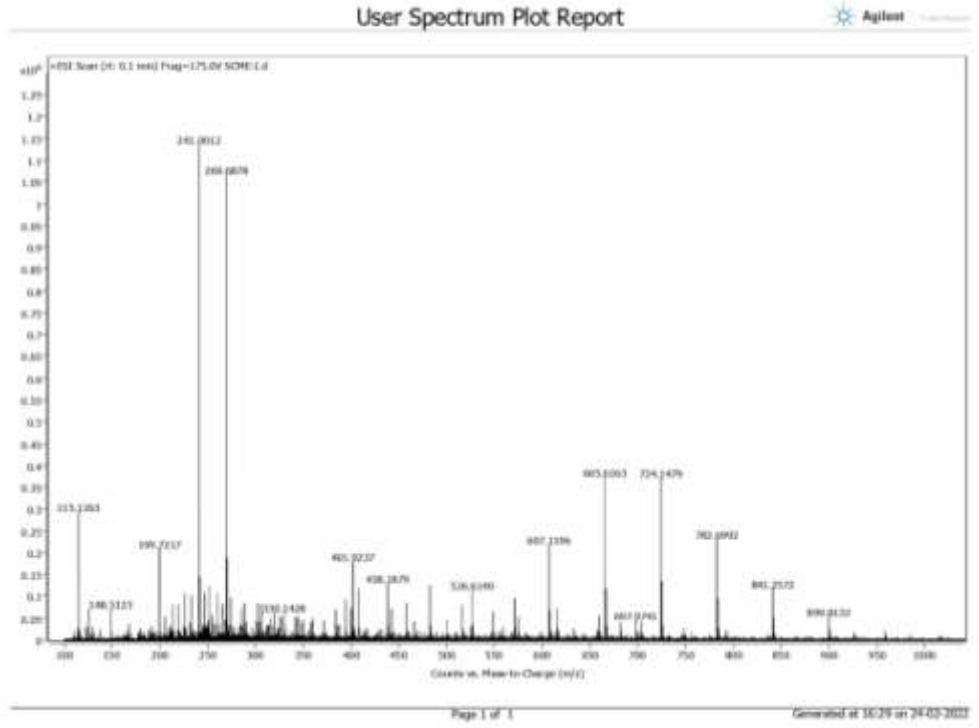

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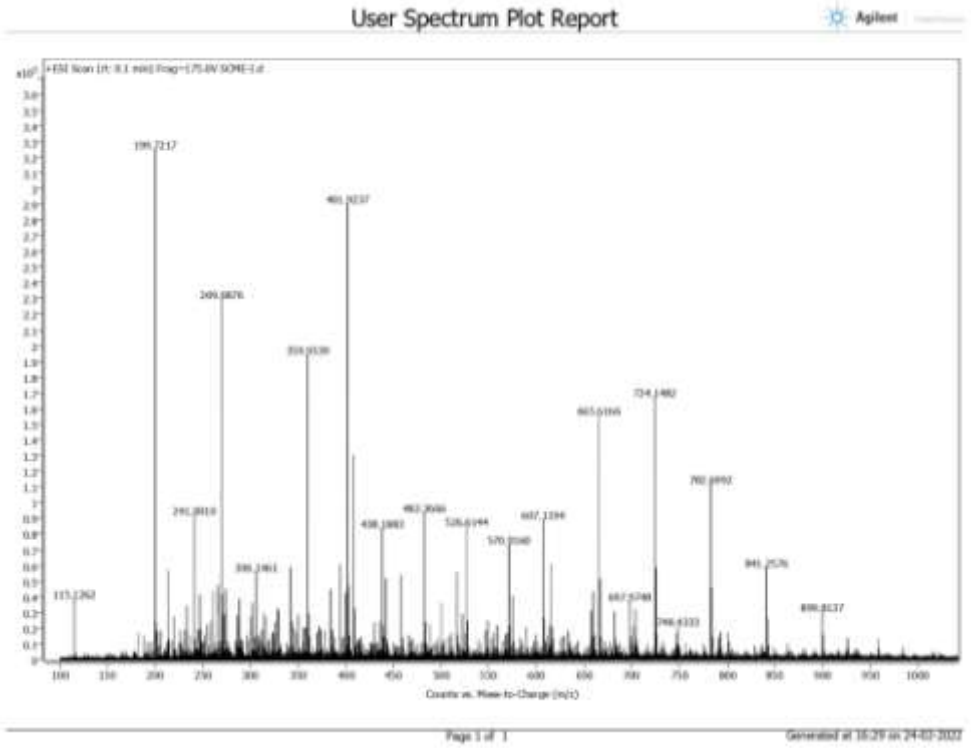

