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High-Performance Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (HPLC-QTOFMS) Analysis on the Ethanol:Water (80:20) Extract of *Lawsonia inermis* Leaves

(Analisis Cecair Kromatografi Berprestasi Tinggi Kuadrupol Spektrometri Jisim Masa Penerbangan (HPLC-QTOFMS) pada Etanol:Air (80:20) Ekstrak daun *Lawsonia inermis*)

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ABSTRACT

Lawsonia inermis *(Henna), (Lythraceae), contains a high amount of phenolic compounds which could activate antioxidants to help reduce free radicals. In this study, the compound content found in the ethanol:water (80:20) extract of local* Lawsonia inermis *was determined using HPLC-QTOFMS. The* ^{1}*H-NMR results were used to determine the peak that exists for the group compounds. Chromatographic peaks were detected and integrated by the MassHunter Acquisition B.07.00 for the Agilent TOF and QTOF and MassHunter Qualitative Analysis B.07.00. The ethanol:water (80:20) extract of* L. inermis *have shown, predominantly, the presence of phenolic compounds (coumarins, flavonoids, naphthalene, and gallic acid) which are highly glycosylated. The presence of compounds such as apiin, lawsone, apigenin, luteolin, cosmosiin, and p-coumaric acid were also found.*

Keywords: Ethanol:water (80:20); ^{1}*H-NMR; HPLC-QTOFMS; leaves* Lawsonia inermis *Malaysia*

ABSTRAK

Lawsonia inermis *(inai), (Lythraceae), mengandungi sejumlah besar sebatian fenolik yang boleh mengaktifkan antioksidan dalam membantu mengurangkan radikal bebas. Dalam kajian ini, kandungan sebatian yang terdapat dalam etanol:air (80:20) ekstrak* Lawsonia inermis *tempatan ditentukan menggunakan HPLC-QTOFMS. Hasil* ^{1}*H-NMR digunakan untuk menentukan puncak kumpulan sebatian. Puncak kromatografi dikesan dan diintegrasikan oleh MassHunter Acquisition B.07.00 untuk Agilent TOF dan QTOF dan Analisis Kualitatif MassHunter B.07.00. Daripada etanol:air (80:20) ekstrak* L. inermis *menunjukkan sebahagian besarnya, kehadiran sebatian fenolik (kumarin, flavonoid, naftalena dan asid galik) yang sangat glikosilasi. Kehadiran sebatian seperti apiin, lawson, apigenin, luteolin, kosmosiin dan asid-p-kumarit turut dijumpai.*

Kata kunci: Daun Lawsonia inermis *Malaysia; etanol:air (80:20);* ^{1}*H-NMR; HPLC-QTOFMS*

INTRODUCTION

Lawsonia inermis (family *Lythraceae*) is a plant that has been widely studied and used throughout the world for traditional medicinal and cosmetic. Globally, *Lawsonia inermis* is used as a traditional or folk medicine for the treatment of a wide range of seemingly unrelated ailments such as induce abortion (Africa) diuretic, gonorrhoea and bronchitis (Cambodia), pain and skin affections intestinal amoebiasis (Egypt), headache, burning of skin, decoction used for sore throat (Ahmad & Beg 2001) jaundice and other liver disorders, itching and other skin disorders (India, Pakistan), hair tonic (India, Jordan), fever, malaria, as a blood tonic (Nigeria), boils, conjunctivitis, pimples, dandruff, scabies, baldness and other scalp disorders (Rusia, Asia) (Semwal et al. 2014). In Malaysia, people call it 'Inai' and it is used as coloring fingernails especially in weddings but not extensively used in medicine such as fungal pathogens treatment, head lice treatment, reduce dandruff, and also for digestive disorders, treating diabetics and ulcers (Othman et al. 2020).

Lawsonia inermis contain high amount of phenolic compounds which activate antioxidants to help reduce free radicals (Oroian & Escriche 2015). Many studies on *L. inermis* have found nearly 70 phenolic compounds that have been isolated from various parts of *L. inermis* include root, bark, flower, and leaves where it is proven to have various active compounds (Semwal et al. 2014). Lawsone (2-hydroxy-1, 4 naphthaquinone) is a natural pigment present in the leaves of *L. inermis*. It is the principal active ingredient of the henna plant

(Saeed 2013). Lawsone, also known as hennotannic acid chemically, lawsone is similar to juglone, which is found in walnuts (Babula et al. 2005; Ebrahimi & Parvinzadeh Gashti 2016).

Throughout the world, proactive studies have proven scientifically the effectiveness of inorganic *L. inermis* pharmacological activities such as antibacterial (Jabborova et al. 2019), virucidal, antiparasitic, anti-inflammatory, analgesic, and anticancer properties, as well as hepatoprotective, immunomodulatory, anthelminthic, antitrypanosomal, and antioxidant activities (Chaudhary et al. 2010; Pasandi Pour & Farahbakhsh 2019; Semwal et al. 2014). For compounds identification, we analysis with high-performance liquid chromatography (HPLC) (Acquaviva et al. 2018). Then, ^{1}H-NMR result produced was used to determine the peak that exists for the group compounds (Chandrakalavathi et al. 2018). The objective of this study was to analyze the compounds content found in the local *L. inermis* using ethanol-water mixtures. This is the most suitable solvent system for the extraction of *L. inermis* due to the different polarities of the active constituents, and the acceptability of the solvent system for human consumption (Alothman 2009; Gull et al. 2013) even though the best solution was acetone followed by ethanol (Tan 2013).

Materials and Methods

Sample collection

Lawsonia inermis leaves were collected from the residential area of Sentosa, Bandar Baru Bangi, Selangor, Malaysia. The collection was led by Mr. Din Bin Md Nor and the botanical identification was made by Mr. Teoh Leong Eng from the Department of the Chemistry University of Malaya. A voucher specimen KL5824 was deposited to the herbarium of Chemistry, Faculty of Science, University of Malaya.

Preparation of the extracts

Dried and ground of *Lawsonia inermis* leaves (300 g) was soaked in 80% ethanol and 20% distilled water. The sample was macerated under room temperature for three consecutive days. Then, the suspension was filtered using filter paper. The eluent was first concentrated using rotary evaporator to remove the ethanol solvent followed with freeze drying method to remove the distilled water to give water extracts (LILEW). About 1.2 g of LILEW were dissolved in distilled water before mix with dichloromethane to form an immiscible solution. The two phases were agitated, by shaking and swirl to bring about substantial physical mixing. After agitation, the phases were allowed to separate. Compounds having different solubilities will be separated base on the polarity of the solvent. Sodium sulfate anhydrous (Na_2SO_4) were use in the organic layer as a drying agent. The dichloromethane solution was concentrated to dryness using rotary evaporator and kept in the fridge until further used (DCM extract). The aqueous solution was then mix with ethyl acetate and liquid-liquid partition were repeated to give EA extract. The LILEW, DCM and EA extracts were weight and recorded (Table 1) and analyze using ^{1}H-NMR as shown in Figures 1 to 3.

Table 1. The yield of DCM partition and EA partition for aqueous extraction LILEW

No.	Extract	Remaining yield
1	LILEW	36 g
2	DCM	97 mg
3	EA	156 mg

Nuclear magnetic resonance spectroscopy (NMR)

One-dimensional ^{1}H-NMR experiments were carried out on JEOL ECA 400 FT-NMR. $CDCl_3$ and MeOD were used as the solvent depending on the polarity. The chemical shifts were recorded with reference to $CDCl_3$ (7.24 ppm) or MeOD (4.78 ppm).

LC-MS chromatography were carried out on Agilent 1260 Infinity series ultra-high-performance liquid chromatography (UHPLC) system and a G6530B accurate-mass Q-TOF LC/MS instrument (Agilent Technologies, Santa Clara, USA). 10 µL of extracts were injected and chromatographed on a ZORBAX Eclipse Plus C8 column (100 × 2.1 mm id, 1.8 µm) column with the mobile phase of A (0.1% formic acid in water) and B (methanol). The mobile phase was performed as follows: Gradient from 5 to 100% B over the next 30 min, isocratic at 100% B for 5 min and re-equilibrated for 5 min at 5% B before the next run. Eluent were monitored using diode array

detector at 220, 256, and 380 nm. ESI-MS was performed in the range 100 to 1,000 in both the positive and negative mode. The temperature was set to 300 °C. Nitrogen gas was used as nebulizer with the flow rate set at 10 L min^{-1}. The ESI source voltage was set at 3.5 kV. Mass Hunter Acquisition B.07.00 software was used for data analysis.

RESULTS AND DISCUSSION

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR)

^{1}H-NMR studies are presented in Figures 1 to 3. In Figure 1, ^{1}H-NMR 400 MHz of aqueous extraction of LILEW, the result shows the chemical shifts of the aromatic group (6.5-8.5 ppm), methoxyl group (CH_2OH/CH-OH) (CH_2O/CH-O) in the range 3.0 to 4.5 ppm. In Figure 2, ^{1}H-NMR 400 MHz of *L. inermis* extract result for DCM partition the chemical shift of the methyl (CH_3), methylene (CH_2), methane (CH) between 0.5 and 2.0 ppm similar with aqueous extraction of LILEW. Figure 3 shows ^{1}H-NMR 400 MHz of *L. inermis* EtOH:water extract result for EA partition show the best and clear chemical shifts of the aromatic group, between 6.5 and 8.5 ppm compare with DCM partition. We decide to run LC-MS profile using EA partition for future investigation of potential compounds from aqueous extraction of LILEW.

LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS) PROFILE ANALYSIS

The LC-MS chromatogram was generated after analysis of the reference standards using the UHPLC-QTOFMS method as described above. Figure 4 shows LC-MS peak/total ion chromatogram (TIC) for LILEW EA partition (negative mode) where Figure 5 shows LC-MS peak number of selected compounds for LILEW extract using EA partition. From the result of LC-MS profile, we manage to identify one major class of compound from phenolic. We have chosen 27 peak number of potential compounds.

TABLE 2. List of compounds, chemical formula, exact mass, [M-H]- and retention time

Peak label	Major classes	Compound name	Chemical formula	Exact mass	Mass detected under negative mode [M - H]-	Retention time (R_t) / min
1	Coumarins	Scopoletin	$C_{10}H_8O_4$	192.0423	191.0208	1.155
2	Tannins	1,2,3,6-Tetra-O-glalloyl-B-D-Glocose	$C_{34}H_{28}O_{22}$	788.1072	787.1068	1.155
3	Trihydroxybenzoic acid	Gallic Acid	$C_7H_6O_5$	170.0215	169.0142	3.163
4	Naphthoquinones	4-Hydroxy-a-tetralone	$C_{10}H_{10}O_2$	162.0681	161.0461	5.586
5	Flavonoids	2,4-Dihydroxybenzoic acid	$C_7H_6O_4$	154.0266	153.0193	7.843
6	Coumarins	Agrimonolide 6-O-β-D-glucopyranoside	$C_{24}H_{28}O_{10}$	476.1682	475.0885	7.926
7	Flavonoids	Acacetin-7-O-glucoside	$C_{22}H_{22}O_{10}$	446.1213	445.0859	8.623
8	Flavonoids	Lawsochrysinin	$C_{20}H_{18}O_4$	322.1205	321.0615	8.955
9	Coumarins	Esculetin	$C_9H_6O_4$	178.0266	177.0194	9.171
10	Coumarins	Fraxetin	$C_{10}H_8O_5$	208.0372	207.0307	9.436
11	Alkylphenones	Lalioside	$C_{14}H_{18}O_{14}$	346.09	345.0835	11.594
12	Flavonoids	3',4' dimethoxyflavone	$C_{17}H_{14}O_4$	282.0892	281.2482	11.992
13	Flavonoids	Lawsochrysin	$C_{25}H_{30}O_4$	394.2144	393.0457	12.208
14	Tannins	1,2,3,4,6-penta-O-galloly-B-D-glucose	$C_{41}H_{32}O_{26}$	940.1182	939.1144	12.988
15	Flavonoids	Luteolin-7-o-glucoside	$C_{21}H_{20}O_{11}$	448.1006	447.0929	14.366
16	Flavonoids	Apiin	$C_{26}H_{28}O_{14}$	564.1479	563.0951	14.432

17	Naphthoquinones	Lawsone	$C_{10}H_6O_3$	174.0319	173.0242	14.598
18	Naphthoquinones	Isoplumbagin	$C_{11}H_8O_3$	188.0473	187.0395	14.847
19	Coumarins	Daphnorin	$C_{25}H_{22}O_{12}$	514.1111	513.1401	15.411
20	Flavonoids	Cosmosin/Apigenin-7-O-ß-D-glucopyranoside	$C_{21}H_{20}O_{10}$	432.1056	413.1001	15.610
21	Flavonoids	Scutellarin	$C_{21}H_{18}O_{12}$	462.0798	461.1117	16.407
22	Flavonoids	Luteolin	$C_{15}H_{10}O_6$	286.0477	285.0408	18.631
23	Flavonoids	Apigenin	$C_{15}H_{10}O_5$	270.0528	269.0461	20.025
24	Coumarins	Daphneside	$C_{21}H_{26}O_{14}$	502.1323	501.1062	22.763
25	Flavonoids	Acacetin	$C_{16}H_{12}O_5$	284.0685	283.1391	25.004
26	Flavonoids	3',4' dimethoxy flavone	$C_{17}H_{14}O_4$	298.0841	297.1536	25.651
27	Coumarins	Lacoumarin	$C_{12}H_{10}O_4$	218.0579	217.0489	38.198

This work describes a comprehensive and inclusive technique for the characterization of major constituents for the ethanol:water (80:20) extract of the leaves of the Malaysian *L. inermis*. Twenty-seven compounds were identified to be phenolic compounds with exact mass, molecular weight, chemical formula, and retention time.

This paper also provides information on nuclear magnetic resonance spectroscopy (NMR) and followed by high-performance liquid chromatography (HPLC) methods to make comprehensive studies. This finding also suggested the mixtures of 80% ethanol with 20% water suitable solvent system for the extraction of *L. inermis* due to the different polarities of the active constituents, and the acceptability of the solvent system for human consumption (Alothman 2009).

Peak no. 1 shows compound scopoletin type of coumarin (Figure 6) (Agarwal et al. 2014). Figure 7 shows peak no. 2 for compound 1,2,3,6-tetra-O-glalloyl-B-D-glucose might be an anti-hepatitis B virus (HBV) agent (Xiang et al. 2010). Figure 8 shows peak no. 3 for compound gallic acid. Gallic acid has been reported to have therapeutic activities in gastrointestinal, antioxidant, and anti-inflammatory (Kahkeshani 2019). In Figure 9, peak no. 4 indicated compound 4-hydroxy-a-tetralone and peak no. 5 shows the fragmentation pattern of compound 2,4-dihydroxybenzoic acid (Figure 10) of the major peak with m/z value 153.0193.

Peak no. 6 indicated of compound agrimonolide 6-O-β-D-glucopyranoside shown in Figures 11 and 12 show peak no. 7 with m/z value 445.0859 indicate mass detected under negative mode [M - H]- of compound acacetin-7-O-glucoside (Muhammad & Muhammad 2005; Sharma et al. 2016). Figure 13 indicated the compound name lawsochrysinin (peak no. 8) (Uddin et al. 2013).

Figure 18 shows compound lawsochrysin (peak no. 13) (Brahmachari 2018). Figure 19 shows compound name 1,2,3,4,6-penta-O-galloly-β-D-glucose indicated in peak no. 14. Peak no. 19 for compound daphnorin has shown in Figure 24. We also manage to identify compound daphneside (peak no. 24) (Figure 29) and for peak no. 27 indicated compound lacoumarin (Figure 32).

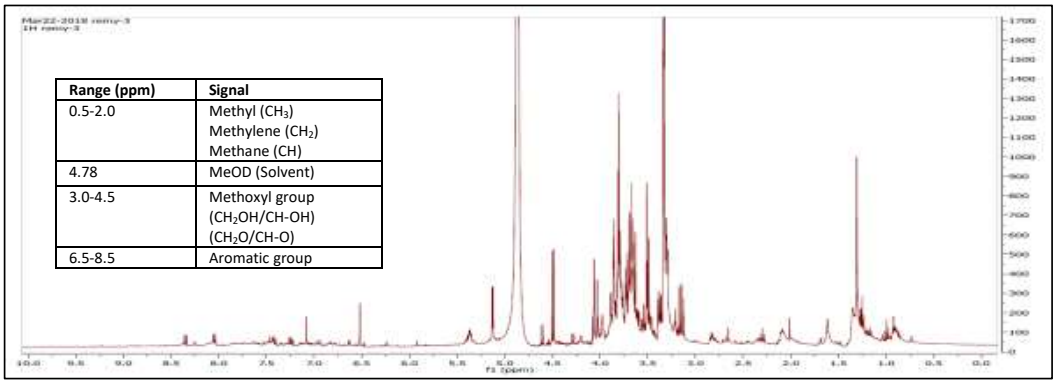
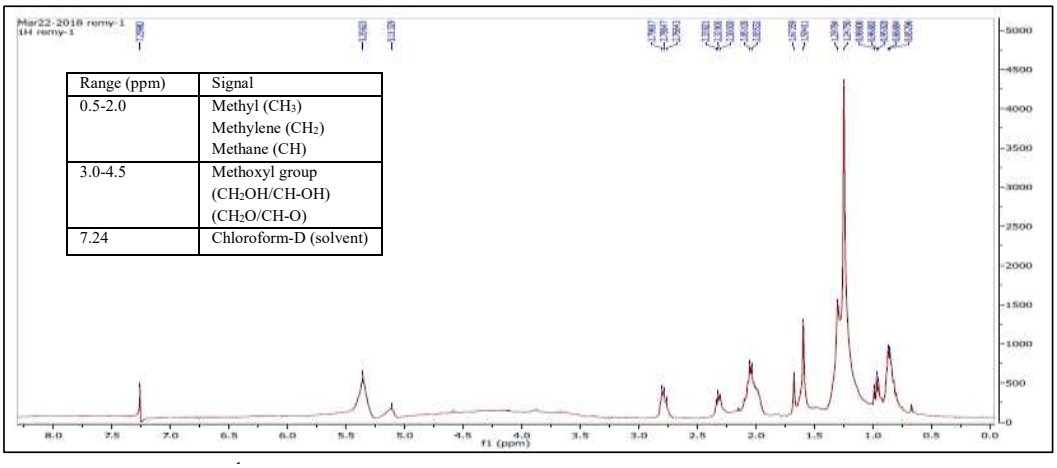
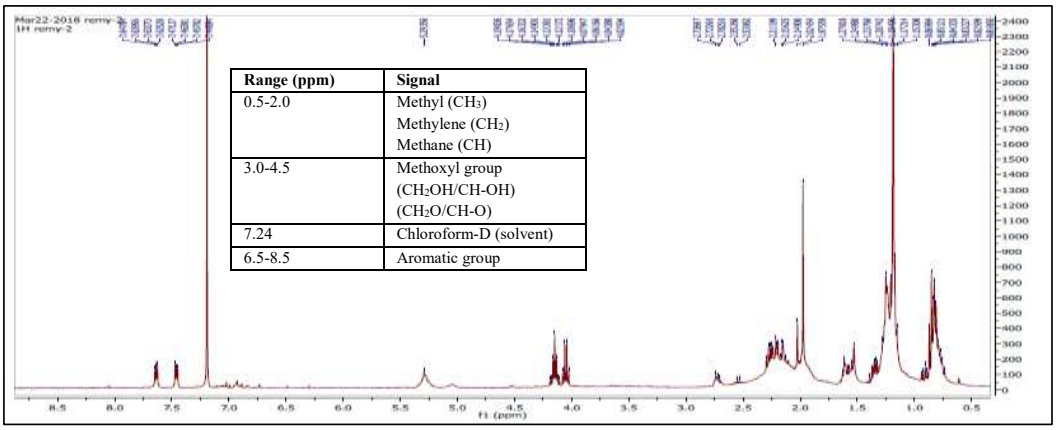


