Comparative study of greener and traditional extraction for the identification of sustainable phytocompounds from Simarouba glauca leaves for health benefits

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Abstract. Emerging advancement in the discovery of novel phytochemical compounds from natural sources is a significant milestone in modern healthcare, aiding in the prevention and management of diseases for a healthy well being. One of the biggest issue currently experienced is the climatic change and its impact on human illnesses can be overcome by the sustainability of potential phytochemicals. Simarouba glauca DC is an evergreen flower plant belonging to the family Simaroubaceae. The leaf decoction of SG has been reported to have anticancer, antimalarial, antiviral, antibacterial, and antihelminthic properties in traditional medicine due to the presence of quassinoids, alkaloids, flavonoids, glycosides, phenolic compounds, saponins, and fixed oils. The current study compares the best extraction efficiency from greener extraction compared to conventional extraction. Methanolic and aqueous leaf extracts of Simarouba glauca were prepared by greener extraction as well as conventional extraction method using 23 full factorial designs in order to optimize the maximum extraction efficiency in both the methods. The study found that the Ultrasonic Assisted Extraction had the highest extraction responses for both methanol (16%w/w) and aqueous (23%w/w) phases. The Soxhlet method produced good extraction responses for both methanol (13%w/w) and water (22%w/w). LC-MS/MS analysis identified the potential phytochemicals such as flavonoids and phenolic compounds (Hesperetin, Kaempferol, Fisetin and Dicaffeoyl quinolactone) in SG aqueous leaf extract. Similarly potential phytochemicals such as flavonoids, alkaloid, terpenoids, steroids and carotenoids were identified from SG methanol leaf extract. Names of compounds are as follows: Rotenone, Silybin B, Oleuropein, Okaramine C, Adonixanthin, Ginsenoside Rh3, 5, 6-Dihydroxylutein, Bovoside A, Germine, Apigenin 6-C-glucoside 8-C-arabinoside and Isofucoxanthinol. In futuristic study, the biological response of the identified potential phytochemicals from Simarouba glauca needs to be assessed for health benefits.

Keywords: Greener extraction technique, climatic change, phytochemicals, LC-MS/MS analysis, Simarouba glauca.

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1 Introduction

The effects of climatic change are a concern for everyone, they are especially important in India, where a larger portion of the population is permanently vulnerable to natural disasters [1]. Plants create bioactive molecules known as phytochemicals to defend themselves. There are many different sources of phytochemicals present in whole grains, fruits, vegetables, nuts, leaf herbals and herbal products. To date, over a thousand phytochemicals have been identified for consumption like flavonoids, alkaloids, carotenoids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibres, and certain polysaccharides etc. In addition to having potent antioxidant properties, these phytochemicals have antiviral, antibacterial, anti-diarrheal, anthelmintic, and antiallergic properties. In recent studies, to identify the health benefits, potential phytochemicals are utilized to help in preventing and managing various ailments like diabetes, obesity, cancer, cardiovascular diseases, dermatitis, immunological disorders, neurological disorders and respiratory disorders etc. At the outset, a variety of disorders can be prevented and treated with natural sources of food supplements that have phytochemicals as functional foods or nutraceuticals [2].

Simarouba glauca DC (S. glauca, abbreviated as SG), commonly known as ‘Laxmitaru’ or ‘Paradise tree’ or dysentery bark is an eco-friendly tree which belongs to the family Simaroubaceae [3]. In traditional system of medicine, the leaf decoction of SG has been reported to exhibit anticancer, anti-malarial, antiviral, antimicrobial and anti-helminthic activities due to presence of quassinoids, alkaloids, flavonoids, glycosides, phenolic compounds, saponins and fixed oils [4]. The health benefits of these phytochemicals depend on their sustainability of the yield of extracted phytochemicals, their purity and quantity that is also dependent on the method of extraction, the solvent used, temperature, and the time of extraction [2]. The green extraction process is linked to the greenhouse effect to retain the sustainability and prevent the molecular degradation of health benefited potential phytochemicals from natural sources [5]. This novel idea has been brought forth to preserve the phytochemicals worldwide and extract phytochemical potential sustainable molecules from natural sources. Enhancing the extraction efficiency in addition to assessing the sustainability of phytochemicals obtained from Simarouba glauca leaves is the main objective of the research work.

2 Materials and Methods

2.1 Plant Material

Fresh healthy leaves of Simarouba glauca have been collected from the PSGCP Herbal Garden at the PSGIMSR & Hospital Campus in Peelamedu, Coimbatore, Tamil Nadu. Plant taxonomist, Dr. M. U. SHARIEF, Botanical Survey of India (BSI), TNAU Campus, Coimbatore - 03 has recognized and verified SG plant leaves (No.: BSI/SRC/5/23/2021/Tech./328).The collected SG leaves were cleansed with tap water, and then shade dried, ground in a Preethi Aries MG 216 mixer grinder (750 watt, green) for extraction purpose.

2.1 Apparatus

For extraction, a Superfit Vacuum Rotary Evaporator, a Microwave Instrument (LG Electronics Pvt Ltd, model no. MC 7688DP), a Digital Ultra Bath Sonicator with a digital timer and temperature controller (Labman Scientific Instruments), a Preethi Aries MG 216 mixer grinder (750 watt, green) and Acquity UPLC (Waters, USA) system coupled with Waters Xevo G2-XS Q–TOF-MS mass spectrometer (Waters, USA) were used. BEH C18 column (50 x 1.0, 1.7u) was used for the separation of chemical compounds.

2.1 Chemicals

Petroleum ether (40-60°C) & Chloroform LR grade (Loba Chemie PVT.LTD), Methanol LR grade (SD Fine Chemical, Mumbai), Distilled water, Ash less Whatman filter paper No.42, diameter 125mm, formic acid (ACS reagent, 96.0%), acetonitrile (analytical grade) (Merck KGaA, Darmstadt, Germany) and certain borosilicate glassware were used.

3 Experimental Design

3.1 Preparation of Simarouba glauca leaf extracts [6, 7, 8 and 9]

A full factorial design (2k) was used to investigate the effect of all the factors and their interactions in extraction efficiency. A common experimental design is one, where all input factors are set at two levels, high and low or + 1 and − 1, respectively and it would have eight runs. In order to maximize the estimated response (Y) in aqueous and methanol SG leaf extracts, a (23) factorial design (2-levels, 3-factors) was constructed, with three factors: time (A), temperature (B) and solvent type along with volume (C) as independent variables. Two levels: (1) and (+1) for low and high levels were selected. By focusing greater attention to the solvents employed in plant extraction, a higher percentage yield of extract was achieved. Therefore, it is recommended to stay away from utilizing poisonous, flammable, or environmentally hazardous solvents.

Potential phytocompounds were identified from SG leaves through LC-MS/MS Analysis. In this full factorial design, it would generate 8 experiments to run and the weight (25g) of sample (Simarouba glauca leaf) was kept constant during all the experiments. Efficiency of extraction was compared with the rapid recovery of extract yield (%) through modern methods of extraction (MAE & UAE) and conventional extraction techniques (Soxhlet & Cold maceration).

Fig. 1 Simarouba glauca DC leaves

3.1.1 MAE & UAE Extraction Methods [6]

To evaluate the influence of factors in MAE (Microwave assisted extraction), three independent variables, including irradiation duration from 1 and 10 min and extraction temperature (30ºC and 90ºC), were used to optimize extraction efficiency. Additionally, in UAE (Ultrasonic Assisted Extraction), a factorial design was created using three independent variables, including the usage of ultrasonic waves with contact time from 10 and 40 min and temperature range of 25°C and 45°C. To optimize the extraction efficiency of SG leaf extracts as effectively as possible, solvents such as methanol and water (50 and 250ml) were utilized.

3.1.2 Cold Maceration & Soxhlet Extraction Methods

In the traditional extraction method, the experimental design conditions were established with three independent variables, such as the cold maceration period, which ranged from 24 and 72 hours, and the soxhlet method [10], which needed 3 and 6 hours. To achieve the highest extraction efficiency, additional variables such as temperature (between 25ºC and 30ºC) and solvents (between 150 and 450 ml each of methanol and water) were utilized.

Before the extraction, shade-dried SG leaves were defatted with petroleum ether (40–60°C) before the extraction. Superfit vacuum rotary evaporators were used to concentrate the prepared SG leaf extracts (methanol and aqueous). After calculating the percentage yield, a phytochemical analysis (Kokate CK et al., 2007; Harborne., 1973) was carried out. Potential phytochemical was identified through LC-MS/MS analysis.

4 Phytochemical analysis of Simarouba glauca leaf extracts

The qualitative phytochemical tests were carried out for the prepared methanol and aqueous leaf extracts of Simarouba glauca to determine the presence of sterols, terpenoids, flavonoids,

phenols, proteins, alkaloids, glycosides, and tannins. Table 6 presents the results of the phytochemical analysis [11,12].

5 Chromatographic conditions

5.1 LC-MS/MS Analysis conditions [13]

LC-MS/MS phytochemical profiling was assessed for Simarouba glauca leaf extracts (methanol and aqueous) which was denoted as SGME and SGAE using Acquity UPLC Mass Spectrometer (Waters, USA). Compounds were separated by using BEH C18 column (50 x 1.0, 1.7u). Binary mobile phase system with solvent A: 0.1% formic acid in water and solvent B as acetonitrile with a gradient elution of 0-1 min (A: 98% & B:2%), 1–6 min (A:50% & B:50%), 12–16 min (A:5% & B:95%), 17–20 min (A: 98% & B: 2%) was used for the elution. The injection volume of the sample was set at 5µL with a flow rate of 0.2 mL/min. Compounds were detected at m/z value 50–80. Phytocompounds were identified in positive and negative ionization mode using the following instrument conditions; gas flow: 800L/Hr; Capillary Voltage: 3.0KV, Collision Energy: 20V, Ramp Collision Energy: 30-90V, Source Temp: 150oC, Desolation Temp: 450oC, Cone gas: 50L/Hr. LC particulars and MS spectrum for sample A, sample B and sample C were showed in Table 14, 15 & Figure 8, 9: The phytocompounds were identified primarily based on the matching of Mass data against MSBNK-RIKEN data base.