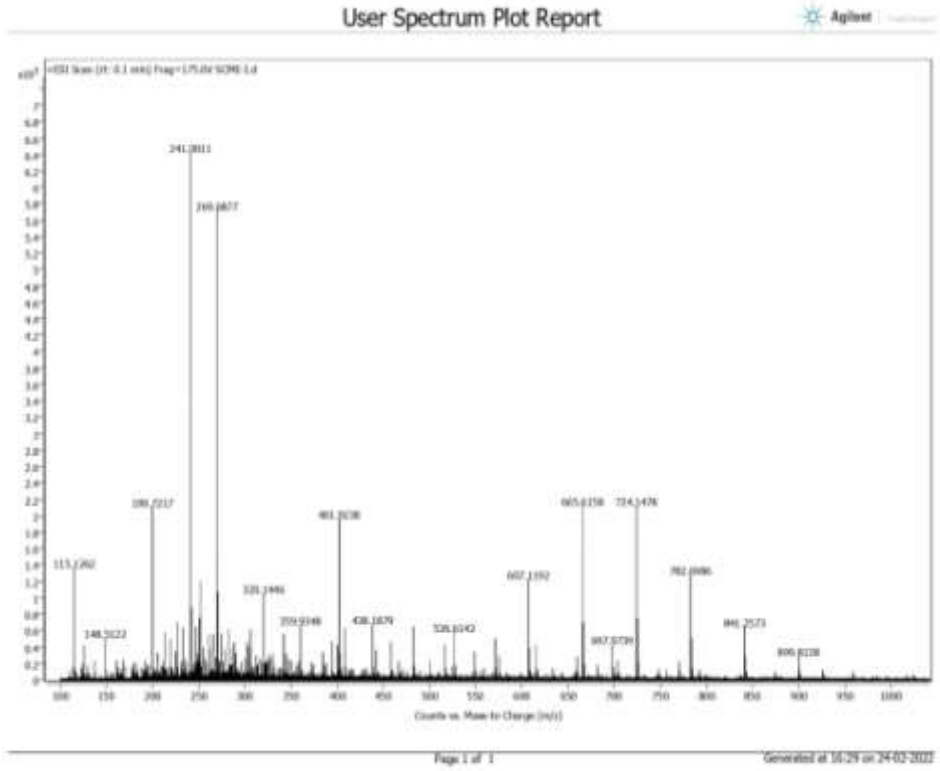

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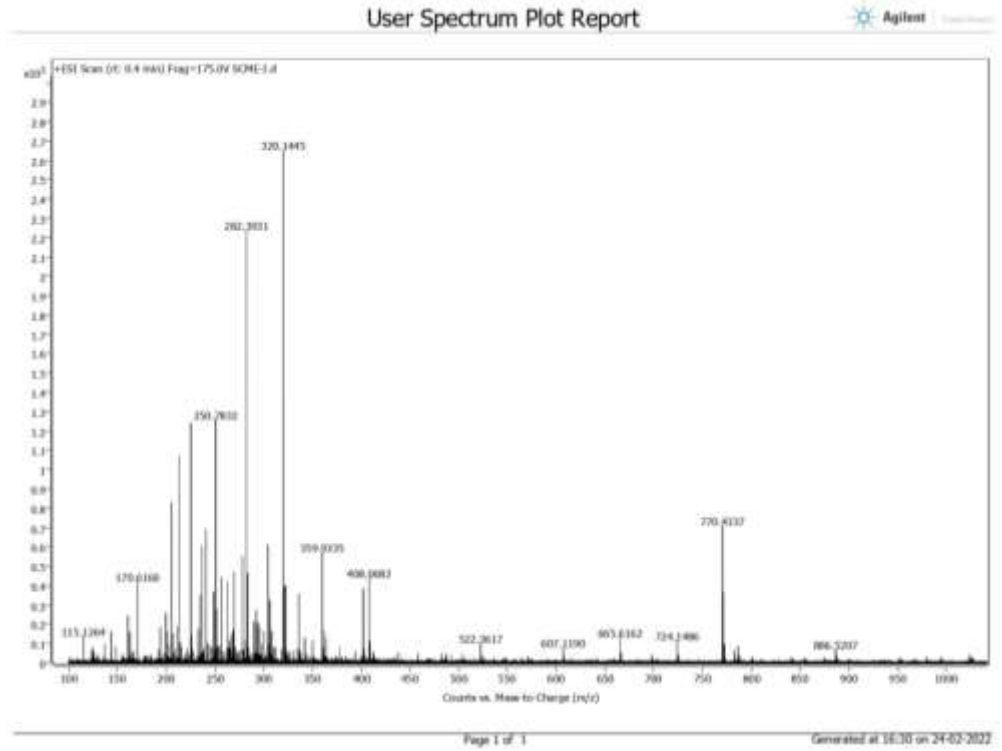
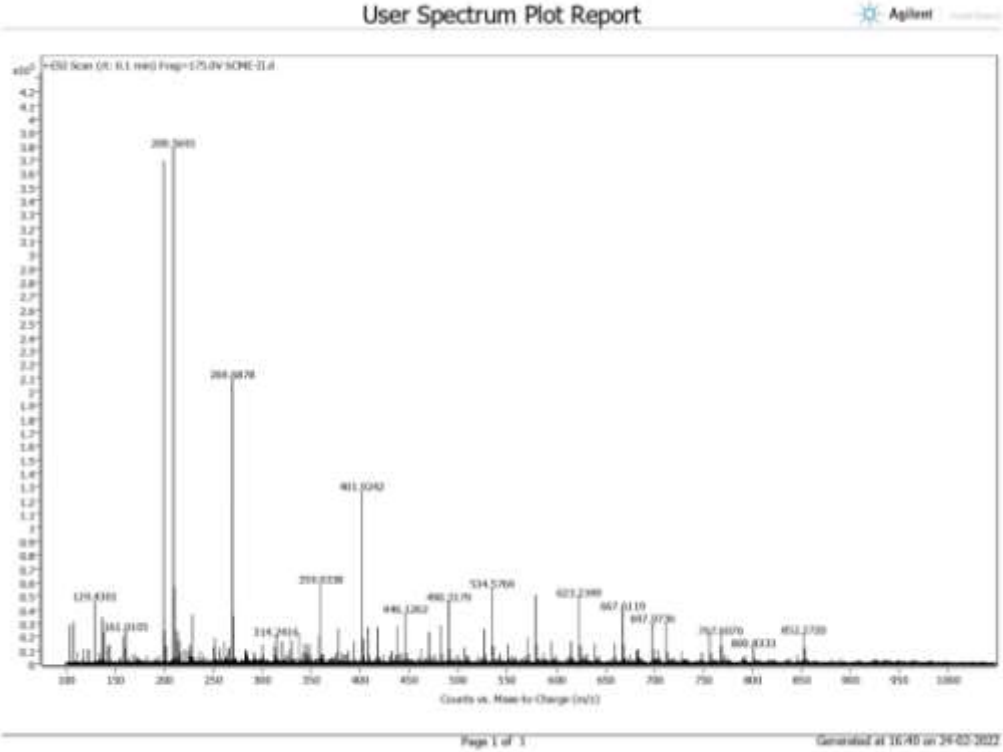
Figures 6: The graphical representation portraying L.C-M.S outcomes of SC-II