FIGURE 1. ^{1}H-NMR 400 MHz of aqueous extraction of LILEW



Range (ppm)	Signal
0.5-2.0	Methyl (CH_3) Methylene (CH_2) Methane (CH)
3.0-4.5	Methoxyl group (CH_2OH/CH-OH) (CH_2O/CH-O)
7.24	Chloroform-D (solvent)

FIGURE 2. ^{1}H--NMR 400 MHz of LILEW extract result for DCM Partition



Range (ppm)	**Signal**
0.5-2.0	Methyl (CH_3) Methylene (CH_2) Methane (CH)
3.0-4.5	Methoxyl group (CH_2OH/CH-OH) (CH_2O/CH-O)
7.24	Chloroform-D (solvent)
6.5-8.5	Aromatic group

FIGURE 3. ^{1}H-NMR 400 MHz of LILEW extract result for EA Partition

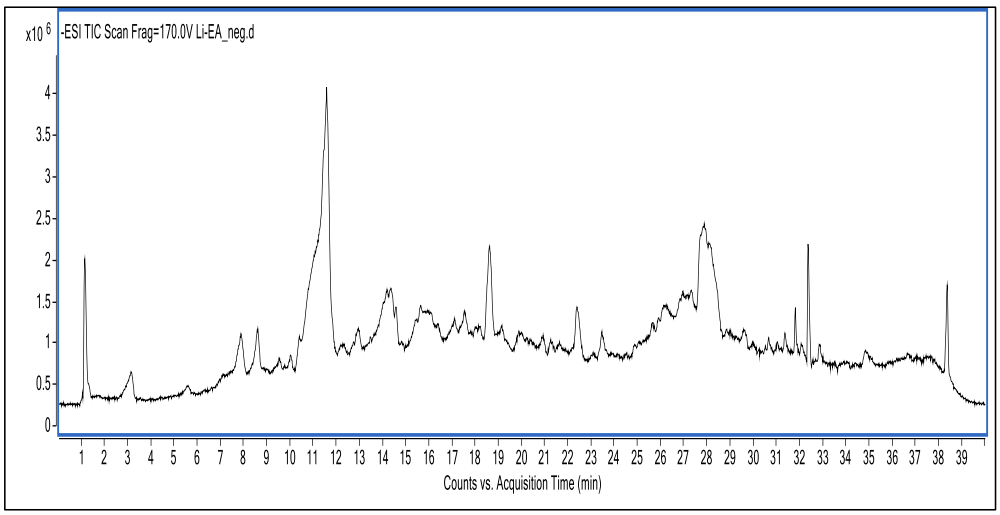


FIGURE 4. LC-*MS Peak/Total Ion Chromatogram (TIC) for* Lawsonia inermis EtOH: Water of EA partition (Negative mode

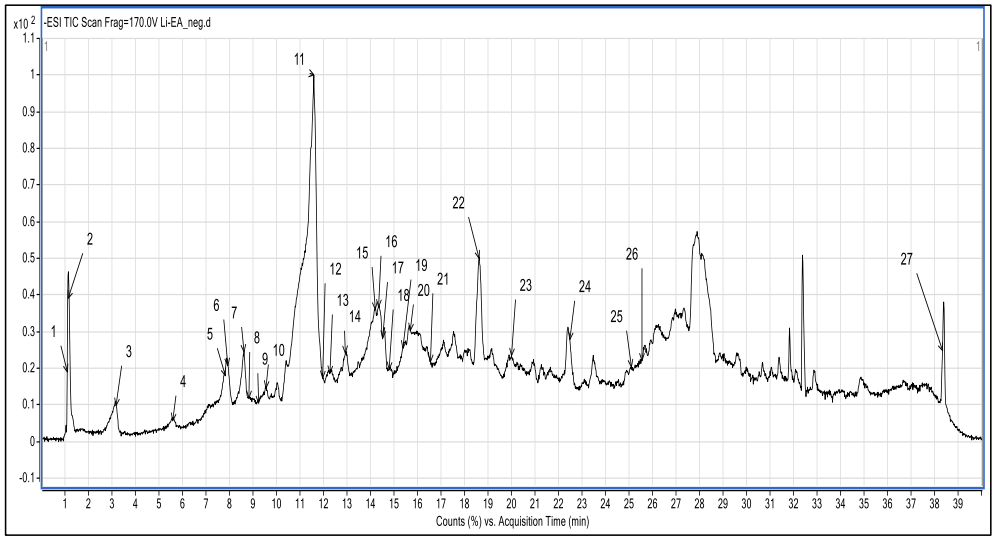
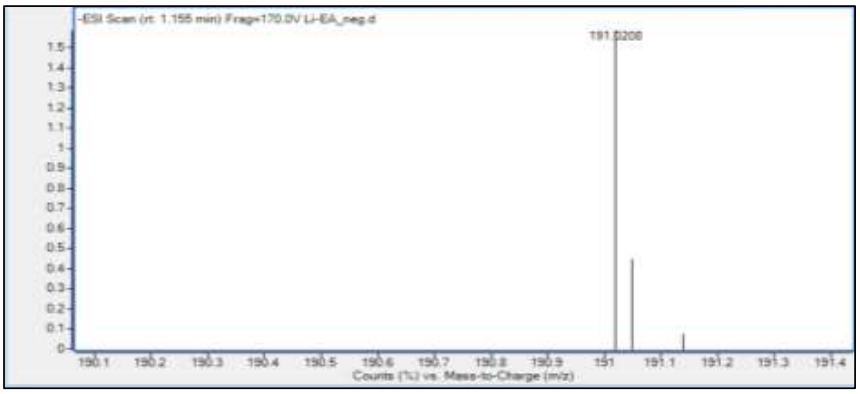


FIGURE 5. LC-MS Peak number of Compounds for EA Partition of aqueous LILEW extract in Negative mode

Peak no. 1, Name: **Scopoletin**
Exact Mass: 192.0423, (m/z-H); [m/z-1]= 191.0432 (RT: 1.1155 min)



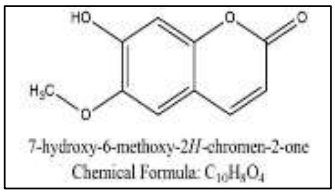
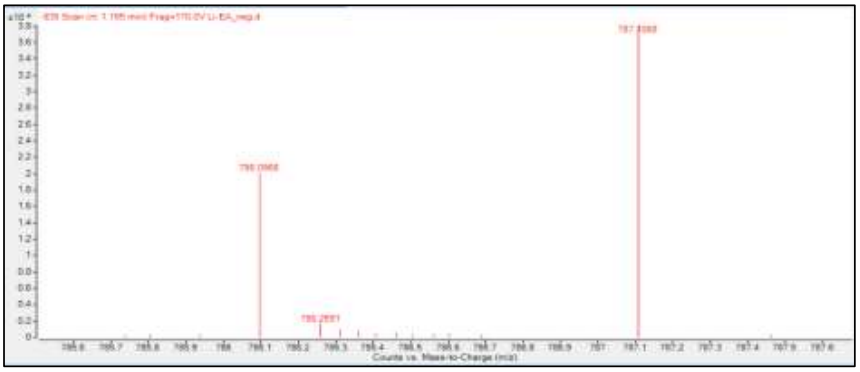


FIGURE 6. Fragmentation pattern from MS/MS spectrum of the major peak label no. 1 with m/z value 191.0208

Peak no. 2, Name: **1,2,3,6-Tetra-O-glalloyl-B-D-Glocose**
Exact Mass: 788.1072, (m/z-H); [m/z-1]= 787.1072 (RT:1.155 min)