5.2 Statistical analysis [14]

Analysis of Variance (ANOVA) was used to evaluate the effect of independent variables on the response. The experimental design and data analysis were carried out using Design Expert trial version 13.

6 Results

6.1 Preparation of extract

Simarouba glauca leaf extract (methanol and aqueous) were prepared by greener extraction technique as well as conventional extraction method using 23 full factorial designs in order to optimize the maximum extraction efficiency in all the methods.

The study found that the UAE had the highest extraction responses for both methanol (16%w/w) and aqueous (23%w/w). However, the Soxhlet method produced good extraction responses for both methanol (13%w/w) and water (22%w/w). The observed outcomes were displayed in Figure 2 and Table 1. The extracted materials were subjected to both phytochemical and LC-MS/MS analysis in order to identify the potential phytocompounds present in the SG leaf extract.

Table 1 Percentage yield of *Simarouba glauca* leaf extracts

Extraction Method	% Yield w/w Methanol extract	% Yield w/w Aqueous extract
MAE	11.28	12.15
UAE	16	23

Cold Maceration	11.5	15.6
Soxhlet	13	22

***** *MAE-Microwave Assisted Extraction, *UAE-Ultrasonic Assisted Extraction*

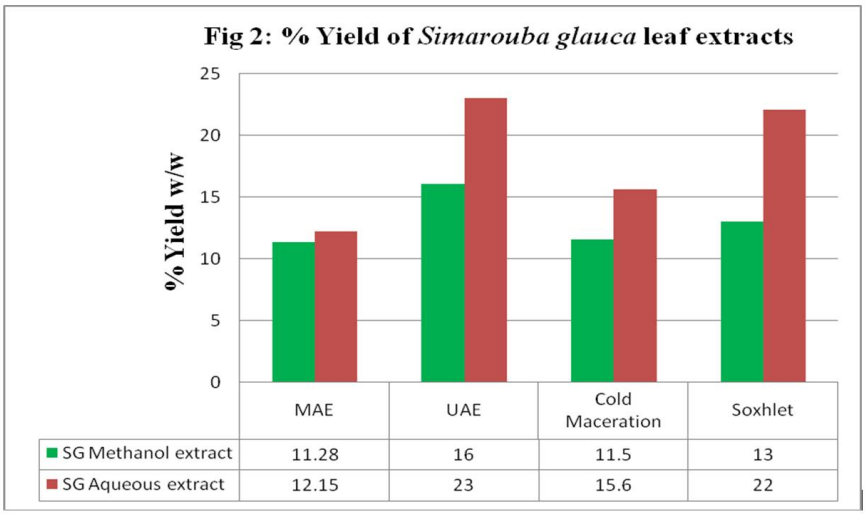


Fig 2. % Yield of *Simarouba glauca* leaf extracts

6.2 Optimization of experimental design

6.2.1 Optimization in Microwave assisted extraction [6]

The summary of both the classic and modern extraction type outcomes has been furnished in tables 2, 5, 8, and 11. In 2^3 full factorial designs, the responses were found to be 11.28% and 12.15%w/w for methanol and aqueous in MAE (Microwave assisted extraction) at 10 minutes of irradiation time with 250ml solvents used, respectively, time (A) and solvent (C) was involved in MAE to optimize the maximum extraction (% yield of SG leaf extracts). The results showed that temperature had little or no effect on the extraction efficiency of phytocompounds. All the extraction responses and the coded components were shown in Table 2

Table 2: 2^3 factorial designs with corresponding response in MAE of SG leaf extract

Std	Run	Factor 1 A:Time min	Factor 2 B:Temperature °C	Factor 3 C:Solvents ml	Response 1 % Yield (Methanol) (%w/w)	Response 1 % Yield (Aqueous) (%w/w)
4	1	10	90	50	1.88	2.91
5	2	1	30	250	1.69	2.72
6	3	10	30	250	11.28	12.15
3	4	1	90	50	1.58	2.64

8	5	10	90	250	9.96	10.12
7	6	1	90	250	2.56	3.68
2	7	10	30	50	0.86	1.92
1	8	1	30	50	1.25	2.25

In the full factorial model in the MAE, analysis of variance (ANOVA) results were calculated and displayed in Table 3 and 4. P - Values ≤ 0.0500 in this model showed that the variable's irradiation time (A) and solvent type (C) were more significant than temperature (B). Coefficient of determination R^2 values for methanol and aqueous SG leaf extract was recorded as 0.9861 and 0.9723 which is desirably high (close to 1).

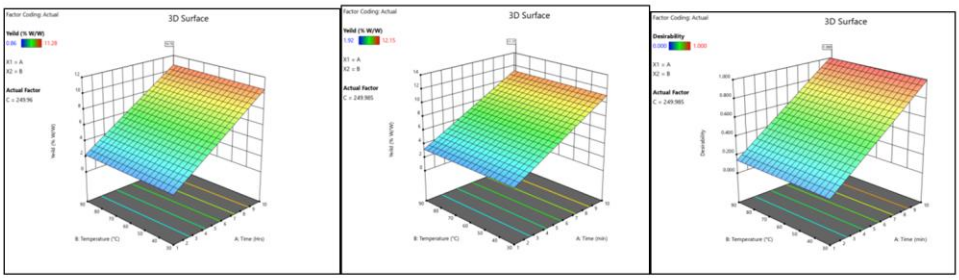
Table 3: Analysis of variance (ANOVA) in MAE (Methanol SG leaf extract)

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	121.87	4	30.47	53.05	0.0041	**Significant**
A-Time	35.70	1	35.70	62.16	0.0043	
B-Temperature	0.1012	1	0.1012	0.1763	0.7028	
C-Solvent	49.60	1	49.60	86.36	0.0026	
AC	36.47	1	36.47	63.49	0.0041	
Residual	1.72	3	0.5743			
Cor Total	123.59	7				

Table 4: Analysis of variance (ANOVA) in MAE (Aqueous SG leaf extract)

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	107.86	4	26.97	26.31	0.0113	**Significant**
A-Time	31.24	1	31.24	30.48	0.0117	
B-Temperature	0.0120	1	0.0120	0.0117	0.9206	
C-Solvent	44.89	1	44.89	43.79	0.0070	
AC	31.72	1	31.72	30.94	0.0115	
Residual	3.08	3	1.03			
Cor Total	110.94	7				