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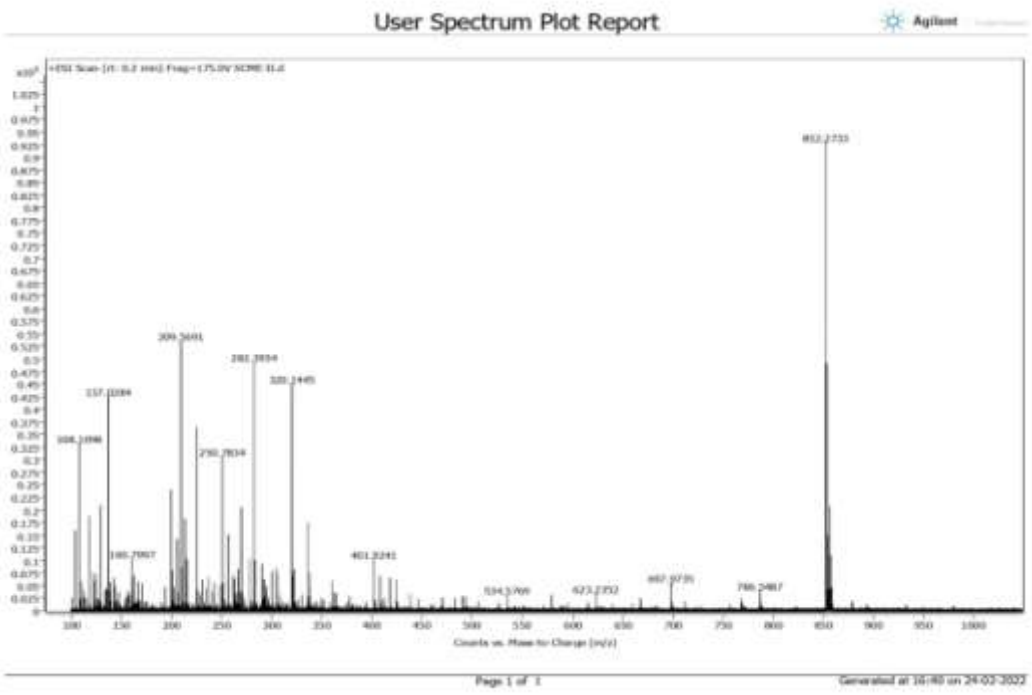