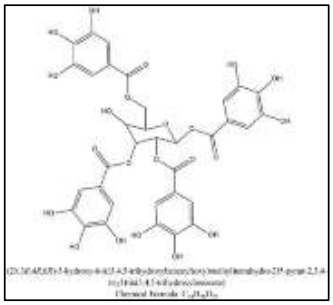
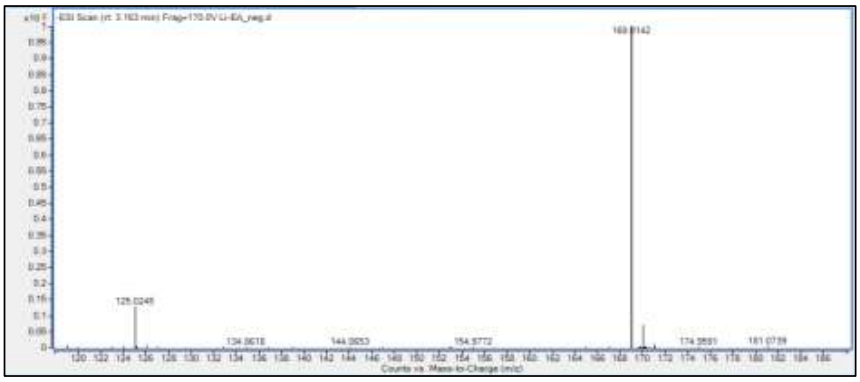


FIGURE 7. Fragmentation pattern from MS/MS spectrum of the major peak with m/z value 787.1068

Peak no. 3, Name: **Gallic Acid**
Exact Mass: 170.0215, (m/z-H); [m/z-1]= 169.0215 (RT: 3.163 min)



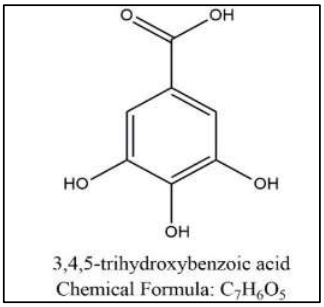
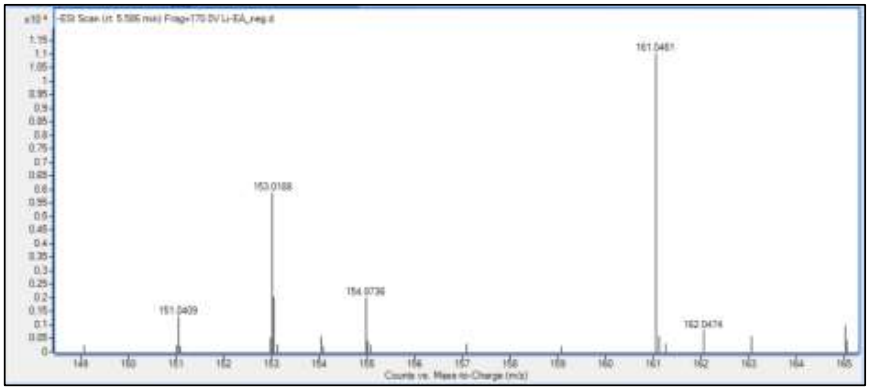


FIGURE 8. Fragmentation pattern from MS/MS spectrum of the major peak with m/z value 169.0142

Peak no. 4, Name: **4-Hydroxy-a-tetralone.**
Exact Mass: 162.0681, (m/z-H); [m/z-1]= 161.0681 (RT: 5.586 min)



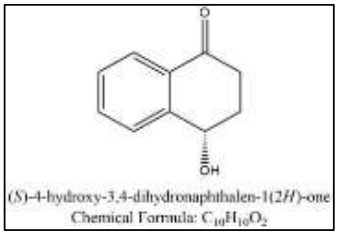
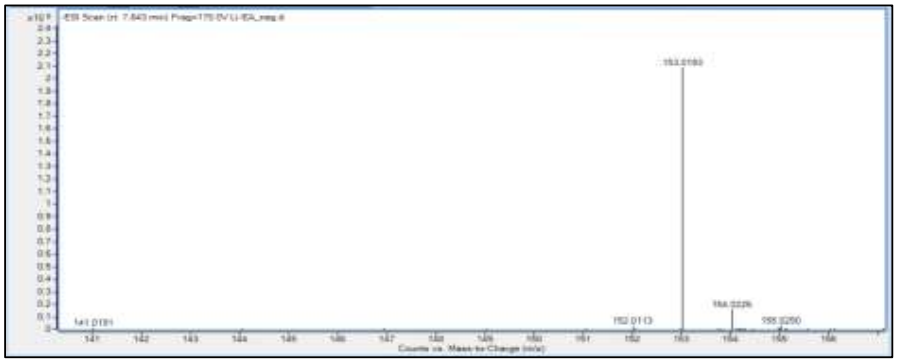


FIGURE 9. Fragmentation pattern from MS/MS spectrum of the major peak with m/z value 161.0461

Peak no. 5, Name: **2,4-Dihydroxybenzoic acid**
Exact Mass: 154.0266, (m/z-H); [m/z-1]= 153.0266 (RT: 7.843 min)



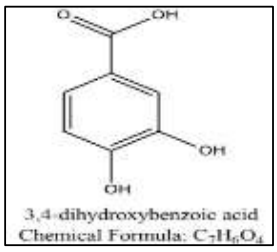


FIGURE 10. Fragmentation pattern from MS/MS spectrum of the major peak with m/z value 153.0193

Peak no. 6, Name: **Agrimonolide 6-O-β-D-glucopyranoside**
Exact Mass: 476.1682, (m/z-H); [m/z-1]= 475.1682 (RT: 7.926 min)

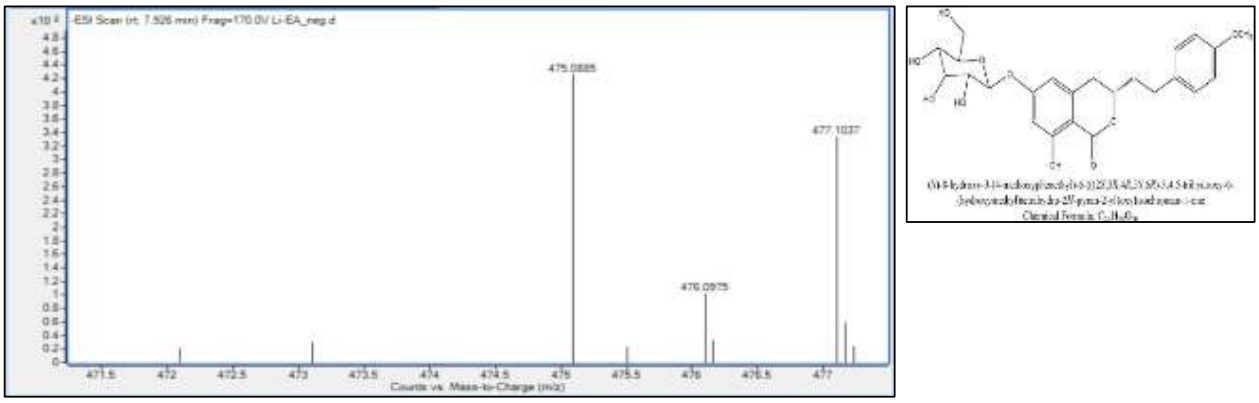


FIGURE 11. Fragmentation pattern from MS/MS spectrum of the major peak with m/z value 475.0885

Peak no. 7, Name: **Acacetin-7-O-glucoside**
Exact Mass: 446.1213, (m/z-H); [m/z-1]= 445.1213 (RT: 8.623 min)

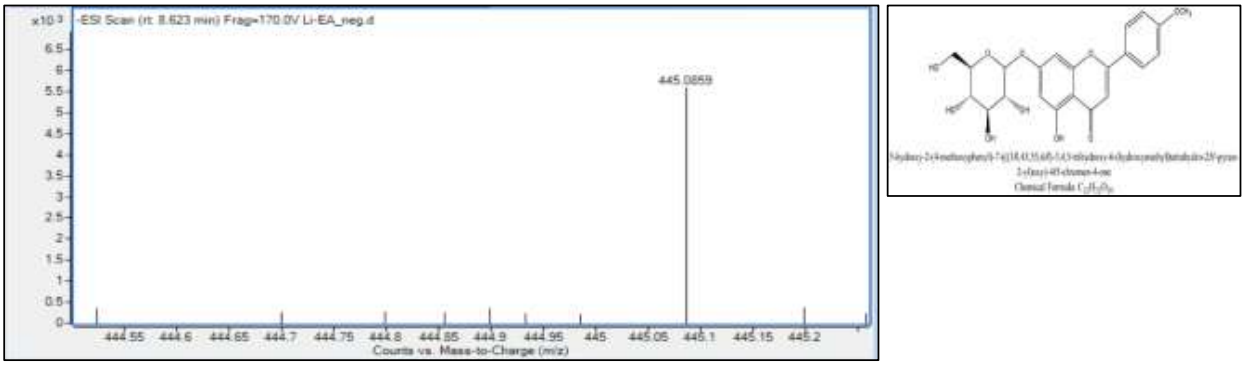


FIGURE 12. Fragmentation pattern from MS/MS spectrum of the major peak with m/z value 445.0859

Peak no. 8, Name: **Lawsochrysinin**
Exact Mass: 322.1205, (m/z-H); [m/z-1]= 321.1205 (RT: 8.955 min)