Figures 3a, 3b and 3c illustrate the 3D surface response for the % yield of the methanol, aqueous SG leaf extracts (%w/w) and desirability was obtained between irradiation time and the type of solvents that were fitted to optimize the best extraction efficiency seen in MAE.




```3a (Methanol SG leaf extract)  3b (Aqueous SG leaf extract)  3c (Desirability)

**Fig. 3.** 3D Surface Response graph for the % yield (%w/w) and Desirability (P-values ≤ 0.05) in MAE

### 6.2.2 Optimization in Ultrasonic Assisted Extraction [6]

In order to determine the greatest extraction (%) yield of SG leaf extracts, a $2^3$-factorial design was created. The coding factors and experimental runs of the maximal responses in UAE were determined to be 16% and 23% w/w for 40 minutes of ultrasonic wave contact length (A), 45°C temperature (B), and 250ml solvents (C) respectively. These results are showed in Table 5.

The ANOVA findings are shown in Tables 6 and 7. P-values of less than 0.0500 demonstrated the significance of the chosen A & B values for both water and methanol. ABC was significant for P values 0.1 in methanol, despite the fact that it was only significant for aqueous solutions with P-values less than 0.0500.

**Table 5**: $2^3$ Factorial design conditions with corresponding response in UAE of *Simarouba glauca* leaf

| Std | Run | Factor 2 A:Time (min) | Factor 1 B:Temperature (°C) | Factor 3 C:Solvent (ml) | Response 1 Yield (Methanol) % w/w | Response 1 Yield (Aqueous) % w/w |
|---|---|---|---|---|---|---|
| **6** | 1 | 10 | 45 | 250 | 1.5 | 2.5 |
| **2** | 2 | 10 | 45 | 50 | 1 | 2 |
| **8** | 3 | 40 | 45 | 250 | 16 | 23 |
| **3** | 4 | 40 | 25 | 50 | 2 | 1.5 |
| **5** | 5 | 10 | 25 | 250 | 14 | 20 |
| **1** | 6 | 10 | 25 | 50 | 4.5 | 7.5 |
| **4** | 7 | 40 | 45 | 50 | 4 | 5 |
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7	8	40	25	250	8	4

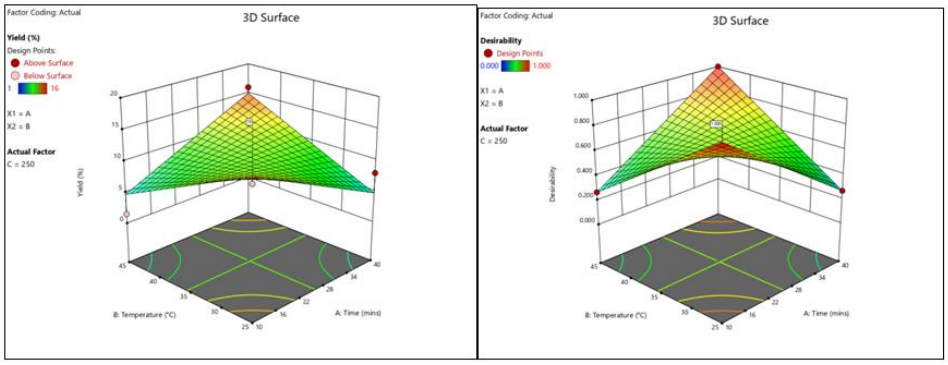
Table 6: Analysis of variance (ANOVA) in UAE (Methanol SG leaf extract)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	210.63	3	70.21	11.82	0.0186	**Significant**
C-Solvent	98.00	1	98.00	16.51	0.0153	
AB	84.50	1	84.50	14.23	0.0196	
ABC	28.13	1	28.13	4.74	0.0951	
Residual	23.75	4	5.94			
Cor Total	234.38	7				

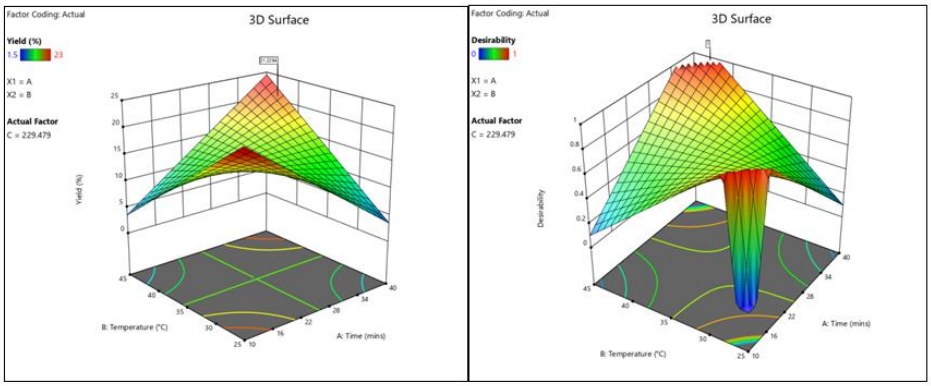
Table 7: Analysis of variance (ANOVA) in UAE (Aqueous SG leaf extract)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	493.59	3	164.53	74.15	0.0006	significant
C-Solvent	140.28	1	140.28	63.23	0.0014	
AB	336.20	1	336.20	151.53	0.0003	
ABC	94.53	1	94.53	42.61	0.0028	
Residual	8.88	4	2.22			
Cor Total	502.47	7				

The 3D surface response and cube diagram for the % yield of the methanol, aqueous SG leaf extracts (%w/w), and desirability were fitted to optimize the best extraction efficiency observed in UAE, as shown in Figures (4a, 4b, 4c, and 4d) and (5a,5b,5c and 5d). R2 values were recorded for the SG leaf extract in water (R2=0.9823) and in methanol (R2=0.8987).

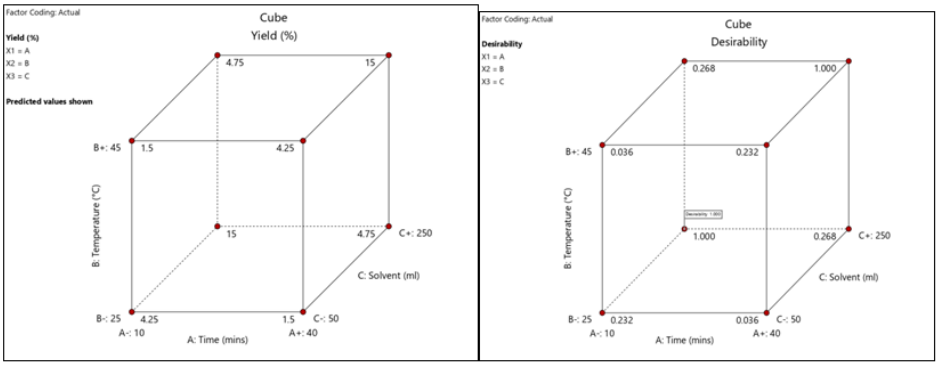


4a (Methanol SG leaf extract) **4b (Desirability-Methanol)**

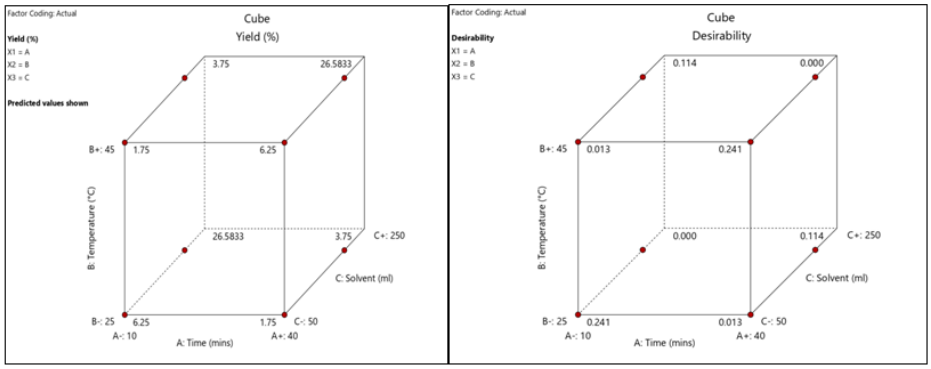


4c (Aqueous SG leaf extract) **d (Desirability-Aqueous)**

Fig.4 3D Surface Response graph for the % yield (%w/w) and Desirability in UAE (P-values ≤ 0.0500)



5a (Methanol SG leaf extract) **5b (Desirability-Methanol)**



5c (Aqueous SG leaf extract) **5d (Desirability-Aqueous)**

Fig. 5 Figure 5a and 5b: Cube diagram for the % yield in %w/w and Desirability in UAE (P values 0.1), 5c and 5d: Cube diagram for the % yield (%w/w) and Desirability in UAE (P-values ≤ 0.05)

6.2.3 Optimization in Cold Maceration Method [10]

In cold maceration, maximum extraction responses were optimized in this model for SG leaf extract at 15.6 % (aqueous) and 11.5% (methanol) at 72 hours (A) time with 450ml of solvent (C) at normal room temperature (25ºC) in the cold maceration method. Table 8 expressed all the extraction responses as well as the coded factors.

Table 8: 2^3 Factorial designs with corresponding response in Cold Maceration of *Simarouba glauca* leaf