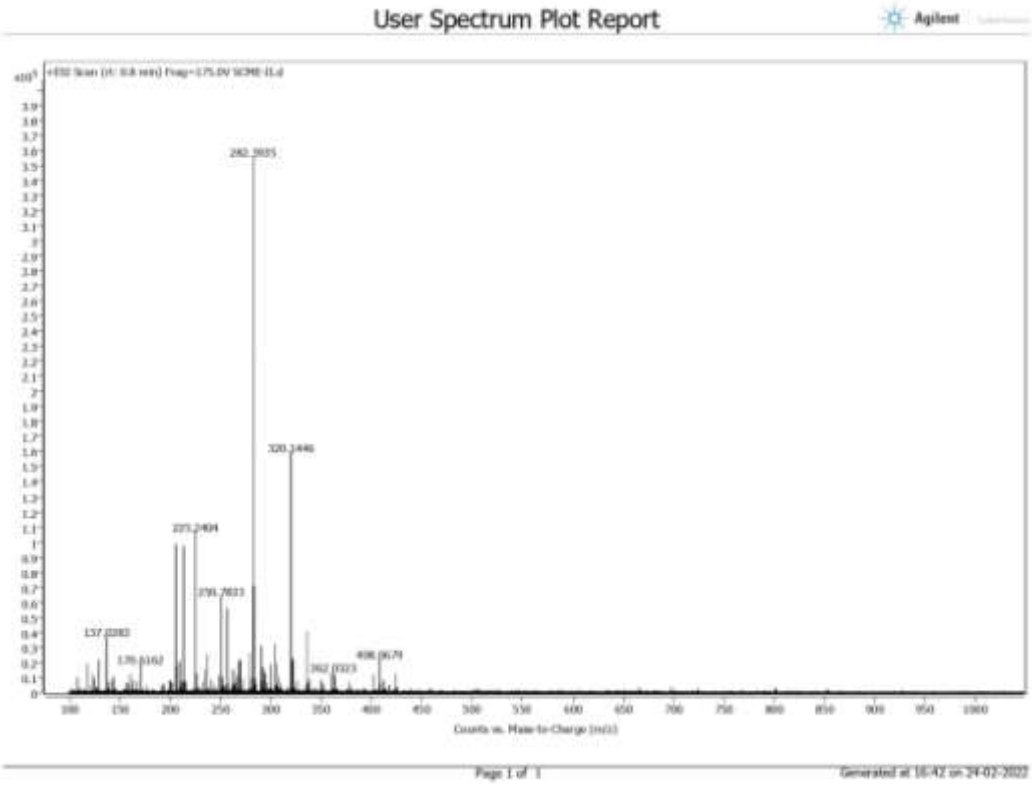

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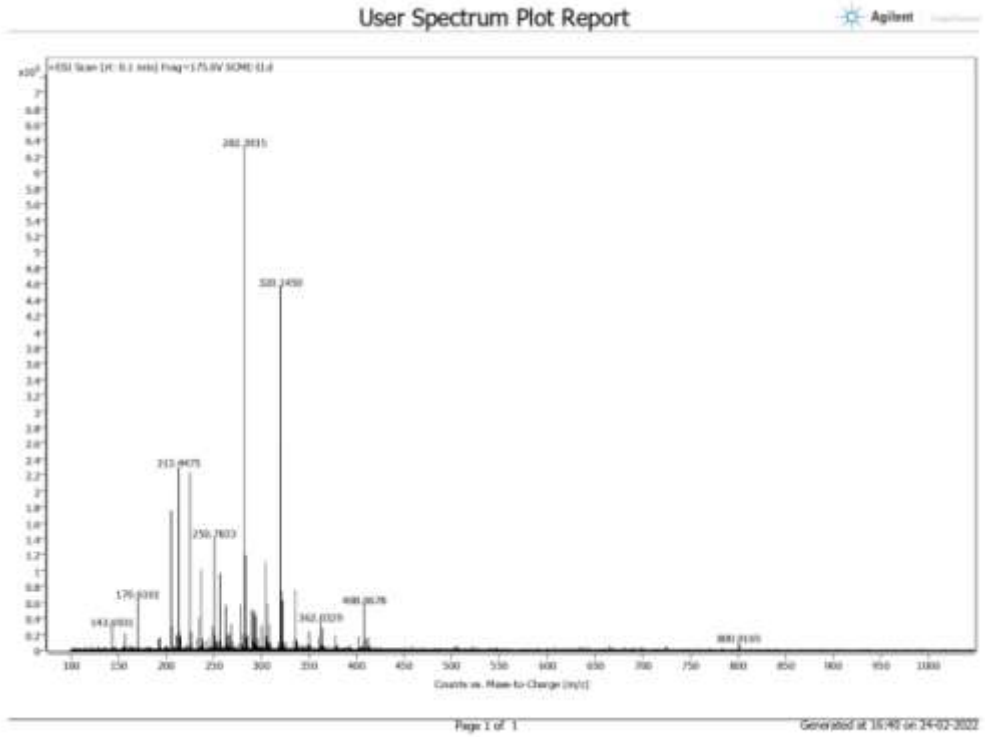