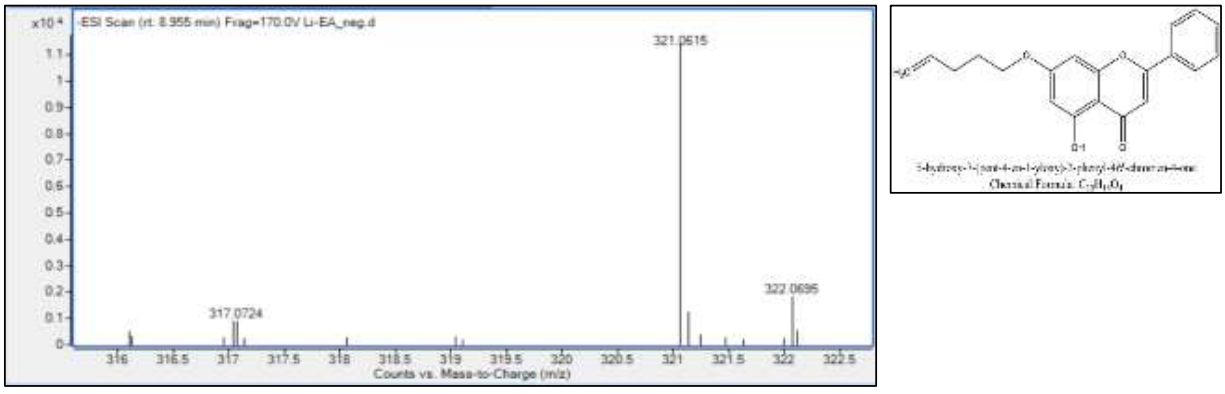
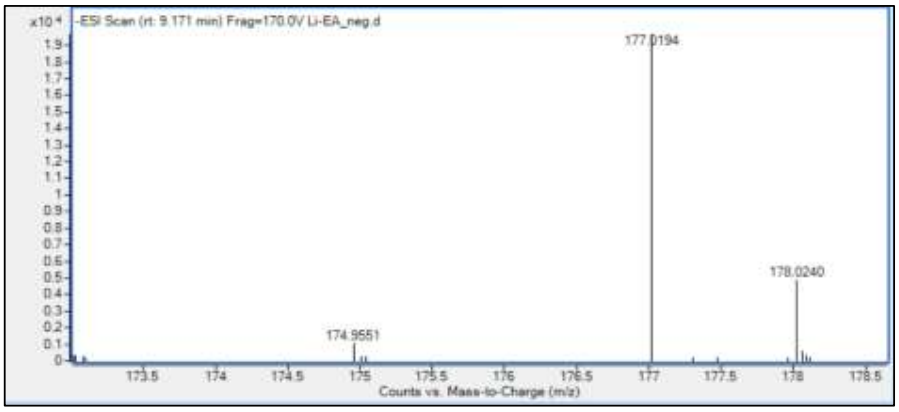


FIGURE 13. Fragmentation pattern from MS/MS spectrum of the major peak with m/z value 321.0615

Peak no. 9, Name: **Esculetin**
Exact Mass: 178.0266, (m/z-H); [m/z-1]= 177.0266 (RT: 9.171 min)



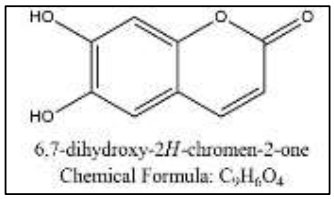
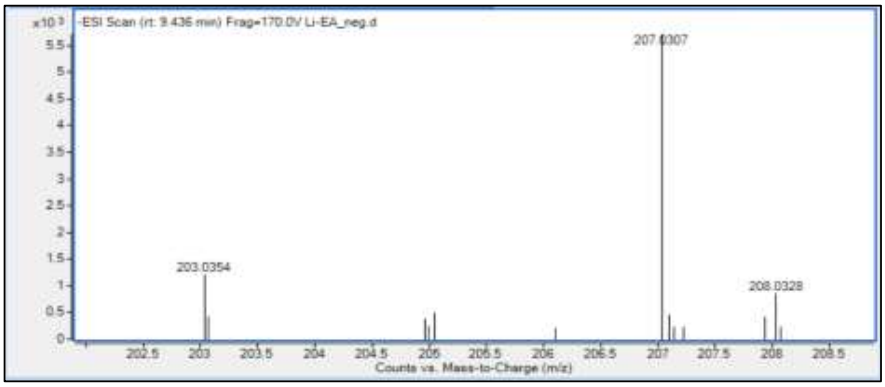


FIGURE 14. Fragmentation pattern from MS/MS spectrum of the major peak with m/z value 177.0194

Peak no. 10, Name: **Fraxetin**
Exact Mass: 208.0372, (m/z-H); [m/z-1]= 207.0372 (RT: 9.436 min)



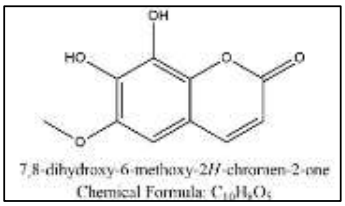
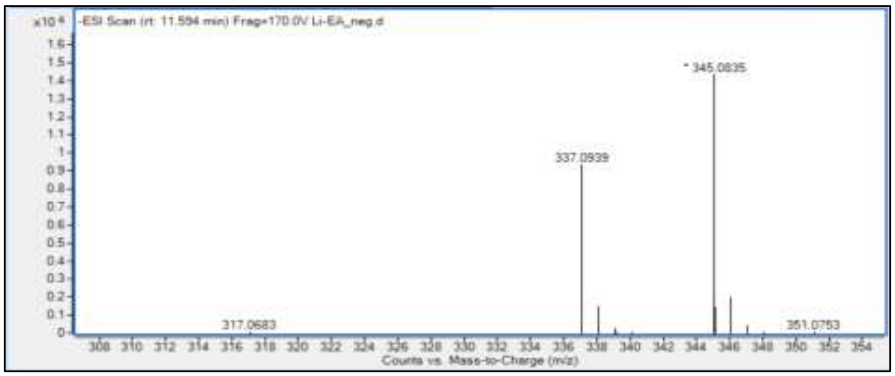


FIGURE 15. Fragmentation pattern from MS/MS spectrum of the major peak with m/z value 207.0307

Peak no. 11, Name: **Lalioside**
Exact Mass: 346.0900, (m/z-H); [m/z-1]= 345.0900 (RT: 11.594 min)



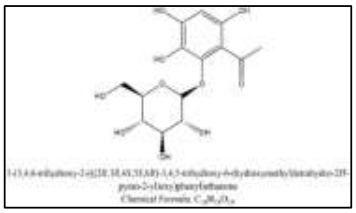


FIGURE 16. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 345.0835

Peak no. 12, Name: **3',4' dimethoxyflavone**
Exact Mass: 282.0892, (m/z-H); [m/z-1]= 281.0892 (RT: 11.992 min)

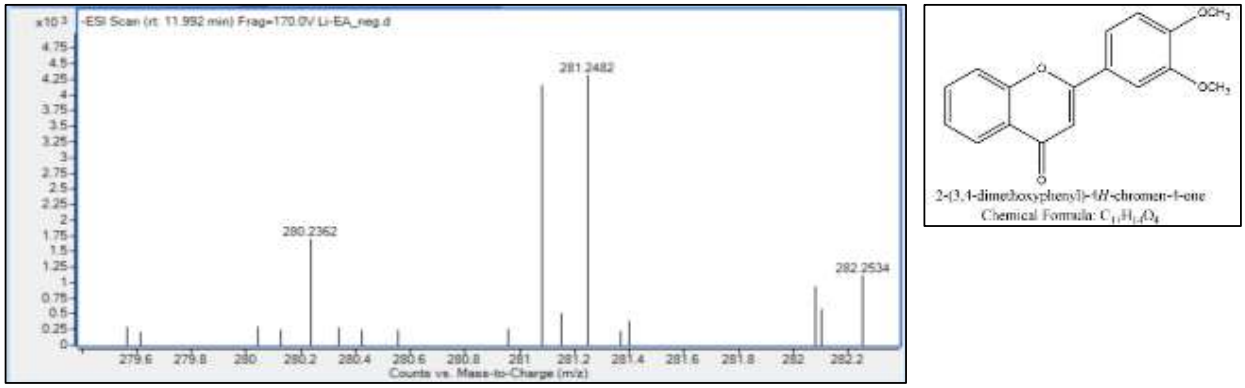


FIGURE 17. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 281.2482

Peak no. 13, Name: **Lawsochrysin**
Exact Mass: 394.2144, (m/z-H); [m/z-1]= 393.2144 (RT: 12.208 min)

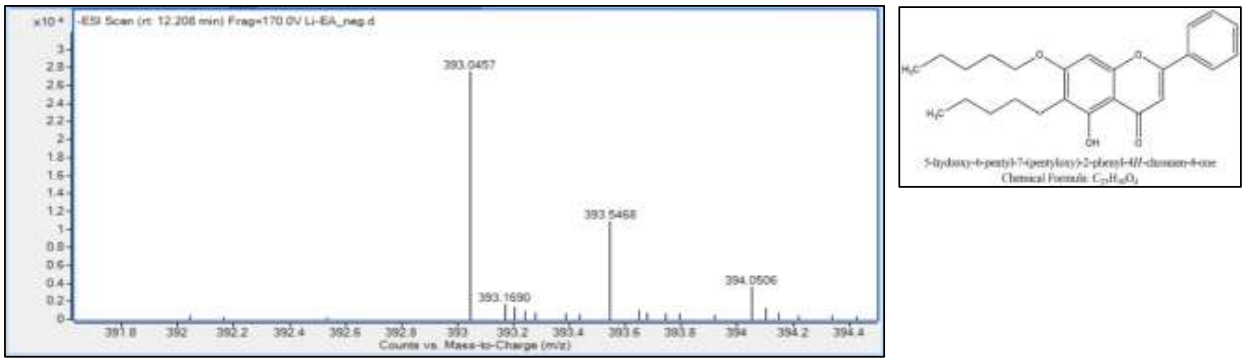


FIGURE 18. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 393.0457

Peak no. 14, Name: **1,2,3,4,6-penta-*O*-galloly-β-D-glucose**
Exact Mass: 940.1182, (m/z-H); [m/z-1]= 939.1182 (RT: 12.988 min)