Std	Run	Factor 2 A:Time Hrs	Factor 1 B:Temperature ºC	Factor 3 C:Solvent ml	Response 1 Yield (Methanol) % w/w	Response 1 Yield (Aqueous) % w/w
6	1	24	30	450	4.3	6
5	2	24	25	450	4.2	6
4	3	72	30	150	4.2	7.5

8	4	72	30	450	12.8	18
2	5	24	30	150	1.5	2.5
7	6	72	25	450	11.5	15.6
1	7	24	25	150	3	5.3
3	8	72	25	150	6	5.8

P values less than 0.0500 in the present case suggested that the selected model AC was significant for methanol, as shown in Table 9. In contrast, P value 0.1 with AB in aqueous was significant, as shown in Table 10. For methanol and water, the coefficient of determination R2 values was found to be 0.9688 and 0.9096, respectively. A 3D surface response graph for the percentage yield and desirability during cold maceration was shown in Figures 6a, 6b, 6c, and 6d.

Table 9: Analysis of variance (ANOVA) in Cold maceration (Methanol SG leaf extract)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	111.48	3	37.16	41.35	0.0018	**Significant**
A-Time	57.78	1	57.78	64.29	0.0013	
C-Solvent	40.95	1	40.95	45.56	0.0025	
AC	12.75	1	12.75	14.19	0.0197	
Residual	3.60	4	0.8988			
Cor Total	115.08	7				

Table 10: Analysis of variance (ANOVA) in Cold maceration (Aqueous SG leaf extract)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	203.98	3	67.99	13.42	0.0148	**Significant**
A-Time	46.56	1	46.56	9.19	0.0387	
C-Solvent	130.41	1	130.41	25.74	0.0071	
AB	27.01	1	27.01	5.33	0.0821	
Residual	20.26	4	5.07			
Cor Total	224.25	7				

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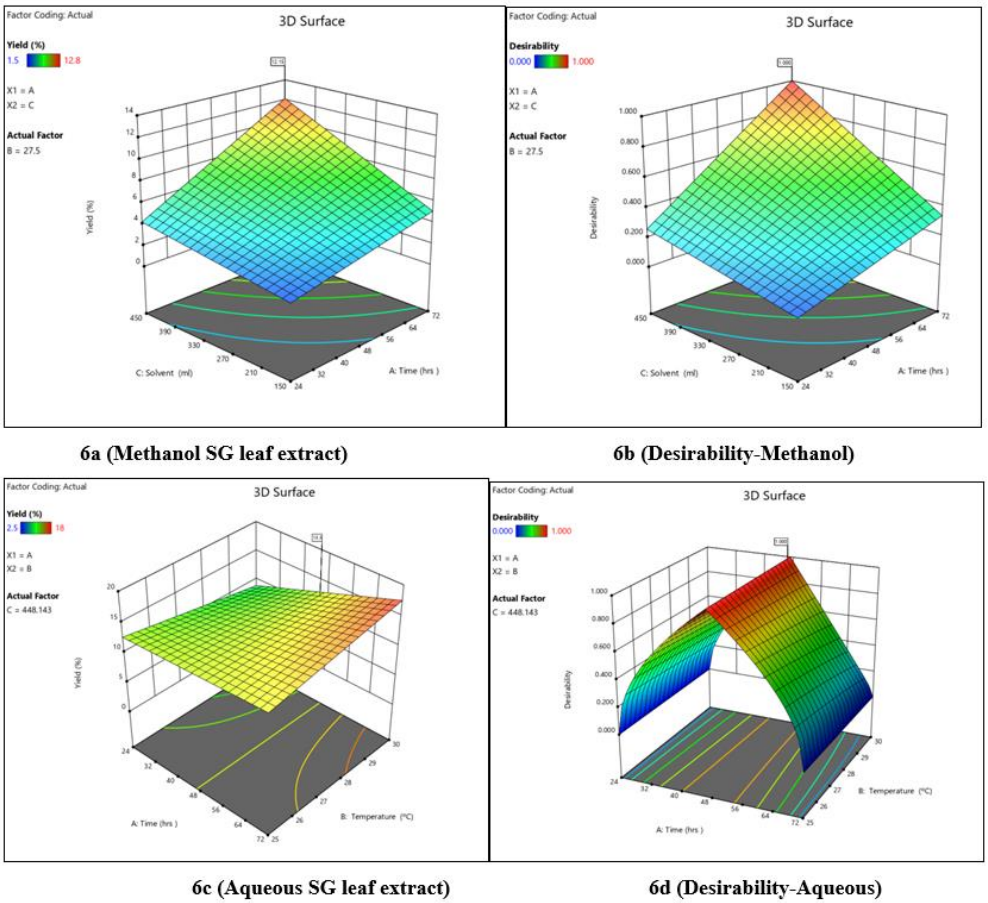


6a (Methanol SG leaf extract) **6b (Desirability-Methanol)**

6c (Aqueous SG leaf extract) **6d (Desirability-Aqueous)**

Fig 6. Figure 6a and 6b: 3D Surface Response graph for the % yield (%w/w) and Desirability in Cold maceration (P-values ≤ 0.0500), Figure 6c and 6d: 3D Surface Response graph for the % yield (%w/w) and Desirability in Cold maceration (P value 0.1)

6.2.4 Optimization in Soxhlet Extraction Method [6]

The maximum extraction responses of SG leaf extract were optimized using the Soxhlet method over a period of 6 hours (A) and 450 ml of solvent (C) at 30°C temperature for aqueous and methanol (22% w/w and 13% w/w) respectively. Table 11 displays all the extraction responses along with the coded variables.

Table 11: 2^3 Factorial designs with corresponding responses in Soxhlet method for *Simarouba glauca* leaf extract

Std	Run	Factor 1 A:Time Hrs	Factor 2 B:Temperature °C	Factor 3 C:Solvent ml	Response 1 Yield (Methanol) % w/w	Response 1 Yield (Aqueous) % w/w
2	1	6	10	150	8	12
8	2	6	30	450	13	22
7	3	3	30	450	6	11
6	4	6	10	450	12	20
1	5	3	10	150	4	6
3	6	3	30	150	2	4
5	7	3	10	450	9	10
4	8	6	30	150	4	7

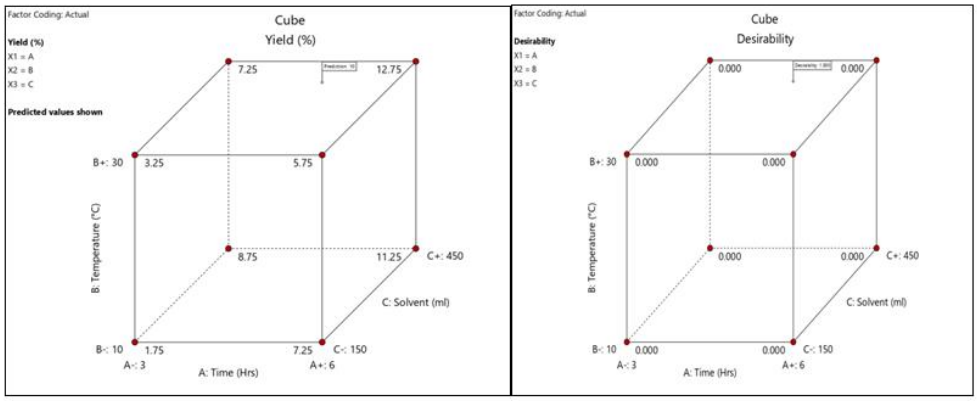
Factorial design, ANONA was computed using the Soxhlet method; The significant P-values less than 0.500 suggested that ABC was primarily responsible for methanol's ability to elicit the greatest number of responses, as shown in Table 13. On the other hand, for aqueous, AC is significant at P values ≤ 0.05 as shown in Table 14. The results showed that the R2 values for methanol and aqueous types were found to be (0.8858 and 0.9418) respectively. A Cube diagram and a 3D Surface Response graph for the yield (%w/w) and desirability of SG leaf extract was made using the Soxhlet method. These are shown in Figures 7a, 7b, 7c, and 8d.

Table 12: Analysis of variance (ANOVA) in Soxhlet method (Methanol SG leaf extract)

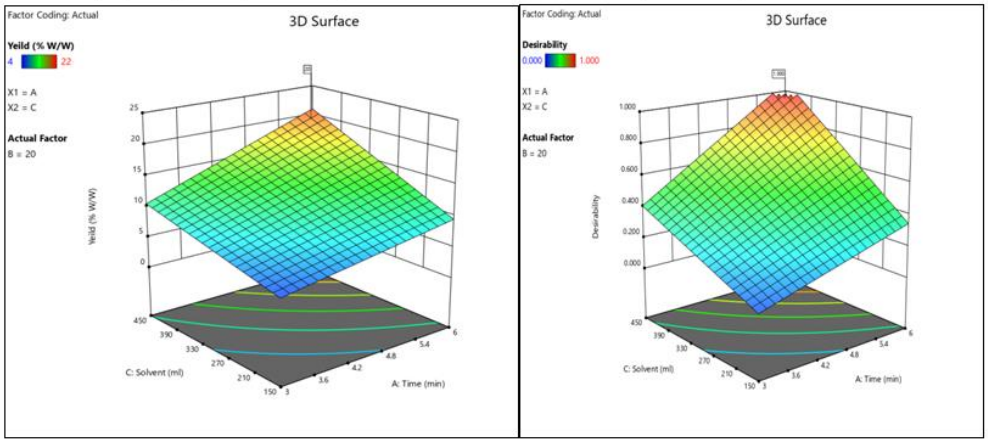
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	97.00	3	32.33	10.35	0.0235	**Significant**
A-Time	32.00	1	32.00	10.24	0.0329	
C-Solvent	60.50	1	60.50	19.36	0.0117	
ABC	4.50	1	4.50	1.44	0.2964	
Residual	12.50	4	3.13			
Cor Total	109.50	7				

Table 13: Analysis of variance (ANOVA) in Soxhlet method (Aqueous SG leaf extract)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	275.00	3	91.67	21.57	0.0062	**Significant**
A-Time	112.50	1	112.50	26.47	0.0068	
C-Solvent	144.50	1	144.50	34.00	0.0043	