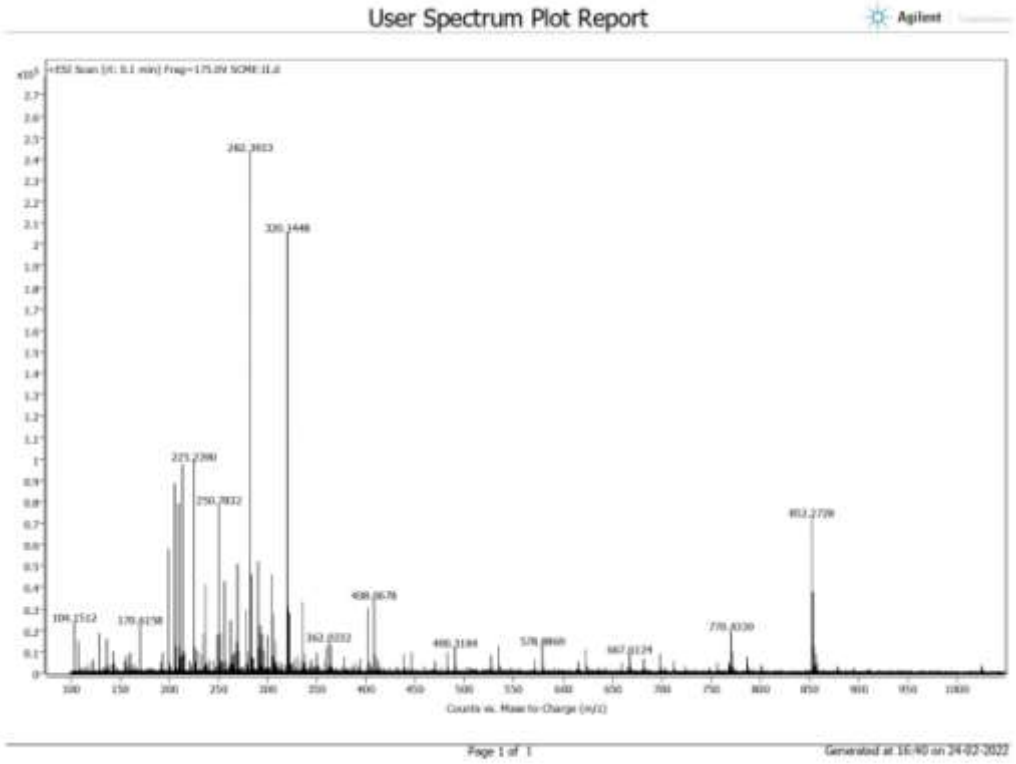

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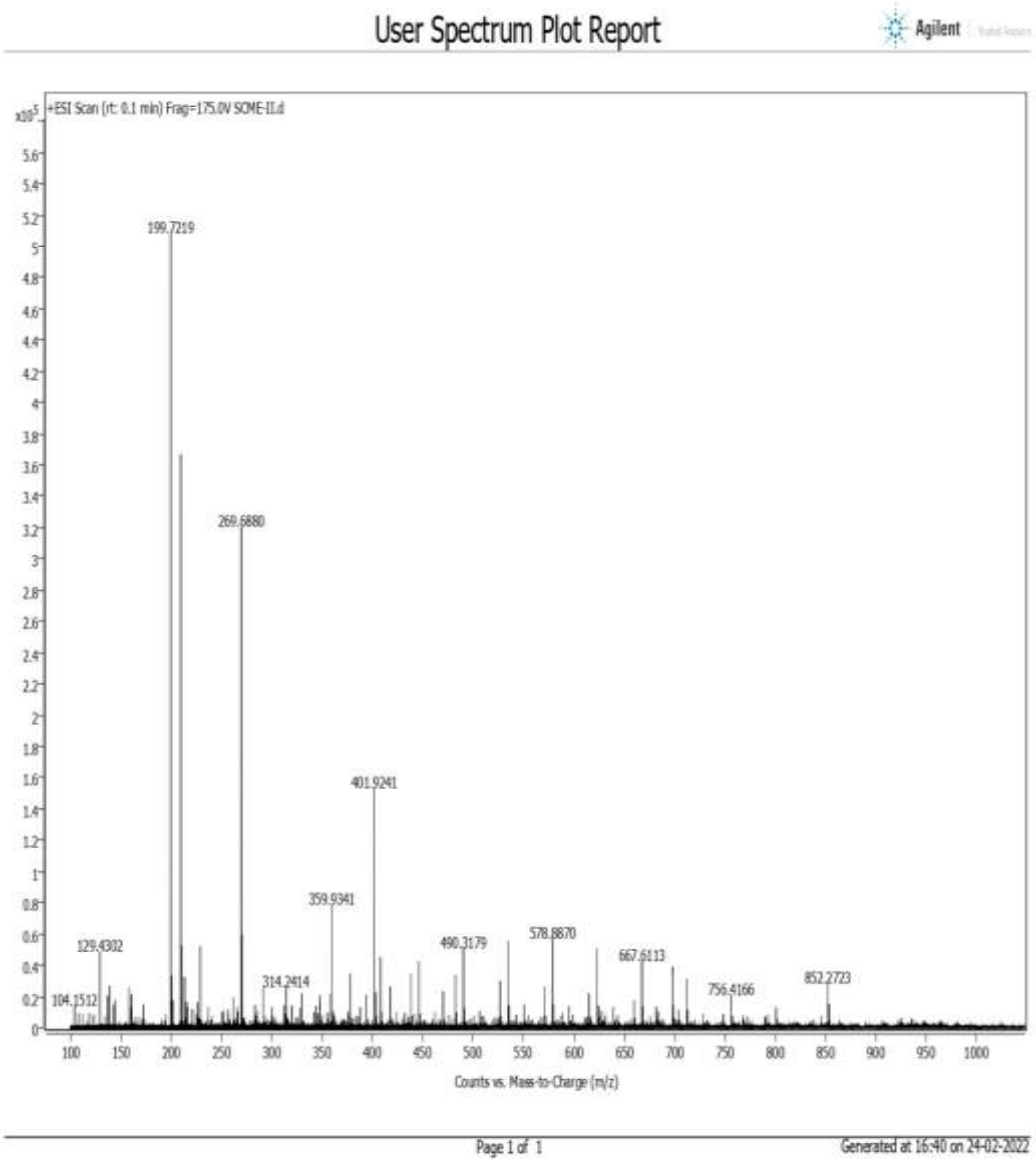