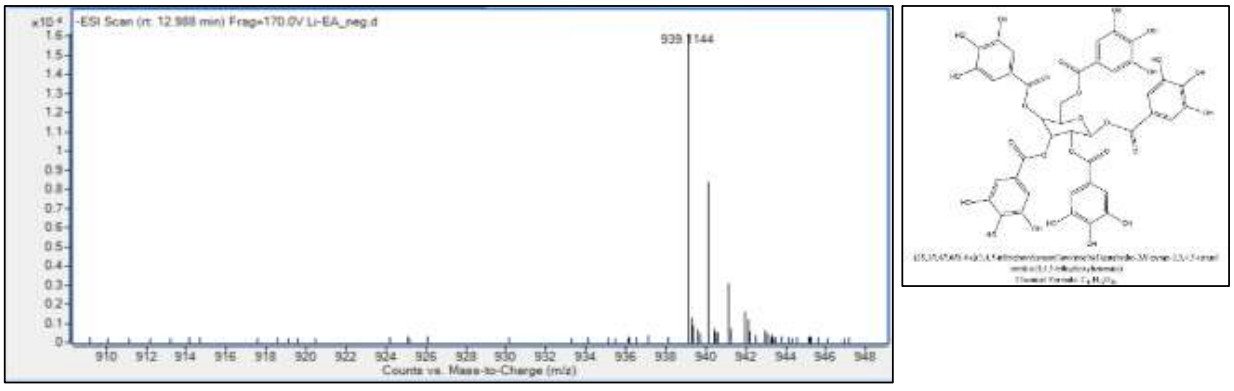


FIGURE 19. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 939.1144

Peak no. 15, Name: **Luteolin-7-*O*-glucoside.**
Exact Mass: 448.1006, (m/z-H); [m/z-1]= 447.1006 (RT: 14.366 min)

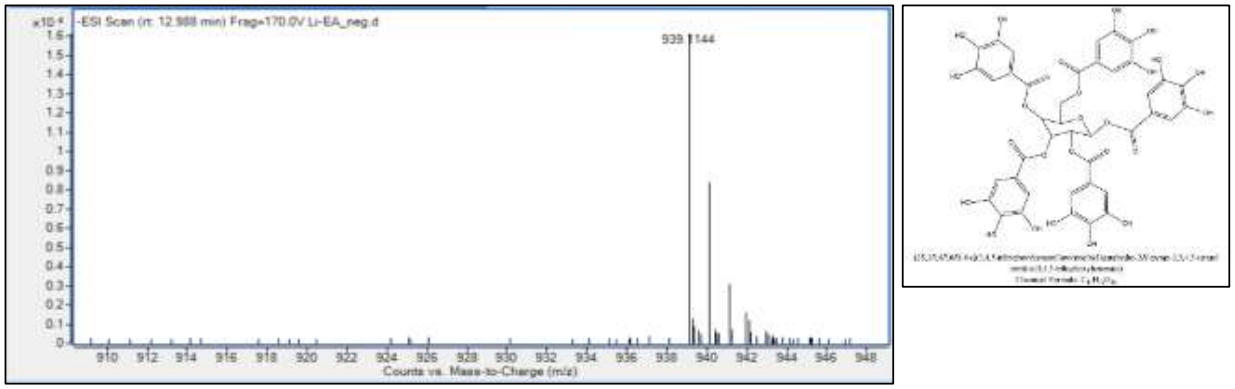


FIGURE 20. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 447.0929

Peak no. 16, Name: **Apiin**
Exact Mass: 564.1479, (m/z-H); [m/z-1]= 563.1479 (RT: 14.432 min)

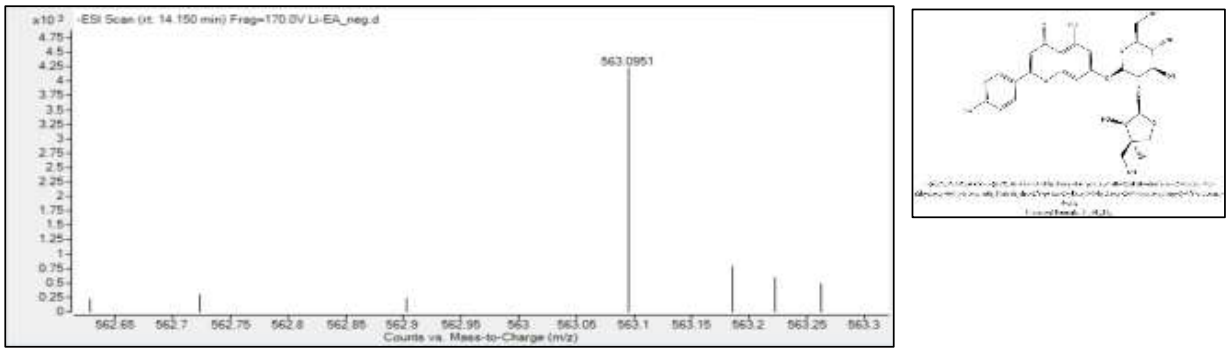


FIGURE 21. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 563.0951

Peak no. 17, Name: **Lawsone**
Exact Mass:174.0317, [m/z-1]= 173.0317(RT 14.598 min)

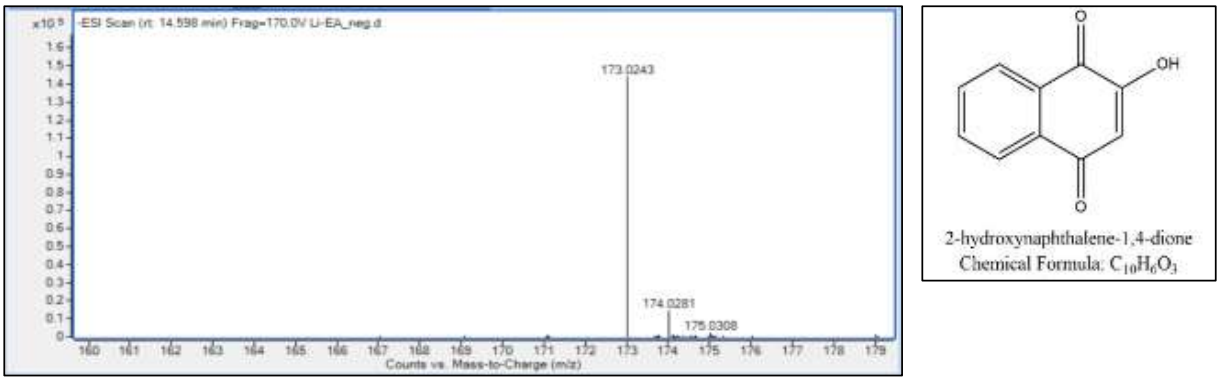


FIGURE 22. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 173.0242

Peak no. 18, Name: **Isoplumbagin**
Exact Mass: 188.0473, (m/z-H); [m/z-1] = 187.0473 (RT: 14.847 min)

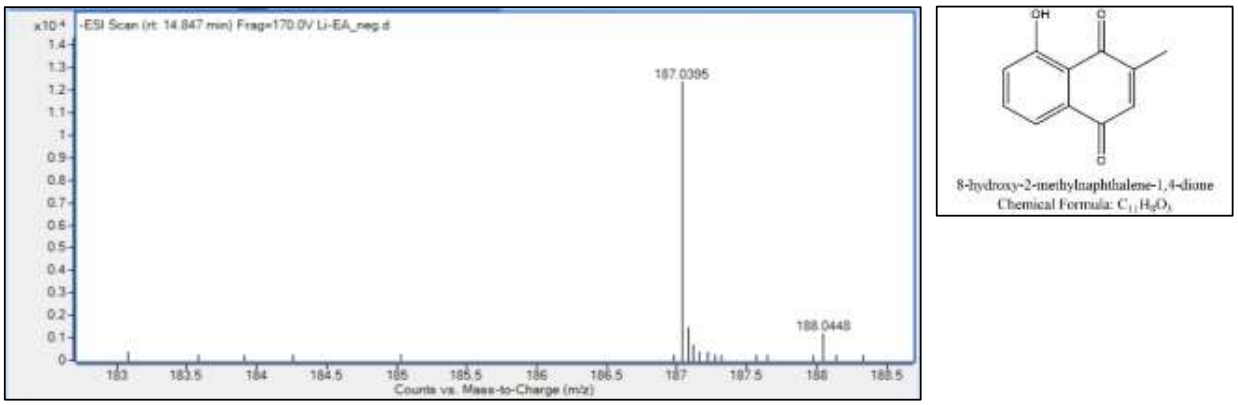


FIGURE 23. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 187.0395

Peak no. 19, Name: **Daphnorin**
Exact Mass: 514.1111, (m/z-H); [m/z-1] = 513.1111 (RT: 15.411 min)