AC	18.00	1	18.00	4.24	0.1087	
Residual	17.00	4	4.25			
Cor Total	292.00	7				



7a (% yield (%w/w)-Methanol) **7b (Desirability-Methanol)**



7c (% yield (%w/w)-Aqueous) **7d (Desirability -Aqueous)**

Fig.7 Figure 7a and 7b: Cube diagram for the % yield (%w/w) and Desirability in Soxhlet method (P value ≤ 0.5 significant for Methanol SG leaf extract), Figure 7c and 7d: 3D Surface Response graph for the % yield (%w/w) and Desirability in Soxhlet method (P value ≤ 0.05 significant for Aqueous)

7 Phytochemical analysis

The qualitative phytochemical analysis of various Simarouba glauca DC. Leaf extracts showed the presence of flavonoids, phenols, alkaloids, glycosides, saponins, terpenoids, tannins, steroids, and proteins. However, alkaloids, terpenoids, and steroids were absent in the aqueous extract of SG. The saponins and carbohydrates were absent in the methanol extract. The qualitative phytochemical results were reported in Table 13.

Table 13: Phytochemical analysis of various leaf extracts of *Simarouba glauca*

S.no	Phytochemicals	SG Methanol extract	SG Aqueous extract
1	Alkaloids	+	-
2	Glycosides	+	+
3	Flavonoids	+	+
4	Phenols	+	+
5	Terpenoids	+	-
6	Steroids	+	-
7	Saponins	-	+
8	Carbohydrates	-	+
9	Tannins	+	+
10	Proteins	+	+

Presence (+); Absence (-)

8 LC-MS/MS Analysis

Prepared *Simarouba glauca* leaf extracts both methanol and aqueous which was denoted as SGME & SGAE were subjected to LC-MS/MS analysis to identify the possible phytocompound responsible for anticancer activity. The MS spectra of all the peaks of the identified phytocompounds through LC-MS analysis were showed in Figure 8 and represented in Table 14. The SGME peak with a retention time between 5-30 min had high intensity was annotated for m/z in positive mode. These m/z values were compared with the reference m/z values of the known phytochemical compounds, and 11 compounds were tentatively identified in positive ion mode. The chemical structures of all the 11 compounds from SGME were represented in Figure 8. The list of all phytocompounds of SGME with their m/z values along with the retention time and other data is represented in Table 14. Four potential phytocompounds were identified from SGAE and results observed m/z particulars and an MS spectrum is showed in Table 15 and Figure 9

From this study. Phytochemicals such as flavonoids and phenolic compounds (Hesperetin, Kaempferol, Fisetin and Dicaffeoyl quinolactone) were identified in SG aqueous leaf extract. Similarly potential phytochemicals such as flavonoids, alkaloid, terpenoids, steroids and carotinoids were identified from SG methanol leaf extract (Rotenone, Silybin B, Oleuropein, Okaramine C, Adonixanthin, Ginsenoside Rh3, 5, 6-Dihydroxylutein, Bovoside A, Germine, Apigenin 6-C-glucoside 8-C-arabinoside and Isofucoxanthinol).

Table 14: LC-MS/MS Analysis for *Simarouba glauca* methanol leaf extract (SGME)

S. No	RT (min)	Compound Name	Chemical Compound Class	Chemical Formula	Measured mass m/z	Exact mass m/z	Fragments (m/z)	Reference
1	6.305	Rotenone	Flavonoids	$C_{23}H_{22}O_6$	395.21	394.14	107.1043, 109.1196, 133.1192, 119.1044, 135.1387, 137.1159, 176.1640, 211.1873, 220.1891, 373.2343, 287.0694, 221.1924	Heinz., 2016
2	9.739	Silybin B	Flavonoids	$C_{25}H_{22}O_{10}$	483.3147	482.12130	484.3186, 485.3281, 501.3242, 544.3315, 543.3293, 465.3035, 439.2903, 393.2497, 307.2183	Markus Kohlhoff., 2020
3	10.851	Oleuropein	Terpenoids	$C_{25}H_{32}O_{13}$	541.3132	540.526	293.2049, 331.1791, 349.1911, 365.2587, 391.2352, 409.2459, 445.2817, 463.2881, 541.3132, 482.2984, 542.3184	Nogawa., 2018
4	11.204	Okaramine C	Indole alkaloid	$C_{32}H_{36}N_4O_3$	525.3193	524.27874	421.2821, 422.2825, 447.2965, 465.3035, 466.3062, 481.2921, 526.3235	Nakayasu., 2021
5	11.406	Adonixanthin	Carotenoid	$C_{40}H_{54}O_3$	583.3603	582.40730	395.2654, 421.2821, 439.2903, 483.3147, 485.3281, 486.3340	Akimoto., 2016
6	11.911	Ginsenoside Rh3	Triterpenoids	$C_{36}H_{60}O_7$	605.3436	604.869	393.2497, 397.2862, 465.3079, 605.3436,	Tsugawa., 2019