Compound A: SC Me I Rf 0.07 Rt 4.65 minutes UV max 262 nm FT IR data CM-1 2941, 2829 C-alkyl, 1450 C=C, 1114 C-O-C , 1020 Ar-H. Mass m/z 282, 320.

Compound B: SC Me I Rf 0.85. UV max 272 nm FT IR data CM-1 2947, 2835 C-alkyl, 1649 C=0 1452 C=C 1112 C-O-C , 1016 Ar-H. Mass m/z 320, 490, 667.

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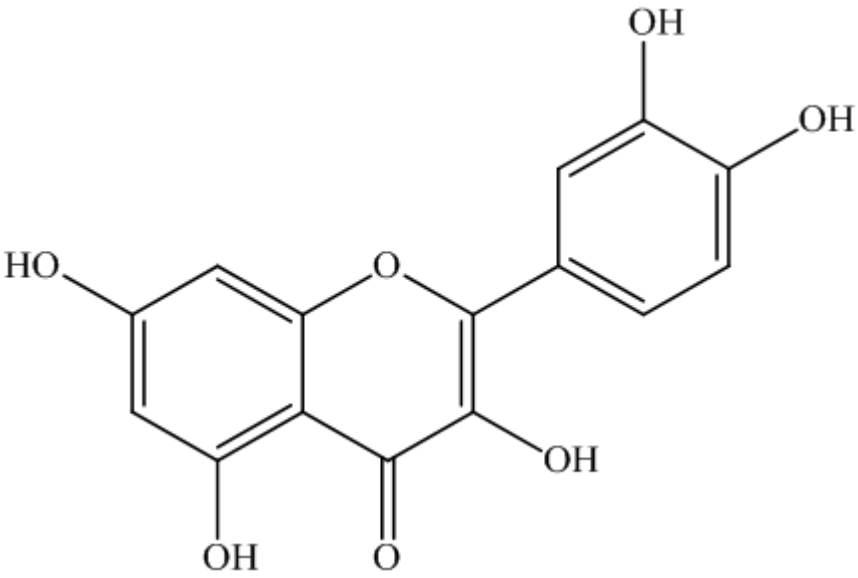

