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UPLC-ESI-MS/MS and Various Chromatographic Technique for Identification of Phytochemicals in *Populus euphratica Oliv.* Leaves Extract

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Abstract

The aim of this study is to screen the phytochemicals found in *Populus euphratica* leaves since this type of trees are used traditionally by many villagers as treatment for eczema and other skin disease and also this plant is poorly investigated for their phytochemicals especially in Iraq. Phytochemical screening of the extracts obtained from the n-hexane and chloroform fraction of leaves of *Populus euphratica* was done by Thin-layer chromatography and various spraying reagents to test if alkaloids, sterols and other compounds are present. UPLC-electrospray ionization –tandem mass spectroscopy along with GC-MS and HPTLC are used to identify the phytochemicals present in the plant leaves.UPLC-ESI-MS/MS method 20 compounds have been identified in various fractions among which are protopine alkaloids. salicin, salicortin, tremulacin. GC-MS showed that the observed data obtained are matched with that in NIST library and confirmed the presence of Hexadecanoic acid trimethylsilyl ester in 43.80% beta-Sitosterol in 37.14% and Diisooctyl phthalate 11.46%.UPLC-ESI-MS/MS is a powerful method for the identification of compounds in mixture based on comparison of their molecular, weight retention time and MS/MS fragmentation. Protopine alkaloid is identified for the first time in *Populus euphratica* and genus Populus. GC-MS is a valuable method for both qualification and quantification of various phytochemicals that are volatile in nature

Keywords: *Populus euphratica*, GC-MS, phytochemical, UPLC-ESI-MS/MS

كشف المواد الكيميائية النباتية في مستخلص اوراق نبات الغرب باستخدام تقنيات كروماتوغرافيا متعددة و كروموماتوغرافيا السائل فائقة الأداء بوجود طيف الكتلة المتتالي والتأين برذاذ الكهرباء

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الخلاصة

تهدف هذه الدراسة إلى اكتشاف المواد الكيميائية النباتية الموجودة في أوراق نبات الغرب، لأن هذه الأنواع من الأشجار تستخدم تقليديًا من قبل العديد من القرويين كعلاج للأكزيما وأمراض الجلد الأخرى وهناك دراسات قليلة حول المركبات الكيميائية الموجودة في هذ النبات خاصة في العراق.

تم اجراء الفحص الكيميائي النباتي للمستخلصات التي تم الحصول عليها من اجزاء الهكسان والكلوروفورم لأوراق الغرب وذلك للتأكد من وجود قلويدات ، ستيرول ومركبات أخرى بواسطة كروماتوغرافيا الطبقة الرقيقة وكواشف الرش المختلفة. كروماتوغرافيا السائل فائقة الاداء مع التأين برذاذ الكهرباء(UPLC-ESI) واستخدام التحليل الطيفي الشامل جنبا إلى جنب مع كروماتوغرافيا الغاز - مطياف الكتلة وكروماتوغرافيا الطبقة الرقيقة عالية الاداء لتحديد المواد الكيميائية النباتية الموجودة في النبات.باستخدام طريقة UPLC-ESI-MS / MS، تم تشخيص ٢٠ مركبًا من المستخلص من بينها قلويد البروتوبين ، الساليسين ، الساليكورتين ، التريميولاسين . أظهرت GC-MS أن البيانات المرصودة التي تم الحصول عليها تتزامن مع تلك الموجودة في مكتبة NIST وأكدت وجود ثلاثي ميثيل سيليل أستر حامض الستاديك في ٤٣,٨٠ ٪، وبيتا سيتوستيرول في ٣٧,١٤ ٪ و ديسوكتيل فثاليت ١١,٤٦ ٪.UPLC-ESI-MS / MS هو وسيلة قوية لتحديد المركبات الموجودة في خليط معين بواسطة المقارنة بين وزنها الجزيئي ووقت الاحتفاظ وتفتيت طيفها الكتلي المتتالي، تم تحديد قلويد البروتوبين لأول مرة في نبات الغرب وفي جنس الصفصافيات GC-MS هي طريقة قيمة لمعرفة الماهية الكمية والنوعية للعديد من المواد الكيميائية النباتية ذات الخاصية الطيارة.