7	12.871	5,6-Dihydroxylutein	Flavonoids	$C_{40}H_{58}O_4$	603.3210	602.43351	484.3186, 499.3100, 603.3210, 606.3410 293.2049, 331.1791, 391.2352, 409.2459, 463.2881, 481.2966, 603.3210, 482.2984, 531.2700, 1205.5939, 604.3268, 1085.5803, 1206.5961, 1207.6060	Sanae Kishimoto., 2004
8	13.729	Bovoside A	Steroids	$C_{31}H_{44}O_9$	561.2888	560.6909	397.2780, 449.3072, 457.2507, 467.3188, 468.3237, 483.3057 501.2739, 503.2737, 504.2797, 563.2883	Nogawa., 2018
9	14.235	Apigenin 6-C-glucoside 8-C-arabinoside	Flavone C,C-glycosides	$C_{26}H_{28}O_{14}$	565.3450	564.496	393.2497, 421.2779, 422.2825, 447.2878, 465.2990, 466.3062, 566.3478	Tsugawa., 2019
10	15.245	Germine	Alkaloids	$C_{27}H_{43}NO_8$	510.3555	509.6457	379.2685, 397.2780, 451.3224, 469.3340, 470.3366, 511.3593	Nogawa., 2018
11	17.366	Isofucoxanthinol	Natural pigment	$C_{40}H_{56}O_5$	617.3875	616.41277	102.1473, 139.1315, 135.1363, 149.1505, 201.1814, 223.1876, 263.2160, 264.2198 301.2638,	Akimoto., 2016

421.3493,
439.3589,
457.3688,
499.3740,
505.3098,
559.3897,
577.4041,
618.3948

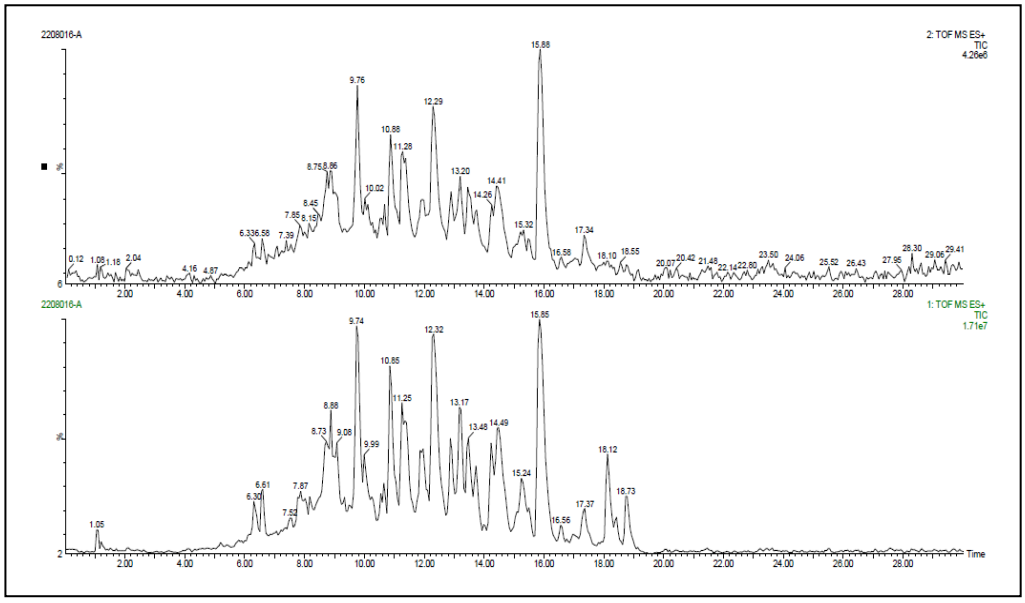
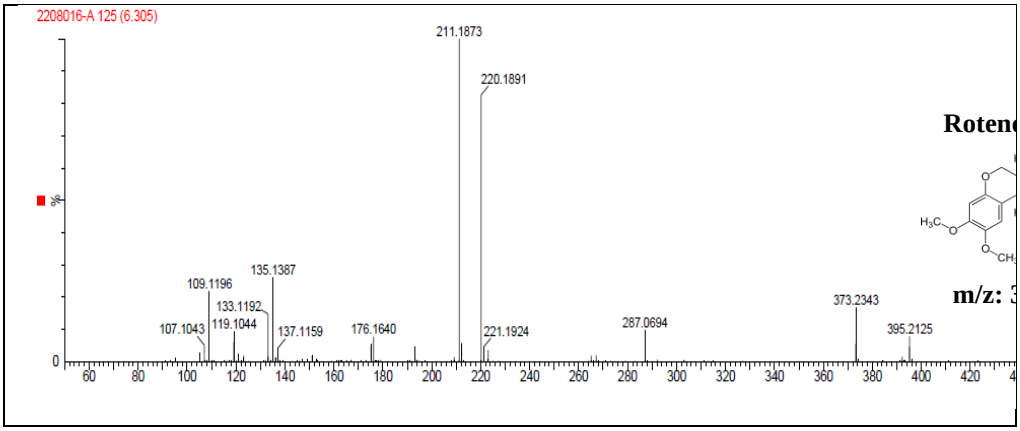
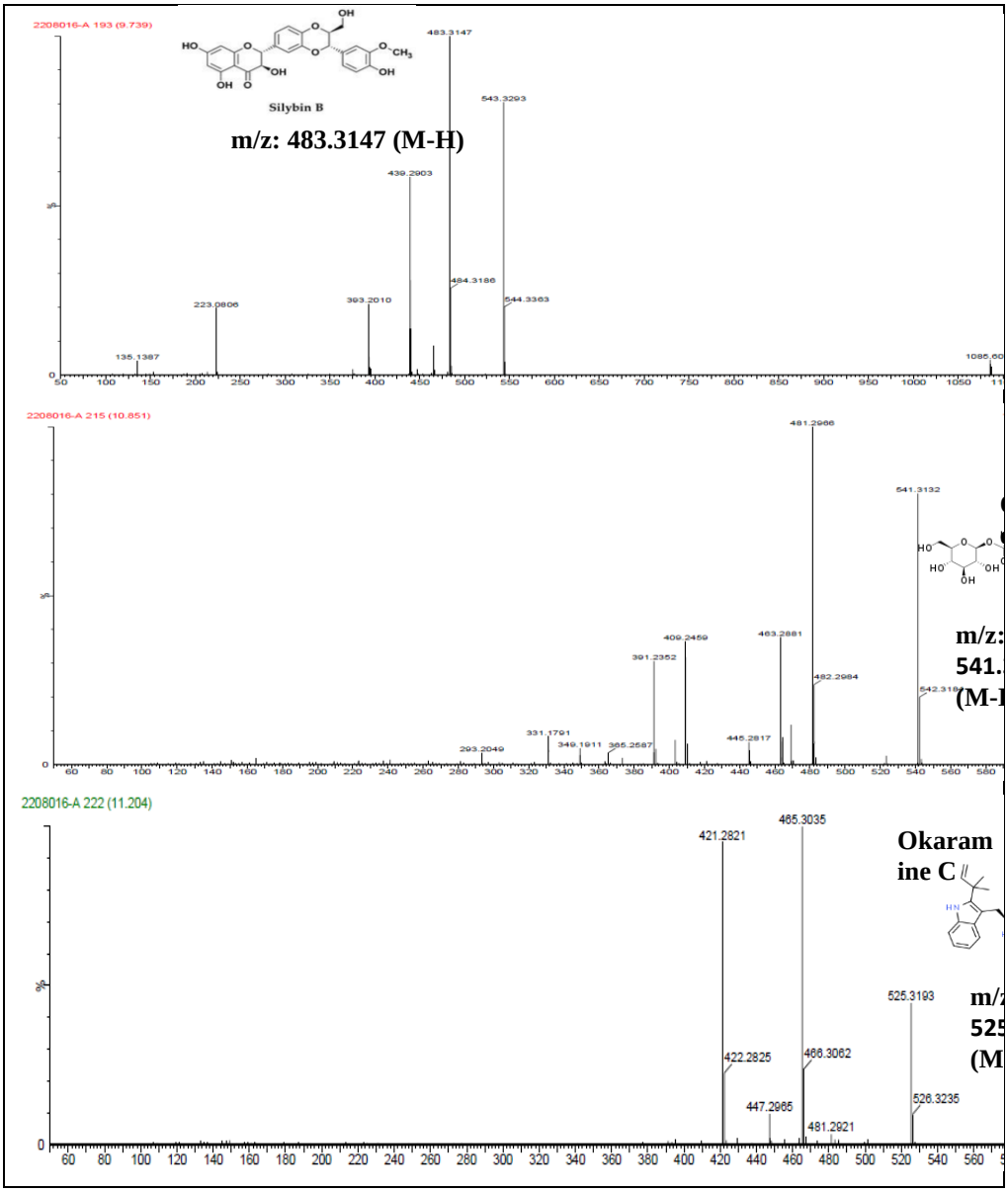
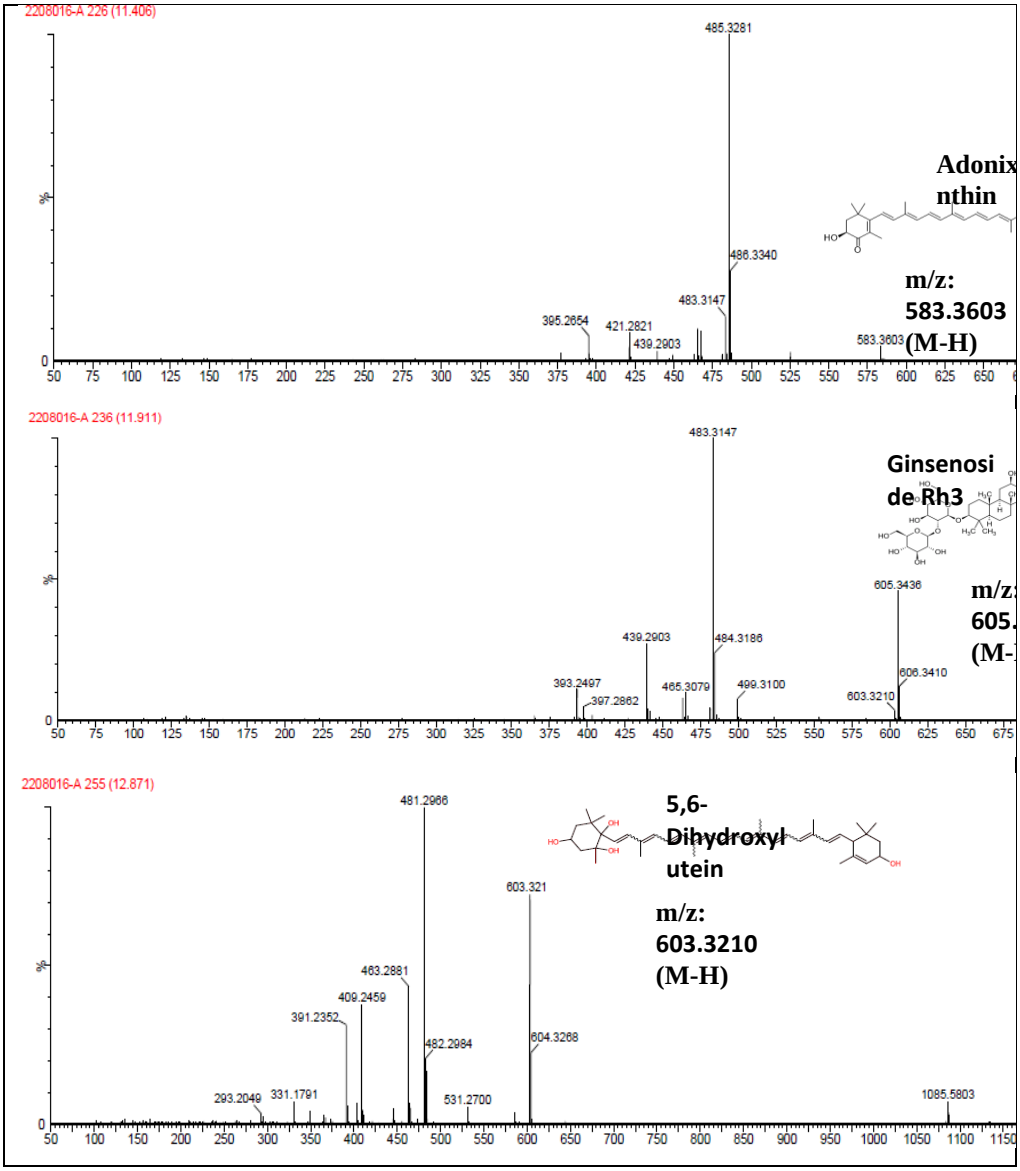
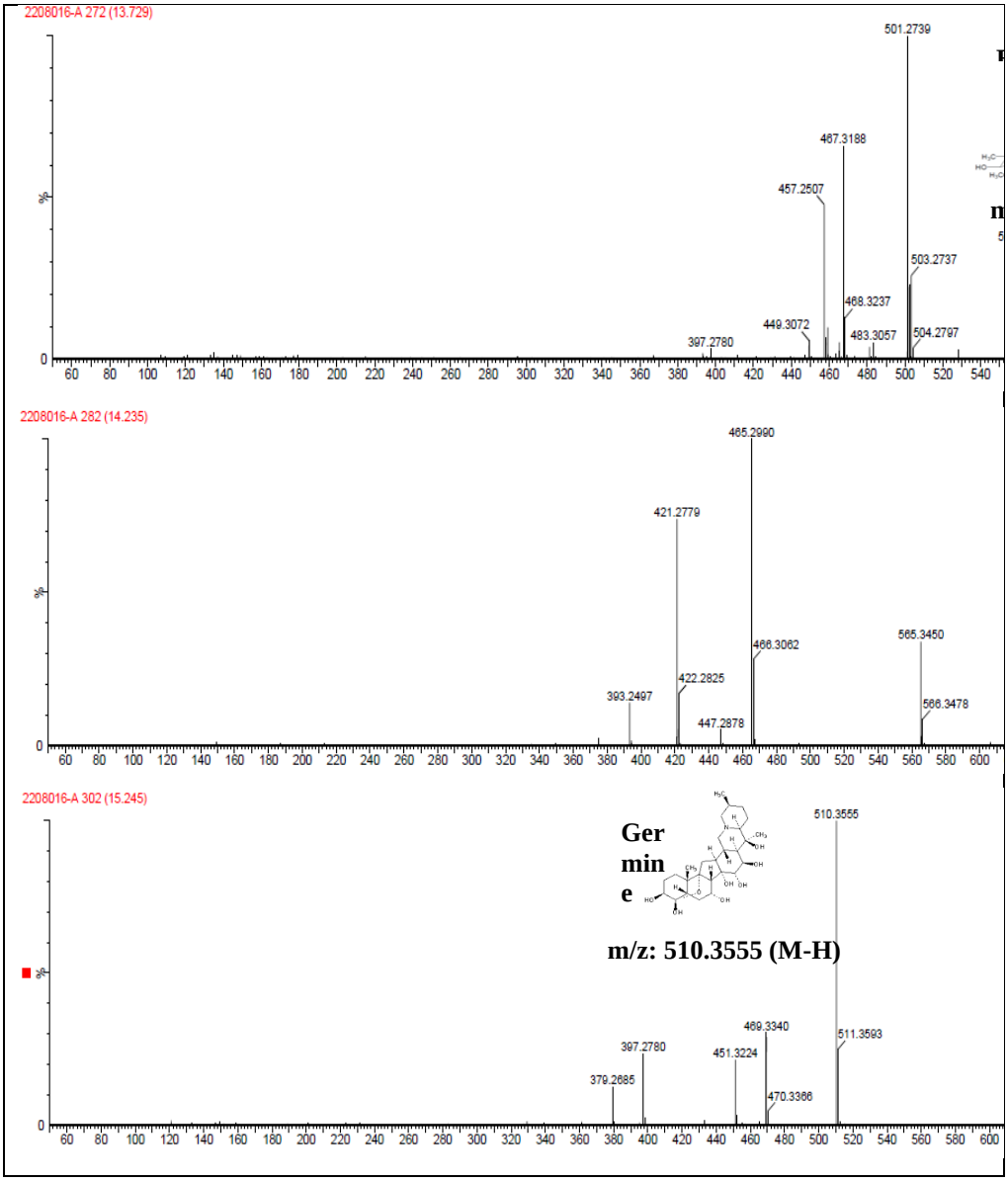


Figure 8: MassBank Database Analysis Report for *Simarouba glauca* methanol leaf extract (SGME)




2208016-A 193 (9.739)
Silybin B
m/z: 483.3147 (M-H)
483.3147
543.3293
439.2903
484.3186
544.3363
223.0806
393.2010
135.1387
2208016-A 215 (10.851)
481.2986
541.3132
409.2459
391.2952
463.2881
482.2984
542.3189
331.1791
349.1911
365.2587
293.2049
445.2817
Okaram
ine C
2208016-A 222 (11.204)
465.3035
421.2821
525.3193
422.2825
466.3062
447.2965
526.3235
481.2921


2208016-A 226 (11.406)
485.3281
486.3340
483.3147
395.2654
421.2821
439.2903
583.3603
Adonixanthin
m/z: 583.3603 (M-H)
2208016-A 236 (11.911)
483.3147
605.3436
439.2903
484.3186
393.2497
397.2862
465.3079
499.3100
606.3410
603.3210
Ginsenoside Rh3
2208016-A 255 (12.871)
481.2966
603.321
463.2881
409.2459
391.2352
482.2984
604.3268
293.2049
331.1791
531.2700
1085.5803
5,6-Dihydroxylutein
m/z: 603.3210 (M-H)


2208016-A 272 (13.729)
501.2739
467.3188
457.2507
503.2737
468.3237
449.3072
483.3057
504.2797
397.2780
%
0
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540
2208016-A 282 (14.235)
465.2990
421.2779
466.3062
565.3450
422.2825
393.2497
566.3478
447.2878
%
0
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600
2208016-A 302 (15.245)
Germine
m/z: 510.3555 (M-H)
510.3555
469.3340
511.3593
397.2780
451.3224
379.2685
470.3366
%
0
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600

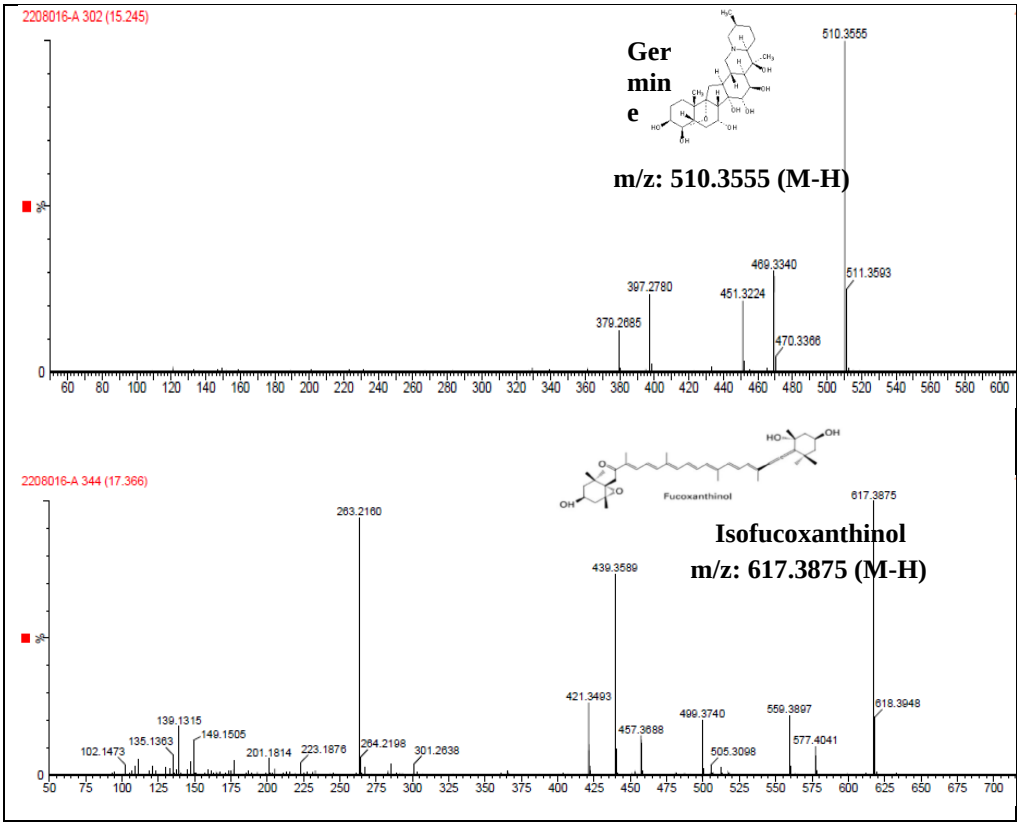


Fig. 9 LC-MS/MS spectrums for *Simarouba glauca* methanol leaf extract (SGME)

Table 15: LC-MS/MS particulars for *Simarouba glauca* aqueous leaf extract (SGAE)

S. No	RT (min)	Compound Name	Chemical Compound Class	Chemical Formula	Measured mass m/z	Exact mass m/z	Fragments (m/z)	Reference
1	6.153	Hesperetin	Flavonoid	$C_{16}H_{14}O_6$	303.0640	302.07904	223.1020, 255.1274, 224.1058	Markus Kohlhoff., 2016
2	6.355	Fisetin	Flavonoid	$C_{15}H_{10}O_6$	287.0694	286.04774	288.0689	Markus Kohlhoff., 2016
3	8.477	Kaempferol	Flavonoid	$C_{15}H_{10}O_6$	287.0659	286.04774	288.0689	Rasche., 2011
4	11.861	Dicaffeoyl quinolactone	Caffeic acid and derivatives	$C_{25}H_{22}O_{11}$	499.3054	498.44	500.3074	Tsugawa., 2019

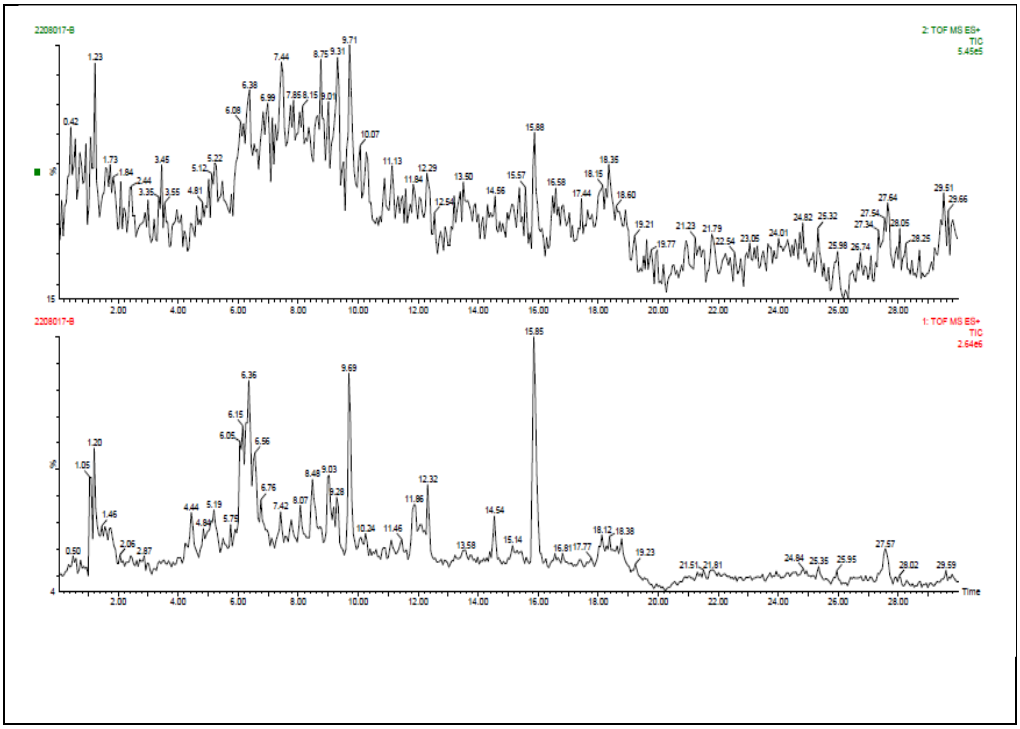
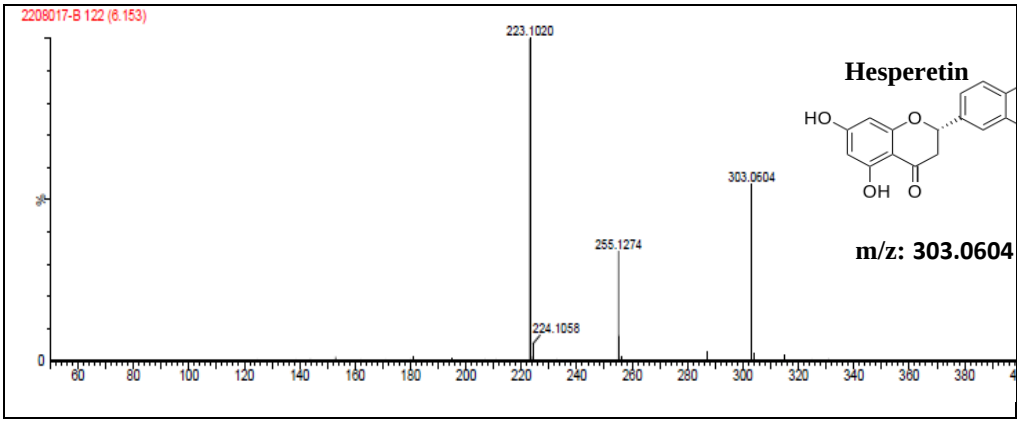


Fig. 9 MassBank Database Analysis Report for *Simarouba glauca* aqueous leaf extract-(SGAE)



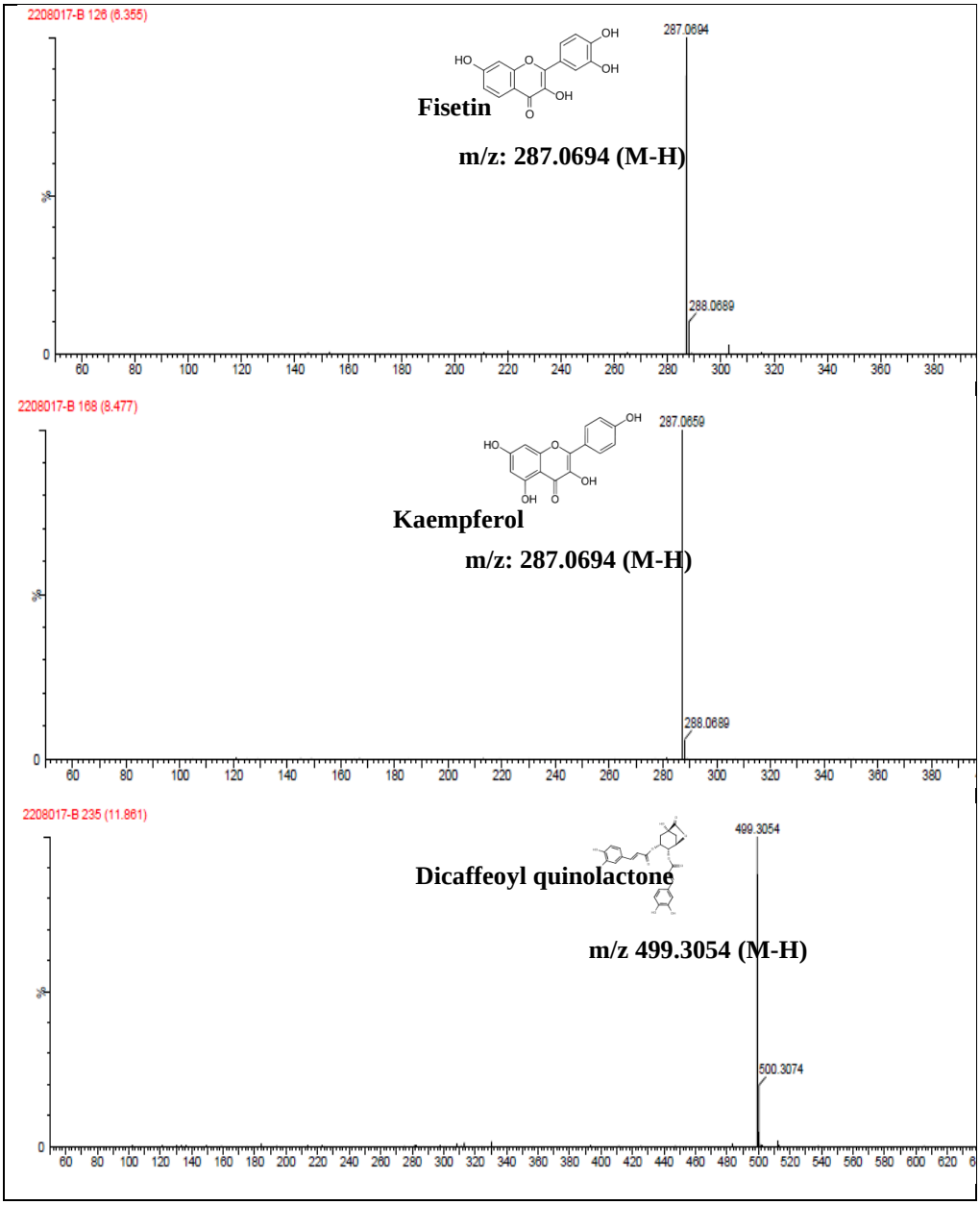


Fig. 9 LC-MS/MS spectrum for *Simarouba glauca* aqueous leaf extract (SGAE)

9 Discussions