Figure 16: Compound A: Quercetin

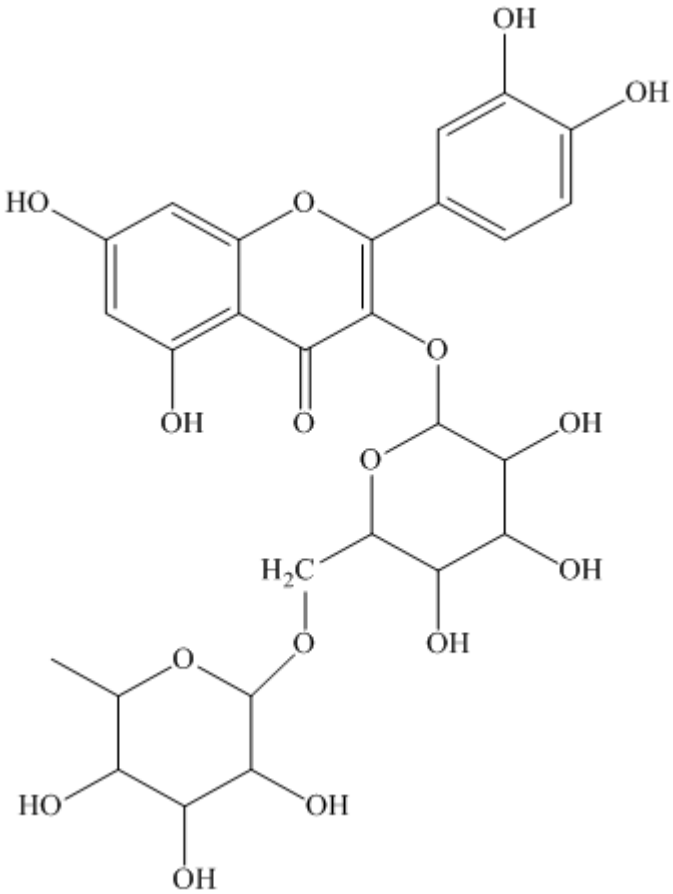

Figure 17: Compound B: Rutin

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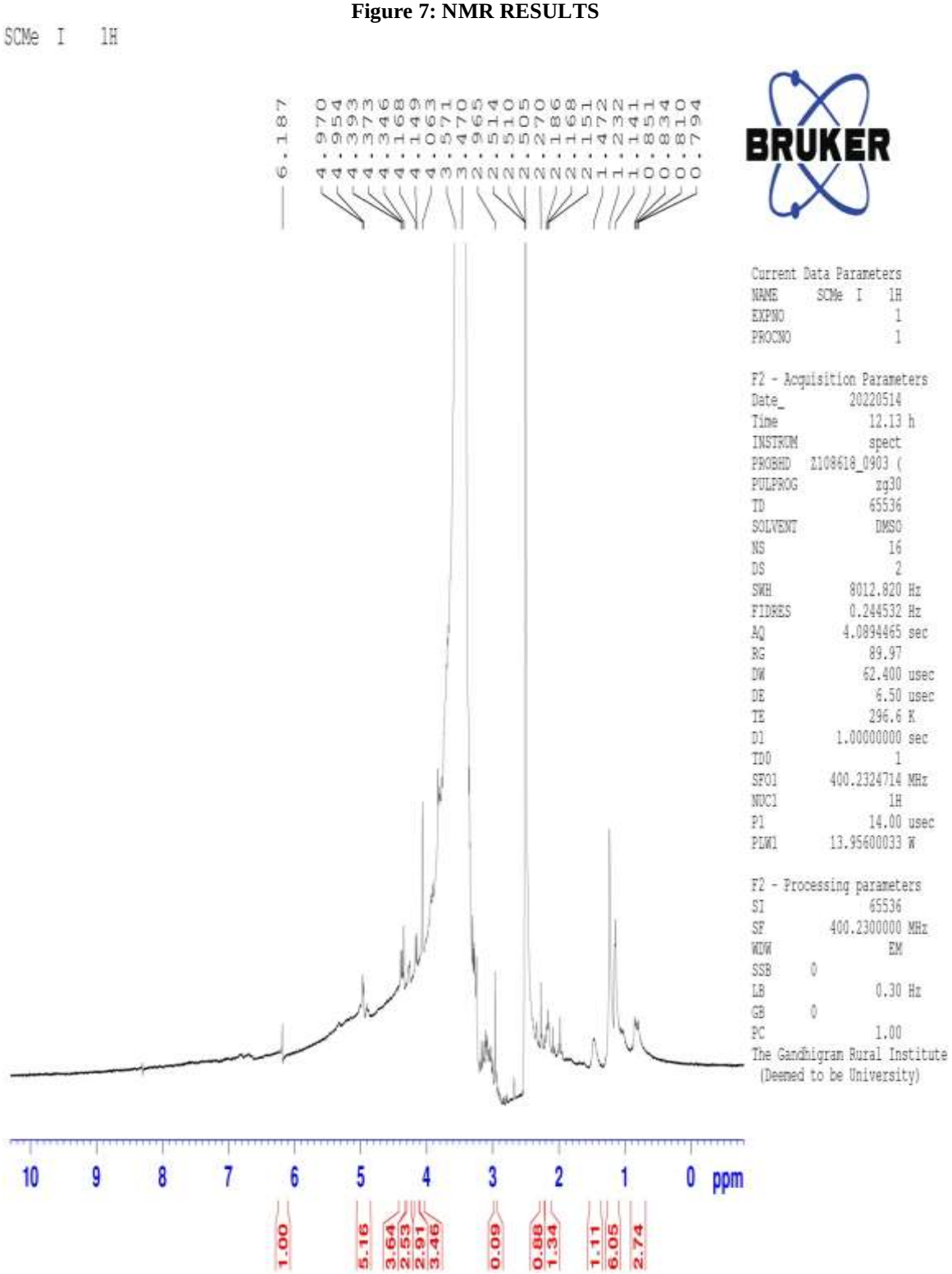
Figure 7: NMR RESULTS