الكلمات المفتاحية: كروماتوغرافيا الغاز - مطياف الكتلة، نبات الغرب، كروماتوغرافيا السائل فائقة الاداء مع التأين برذاذ الكهرباء مع التحليل الطيفي الشامل(UPLC-ESIMS/MS) ، المواد الكيميائية النباتية.

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Introduction

Populus euphratica tree is a member of the Salicaceae family, which abundant of Phenols and its glycosides such as Salicin and populin[(1)].

Previous studies on *Populus euphratica* have reported that some phenolic compounds [(2)] have been identified and volatile oils [(3)] have been detected in this plant. W. Wei et al. (2015) undertook a detailed chemical investigation of the leaves of *Populus euphratica* that afforded 13 compounds, among which 6-O-ciscinnamoylsalicin and 6-O-benzoylsalicortinol were new compounds. The spectral data of 6-O-trans-cinnamoylsalicin and salicortinol were reported for the first time. Meanwhile, nine known compounds were characterized as follows: salicin [(4)] benzyl-O-b-D-glucopyranoside tremulacin[(5)], salireposide [(6)] cinnamrutinose A [(7)], and saligenin [(8)]. Rahimi,et-al (2008) have concluded that the smoke of burnt leaves of the *Populus euphratica* tree was curative in about 66% of patients with warts and there was a low recurrence rate of about 4%. [(9)] .

Populus euphratica extract has been used traditionally by some villagers in some areas of Iraq in the treatment of eczema and various skin condition. So in this study we will investigate the phytochemicals found in the leaves of this naturally abundant tree found in every city in Iraq and trying to resonate this traditional use.

The goal of this study is investigating and screening the phytochemicals and their proportions in hexane fraction of leaves of *Populus euphratica* tree naturally grown in Iraq since there is no phytochemical study had been done previously in Iraq and trying to resonate its traditional use in the treatment of eczema.

Material and Methods

Plant material collection

Leaves of *Populus euphratica* Oliv. was collected from the bank of the Tigris river in the periphery of Tikrit city in April 2018, Authenticated in Iraq Natural History Research Center and Museum by dr. khansaa Rasheed. Leaves were cleaned dried in shade at room temperature and then pulverized and stored for further use.

Method of work

100gm of air-dried powder of the leaves is weighted and defatted with petroleum ether overnight to get rid of chlorophyll and waxy material then extracted in soxhelt with 90% methanol for 9hr, the extract is combined and dried by rotary evaporator the dry extract is weighted and the yield of extraction is calculated to be 37gm.

The dry extract is dissolved in 100ml of distilled water. Then partitioned with 100ml of n-hexane using a separatory funnel for three times all the upper layers are combined together and dried using rotary evaporators, the dried hexane fraction was weighted to be 7.5 gm. The aqueous part is partitioned with chloroform 100ml 3 times to get 17.2gm of chloroform extract. Again the aqueous part is partitioned with butanol to get butanol fraction (12g) symbolized B.B fraction. Thin-layer chromatography and the spraying with Liebermann-Burchard reagent were used to identify the hexane extract containing phytosterols. General identification test for alkaloid has been done with Dragendorff's reagent and Mayer's reagent both gave positive results for alkaloid [(11)] .

B.B fraction is hydrolyzed by reflex for 6 hrs. using 5% HCL to get Butanol after hydrolysis fraction symbolized B.A.

Readymade TLC pre-coated plate of 1mm GF254 for isolation and 0.25 mm GF254 was used for purification of isolated compounds.

Acid-base extraction of alkaloids from the crude extract

Part of the crude extract is suspended in n-hexane and partitioned with water to remove pigment and other non-polar compounds then the aqueous part is treated with NH_4OH to PH10 to liberate free alkaloid then equal volume of chloroform is added to separatory funnel partitioned and the lower organic layer was acidified with 5% H_2SO_4 to PH2 , and again partitioned with equal volume of water ,the aqueous layer now contain all alkaloid as salt, to this layer add NH_4OH to PH10 and partitioned with chloroform the chloroform layer now contain free tertiary alkaloids, this fraction is symbolized as chA fraction [(12)].

High-performance thin-layer chromatography (HPTLC) examination of chA B.B fraction

The presence of flavonoids and phenolic glycoside and alkaloids in the analyzed fractions were confirmed by using the modern technique of HPTLC using Eike-Reich/CAMAG-Laborator/ Switzerland, by comparing retention factor of analyzed sample with that of standards.