The World Health Organisation (WHO) states that 80 % of people use plant extracts and their active compounds as folk medicines in traditional therapies [15]. Recent discovery on the screening of natural phytocompounds from natural sources for treating various diseases were further supported by their benefits, including low toxicity and accessibility. Climate change raises the risk of disease via warming temperatures, variations in precipitation, and increases intensity of some extreme weather events, and rising sea levels are all effects of climate change. Such effects endanger our health by influencing the food we consume, the water we drink, the air we breathe, and the weather we experience [28]. The severity of these health hazards may rises the illness like diabetes, obesity, cancer, cardiovascular diseases, dermatitis, immunological, neurological disorders and respiratory disorders etc, can be prevented and treated with natural sources of food supplements that have phytochemicals as functional foods or nutraceuticals [2]. Researchers were inspired to look for and analyse potential phytochemicals in order to find naturally occurring phytochemical compounds which are compliment for conventional therapy [16].

Simarouba glauca is a flowering tree, native to Florida, South America, commonly known as paradise tree, lakshmi taru, dysentery bark, bitter wood belonging to family Simaroubaceae [3]. The specific name glauca means covered with bloom which refers to the bluish green foliage. In Indian traditional system, all the plant parts of SG has been recognized as medicinal plant due to its wide application of medicine as anticancer, antimicrobial, antiviral and antihelminthic agent [17]. It has a rich source of phytochemical like quassinoids in that glaucarubin, quassinoids, ailanthinone, benzoquinone, holacanthone, melianone, simaroubidin, simarolide, simarubin, simarubolide and sitosterol also their biological properties such as antimicrobial, anticancer, antipyretic and haemostatic activity [18].

GC–MS analysis of unknown compounds spectrum was compared to that using NIST database and antimicrobial activity of various solvent extracts from Simarouba glauca leaves exhibited the presence of fatty acid esters [15]. The quantitative estimation of water soluble vitamins were investigated from Simarouba glauca leaves extracts exhibited the presence of vitamin-A, vitamin-B (B1, B2, and B3) and vitamin-C was determined by GC-MS analysis [19]. GC-MS and HPTLC-based qualitative triterpene identification and quantification of betulinic acid from hexane extract of Simarouba glauca leaves [20]. Squalene is a triterpenoid fraction with anti-inflammatory activities that was isolated from Simarouba glauca and studied using FT-IR and NMR study [21].

The LC-MS analysis of bioactive 'fraction-14' revealed four compounds, eclalbasaponin-v (1), cyanidin-3-O-(2'galloyl)-galactoside (2), kaempferol-3-O-glucoside (3) and kaempferol-3-O-pentoside (4) for the first time in S. glauca in this study [13]. Six canthin-6-one type of anticancer alkaloid constituents were found from bioactivity-guided fractionation of chloroform extract of S. glauca twigs. These included (1) canthin-6-one; (2) 2-methoxycanthin-6-one; (3) 9-methoxycanthin-6-one; (4) 2-hydroxycanthin-6-one; (5) 4,5-dimethoxycanthin-6-one; and (6) 4,5-dihydroxycanthin-6-one; two coumarins, melianodiol, an acyclic squalene-type triterpenoid, 14-deacetyleurylene, two coumarins, scopoletin and fraxidin, and two triglycerides, triolein and trilinolein [18]. Tricaproin (TCN), the chemical acquired from chloroform SG leaves, was identified through structural analysis utilizing GC-MS, FT-IR, and 1H and 13C NMR. [22].

According to the study's conclusions, the greatest extraction responses in the UAE (Ultrasonic Assisted Extraction) were seen for both methanol (16%w/w) and water (23%w/w) by the ultrasonic wave contact period of 40 minutes (A), 45°C temperature (B), and 250ml solvents

(C), respectively. In the Soxhlet method, comparably good extraction responses were obtained for both methanol (13%w/w) and water (22%w/w), even though it required longer time (A) duration 6 hours, 450 ml of solvent (C) at 30°C temperature for the other extraction process such as MAE (Microwave Assisted extraction) and cold maceration method.