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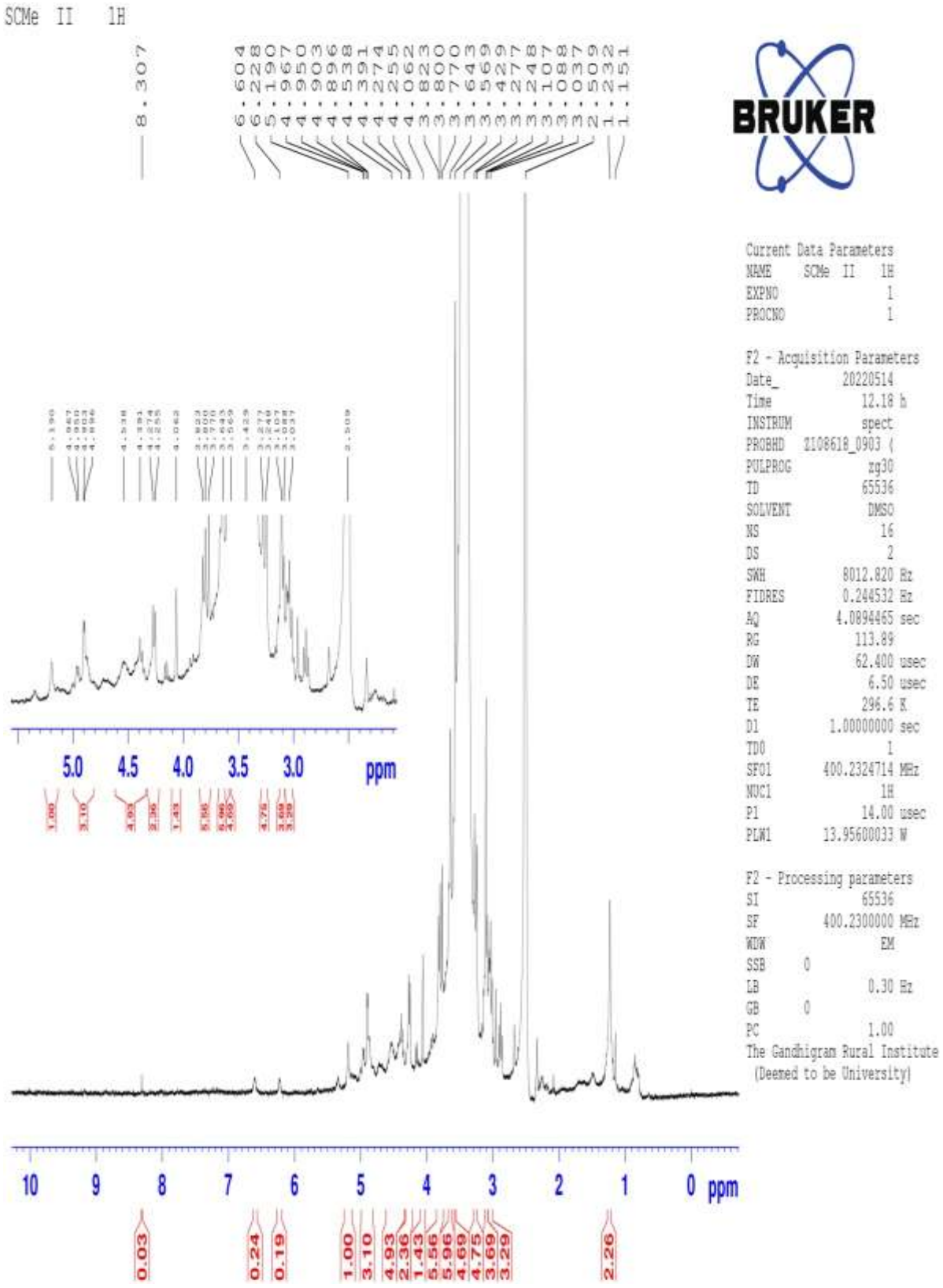

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III. DISCUSSION

The phytochemical analysis and spectral data of smilax china extract shown two key phytochemicals responsible for pharmacological actions. These two are Rutin and Quercetin.

IV. CONCLUSION

This study with multiple spectral analysis helped to identify the important phytochemicals present in smilax china rhizome (Rutin and Quercetin). Characteristics of the compound found in this work which are responsible for biological action of Smilax china rhizome. Further studies may require understanding other phytochemicals.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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