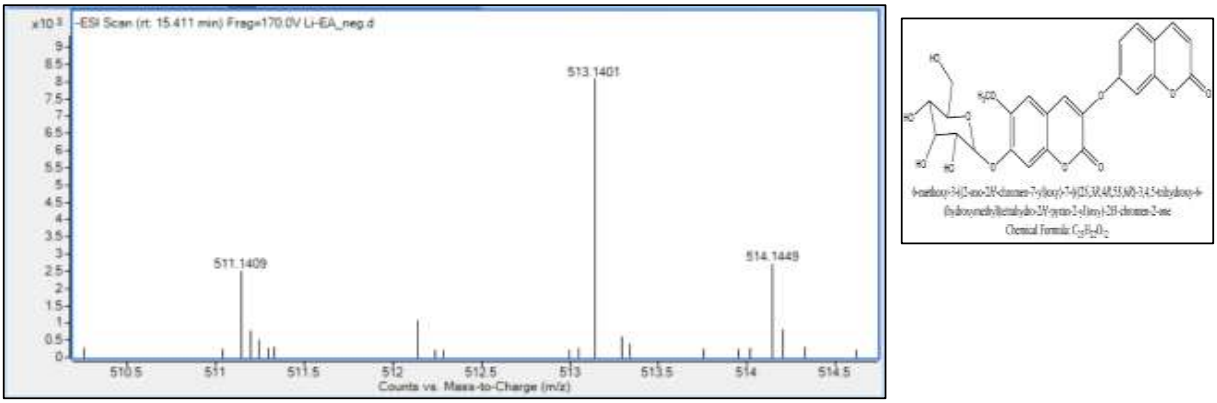


FIGURE 24. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 513.1401

Peak no. 21, Name: **Scutellarin**
Exact Mass: 462.0798, (m/z-H); [m/z-1] = 461.0798 (RT: 16.407 min)

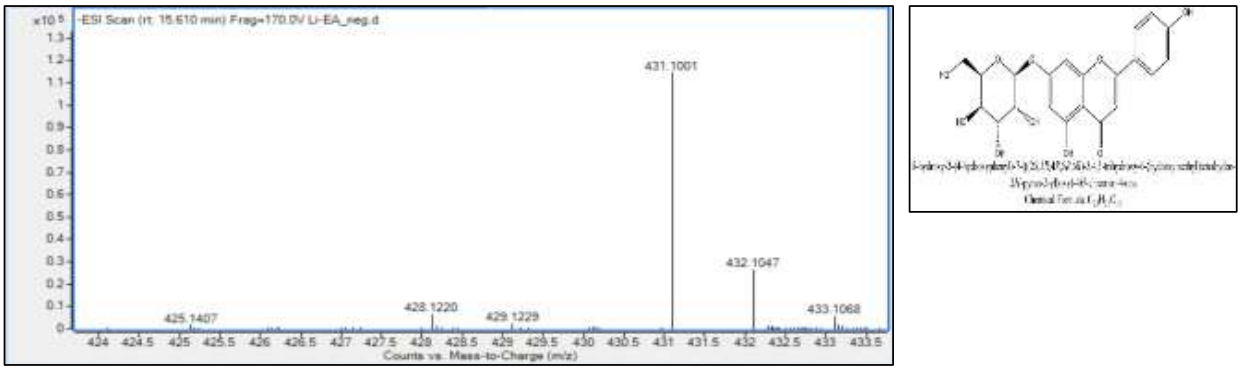
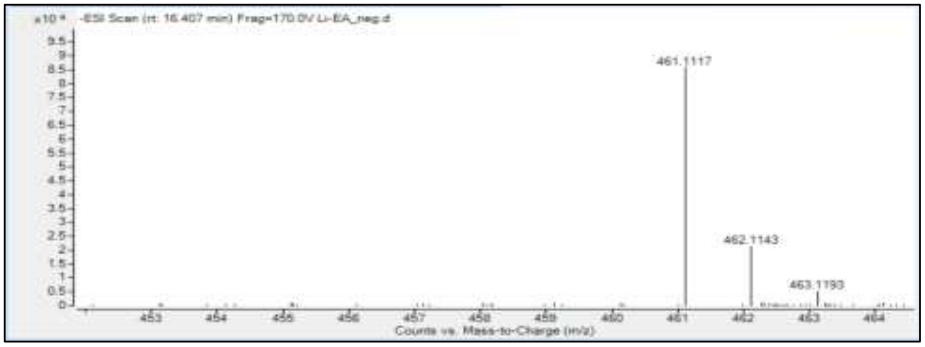


FIGURE 25. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 413.1001

Peak no. 21, Name: **Scutellarin**
Exact Mass: 462.0798, (m/z-H); [m/z-1] = 461.0798 (RT: 16.407 min)



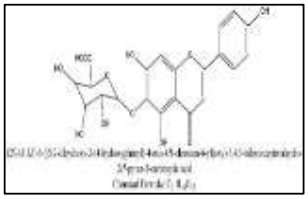
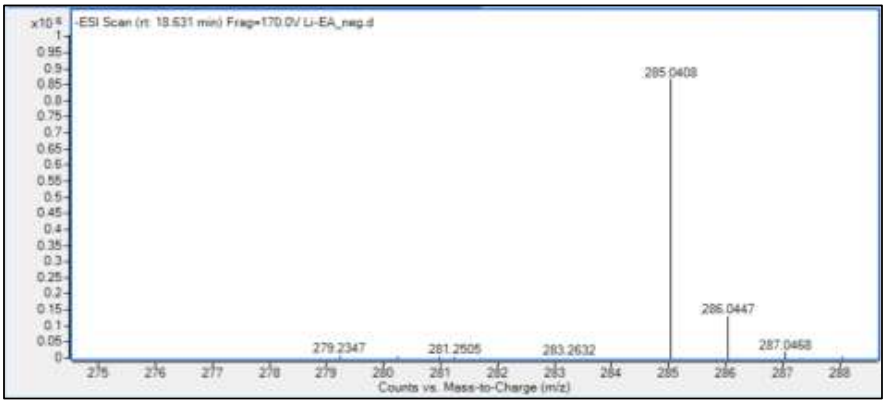


FIGURE 26. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 461.1117

Peak no. 22, Name: **Luteolin**
Exact Mass: 286.0477, (m/z-H); [m/z-1] = 285.0477 (RT: 18.631 min)



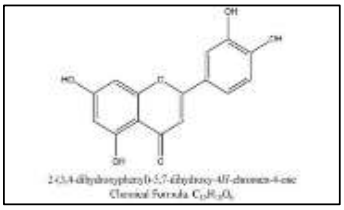
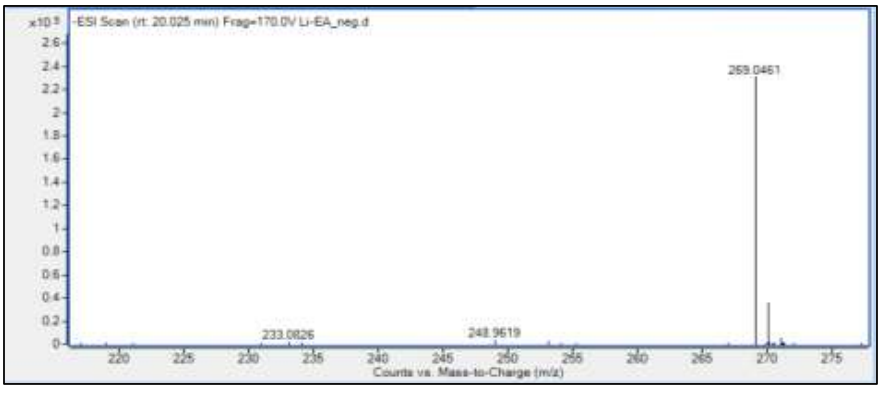


FIGURE 27. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 285.0408

Peak no. 23, Name: **Apigenin**
Exact Mass: 270.0528, (m/z-H); [m/z-1] = 269.0528 (RT: 20.025 min)



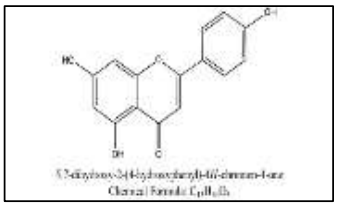


FIGURE 28. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 269.0461

Peak no. 24, Name: **Daphneside**
Exact Mass: 502.1323, (m/z-H); [m/z-1] = 501.1323 (RT: 22.763 min)