In the cold maceration and MAE processes, moderate extraction responses were seen for both methanol (11.5% & 11.28%w/w) and aqueous SG leaf extract (15.6% & 12.15%w/w). However, this process needed a longer extraction period (A), which was roughly 72 hours, using 450ml of solvent (C) at room temperature (25ºC).

From the LC-MS/MS analysis Phytochemicals such as flavonoids and phenolic compounds were indentified (Hesperetin, Kaempferol, Fisetin and Dicaffeoyl quinolactone) in SG aqueous leaf extract. Similarly potential phytochemicals such as flavonoids, alkaloid, terpenoids, steroids and carotinoids were identified from SG methanol leaf extract (Rotenone, Silybin B, Oleuropein, Okaramine C, Adonixanthin,, Ginsenoside Rh3, 5, 6-Dihydroxylutein, Bovoside A, Germine, Apigenin 6-C-glucoside 8-C-arabinoside and Isofucoxanthinol)

Flavonoids [23], alkaloids [24], plant phenolic compounds [25], terpenoids [26], phytosterols [27], and carotenoids [29] are essential chemicals for life and can be found in many fruits, vegetables, plants, leaves, algae, and bacteria. They may have antiviral, anticancer, anti-inflammatory, analgesic, local anaesthetic, neuroprotective, antibacterial, antifungal properties, and aid in the reduction of cholesterol absorption. They also provide protection against free radicals and oxidative stress (antioxidant).

10 Conclusion

In the present work, methanol and aqueous leaf extracts of Simarouba glauca were prepared in both greener extraction technique (MAE and UAE) as well as conventional extraction method (Cold maceration and Soxhlet) using 23 full factorial designs was used in order to optimize the maximum extraction efficiency in first time. According to this study, found that the UAE (Ultrasonic Assisted Extraction) had the highest extraction responses for both methanol (16%w/w) and aqueous (23%w/w). However, the Soxhlet method produced good extraction responses for both methanol (13%w/w) and water (22%w/w) than cold maceration and MAE (Microwave Assisted Extraction). Sustainable potential phytochemical compounds were found using LC-MS/MS analysis such as flavonoids and phenolic compounds (hesperetin, kaempferol, fisetin and Dicaffeoyl quinolactone) in SG aqueous leaf extract. Similarly potential phytochemicals such as flavonoids, alkaloid, terpenoids, steroids and carotinoids were identified from SG methanol leaf extract (Rotenone, Silybin B, Oleuropein, Okaramine C, Adonixanthin, Ginsenoside Rh3, 5, 6-Dihydroxylutein, Bovoside A, Germine, Apigenin 6-C-glucoside 8-C-arabinoside and Isofucoxanthinol). These potential phytochemicals (flavonoids, alkaloids, plant phenolic compounds, terpenoids, phytosterols, and carotenoids) are essential for life and can be found in many fruits, vegetables, plants, leaves, algae, and bacteria. They may have antiviral, anticancer, anti-inflammatory, analgesic, local anaesthetic, neuroprotective, antibacterial, antifungal properties, and aid in the reduction of cholesterol absorption. They also provide protection against free radicals and oxidative stress (antioxidant). According to WHO estimates, 2 billion people lack safe drinking water and 600 million suffer from foodborne infections each year, with children under the age of five accounting for 30% of foodborne fatalities. Climate stressors increase the risk of waterborne, foodborne disease and also illness like diabetes,

obesity, cancer, cardiovascular diseases, dermatitis, immunological, neurological disorders and respiratory disorders etc, can be prevented and treated with natural sources of food supplements that have phytochemicals as functional foods or nutraceuticals. Modern extraction technologies and spectral data are also helping us to recognize and identify the sustainable potential phytochemicals found in natural sources to cure various ailments. In future, biological responses of the identified potential phytochemicals from Simarouba glauca will be evaluated.

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