Method for UPLC-ESI-MS/MS

ESI-MS positive and negative ion acquisition mode was carried out on a XEVO TQD triple quadruple instrument Waters Corporation, Milford, MA01757 U.S.A, mass spectrometer

Column: ACQUITY UPLC - BEH C18 1.7 µm - 2.1 × 50 mm Column

Flow rate: 0.2 mL\min

Solvent system: consisted of

(A) Water containing 0.1 % formic

(B) Methanol containing 0.1 % formic acid

The sample (100 µg/mL) solution was prepared using high performance liquid chromatography (HPLC) analytical grade solvent of/MeOH, filtered using a membrane disc filter (0.2 µm) then subjected to LC-ESI-MS analysis. Samples injection volumes (10 µL) were injected into the UPLC instrument, Sample mobile phase was prepared by filtering using 0.2 µm filter membrane disc and degassed by sonication before injection. The parameters for

analysis were carried out using negative ion mode as follows: source temperature 150 °C, cone voltage 30 eV, capillary voltage 3 kV, desolvation temperature 440 °C, cone gas flow 50 L/h, and desolvation gas flow 900 L/h. Mass spectra were detected in the ESI between *m/z* 100–1000. The peaks and spectra were processed using the Maslynx 4.1 software and tentatively identified by comparing its retention time (Rt) and mass spectrum with reported data.

Results and Discussion

Analysis of fractions by high-performance thin-layer chromatography (HPTLC)

HPTLC is one of the most advanced forms of TLC, efficient for qualitative and quantitative analysis. table 3-1 show HPTLC results of standard flavonoids, alkaloids, phenolic glycoside, and the analyzed fractions.

Table 1. Phenolic glycoside, flavonoids and Alkaloid content of different extract fraction detected by HPTLC and their max Rf value.

fractions	Mobile phase	Isolated compounds	Reference standard	Rf of compound	Rf in fraction
chA	M5	C1	protopine	0.73	0.73
B.B	M4	S1	salicin	0.29	0.28
	M4		NeohesperidinN1	0.17	0.17
	M5	S1	Salicin	0.56	0.56
	M5		Neohesperidin. N1	0.60	0.60

HPTLC of B.B fraction

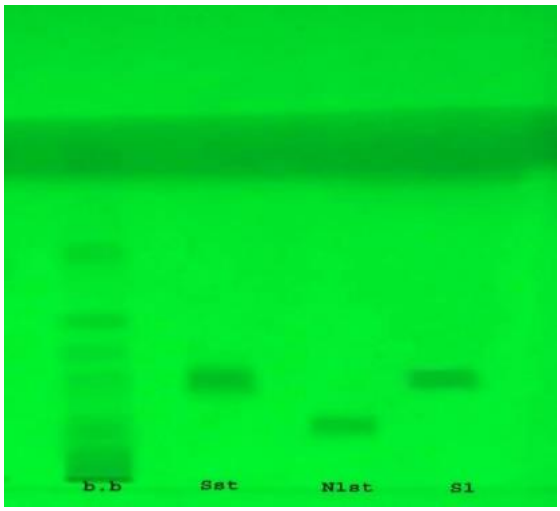


Figure1. HPTLC plate analyzed fraction with reference standard detection under UV light at 256nm (b.b=butanol before hydrolysis fraction, Sst=salicin standard ,N1= neohesperidin standard , S1 = isolated compound from b.b fraction) developed in [chloroform : methanol : acetic acid(70:20:10)] solvent system. symbolized M4.

HPTLC OF chA and B.B fractions

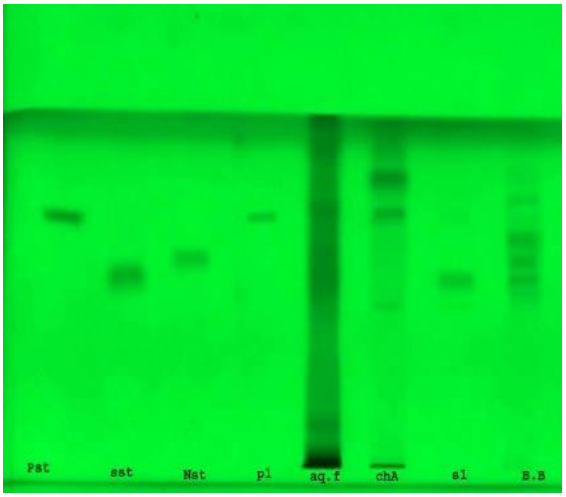


Figure 2 .HPTLC of analyzed fraction and reference standard at 256nm (Pst=protopine standard Sst=salicin standard, Nst=neohesperidin standard, P1=C1=isolated compound from chA fraction, aq.f=aqueous fraction after butanol hydrolysis, S1=isolated compound from B.B, chA=acid-base purification of chloroform fraction. Developed in [ethyl acetate: acetic acid: formic acid: water (70:10:10:10)] solvent system symbolized M5.