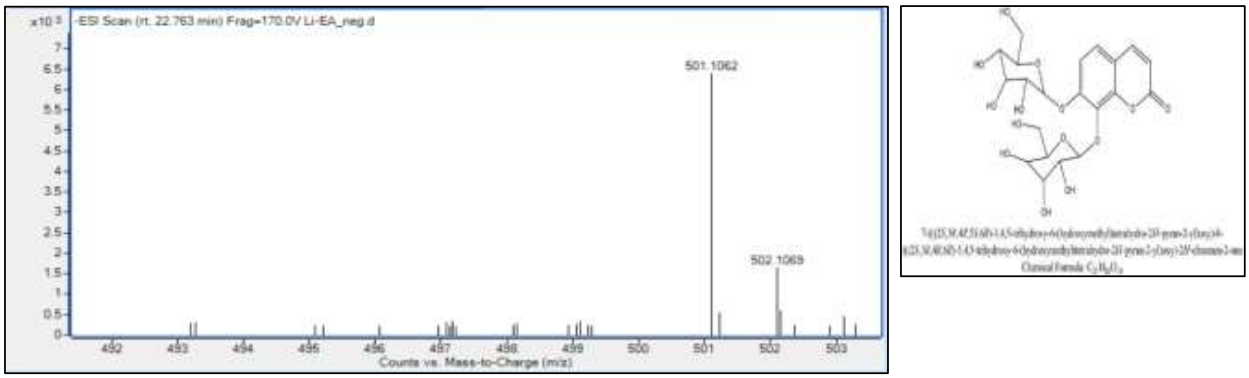


FIGURE 29. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 501.1062

Peak no. 25, Name: **Acacetin**
Exact Mass: 284.0685, (m/z-H); [m/z-1] = 283.0685 (RT: 25.004 min)

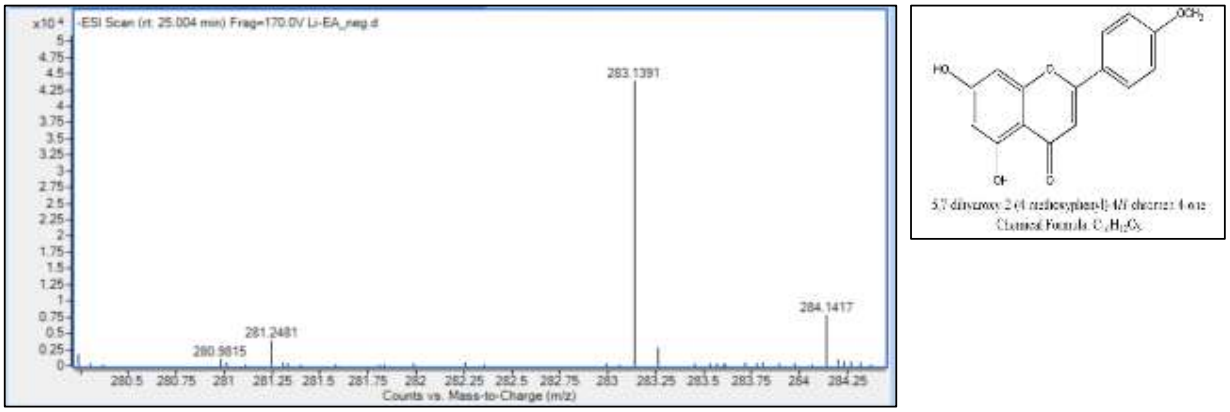


FIGURE 30. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 283.1391

Peak no. 26, Name: **3',4' dimethoxyflavone**
Exact Mass: 298.0841, (m/z-H); [m/z-1] = 297.0841 (RT: 25.651 min)

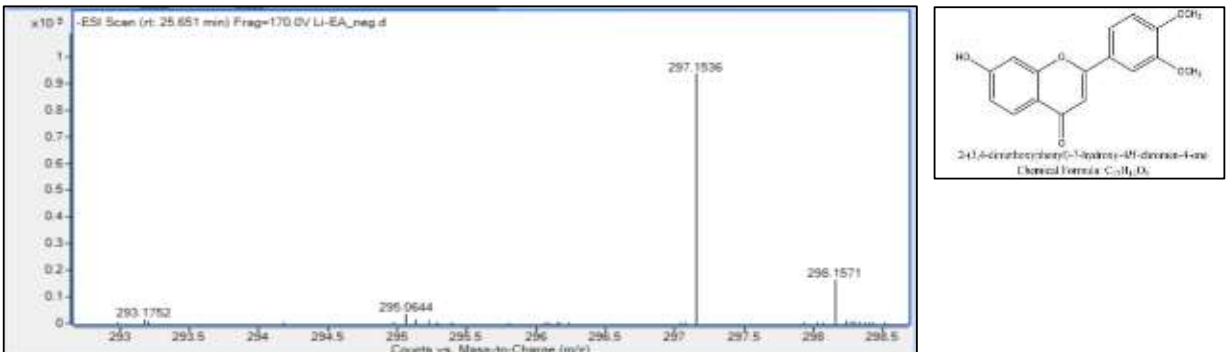


FIGURE 31. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 297.1536

Peak no. 27, Name: **Lacoumarin**
Exact Mass: 218.0579, (m/z-H); [m/z-1] = 217.0579 (RT: 38.198 min)

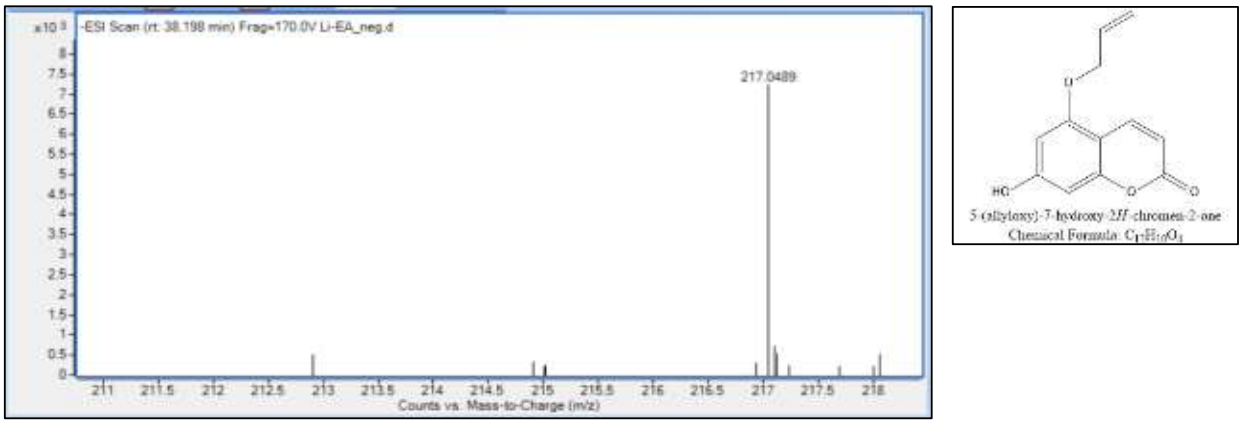


FIGURE 32. Fragmentation pattern from the MS/MS spectrum of the major peak with m/z value 217.0489

CONCLUSION

Many researchers had reported the antioxidant activities of Henna extracts. The antioxidant activities have confirmed the role of the phenolic compounds in the observed activities (Aqil et al. 2006; Hosein & Zinab 2007; Hsouna et al. 2011; Molina-García et al. 2018).

Compounds such as lalioside (peak no. 11) as a potential source of new natural antioxidants (Hsouna et al. 2011), apiin (peak no. 16), lawsone (peak no. 17), apigenin (peak no. 23), luteolin (peak no. 22), cosmosiin (peak no. 20), p-coumaric acid (peak no. 29), 2-methoxy-3-methyl-1,4-naphthoquinone (peak no. 30) produced by the leaves, displayed good antioxidant activities against ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] compared to ascorbic acid (Mikhaeil et al. 2004) as shown in Table 2.

We manage to detect compound esculetin in peak no. 9 (Figure 14), lalioside (Figure 16), apiin (Figure 21), lawsone (Figure 22), cosmosiin (Figure 25), luteolin (Figure 27), apigenin (Figure 28), in the LC-MS analysis which were recorded by Hsouna et al. (2011) as compound possess good antioxidant activity.

Compound fraxetin is a natural coumarin clinically use as anticoagulant agents shown in peak no. 10 (Figure 15) (Liang et al. 2017). Compound 3',4' dimethoxyflavone in peak no. 12 have shown its potential for anticancer properties (Figure 17) (Lee & Safe 2000) and compound 3',4'-dimethoxyflavone in peak no. 26 (Figure 31) also have shown anticancer treatment (Kaur et al. 2013). Compound luteolin-7-*O*-glucoside indicated in peak no. 15 (Figure 20) (Park & Song 2018) and compound isoplumbagin indicated in peak no. 18 (Figure 23) (Ponugoti 2018) were reported to have the potential for anti-inflammatory activity.

Zhu et al. (2017) have demonstrated in clinical studies of compound scutellarin in peak no. 21 show the potential of an anti-tumour agent for cancer treatment (Figure 26). Compound acacetin indicated in peak no. 25 (Figure 30) is an O-methylated flavone that exhibits anti-inflammatory and antiviral activity which were prevented with HIV-1 activation (Kumar & Pandey 2013).

From the investigations, an extract *of L. inermis* have shown predominantly the presence of phenolic compounds such as coumarins, flavonoids, naphthalene, and gallic acid (Figure 8) derivatives which could be glycosylated (Hsouna et al. 2011). Wide range of biological activities have been attributed to henna, including antibacterial, virucidal (inactivate viruses), antiparasitic, anti-inflammatory (Imam et al. 2013), analgesic (relieve pain) and anticancer properties, as well as hepatoprotective, immunomodulatory, anthelminthic, antitrypanosomal (control sleeping sickness) and antioxidant activities (Semwal et al. 2014).

It also provided useful evidence regarding the safety of this traditional medicinal plant as mentioned in the ethnobotany for medicinal bloated and stomach ache in Malay medicine reported in the book medicine in Malay villages vol 2 (Werner 2002). Therefore, this finding is suitable for further research on the gastroprotective activity of *L. inermis.*

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