UPLC-ESI-MS/MS for tentative identification of compounds in fractions

Twenty phytochemicals have been putatively characterized by UPLC-ESI-MS/MS in *Populus euphratica* leaves based on comparison of mass fragmentation pattern, LC retention time and molecular weight with previous study and the literature in phytochemicals mass library such as Respect (http:// spectra.psc .riken. jp/ menta .cgi/ respect/search/keyword?page=1),
MONA (http: //mona .fiehnlab .ucdavis .edu /) ,
GNPS (https://gnps.ucsd.edu/ProteoSAFe /gnpslibrary.jsp?library=GNPS-NIST14-MATCHES),
Meltin (https://metlin.scripps.edu/ landing _page .php?pgcontent=batch_search).
comparing the results of mas fragmentation with the previous study also helpful in confirming the result. Sometime, the precursor ion is not as the molecular weight this is due to adduct formation which is common in ESI – mode especially with phenolic glucoside [11]. All the analysis have done, using XEVO TQD triple quadruple instrument (Milford, MA01757 USA, mass spectrometer)

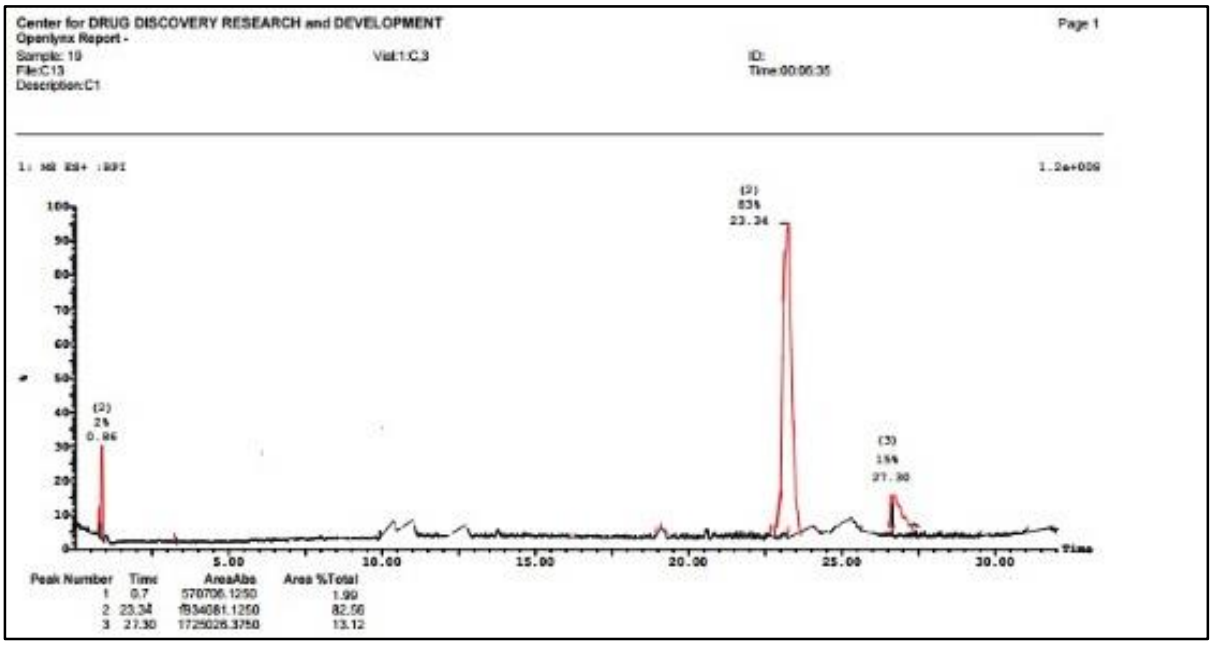


Figure3. UPLC chromatogram of isolated C1

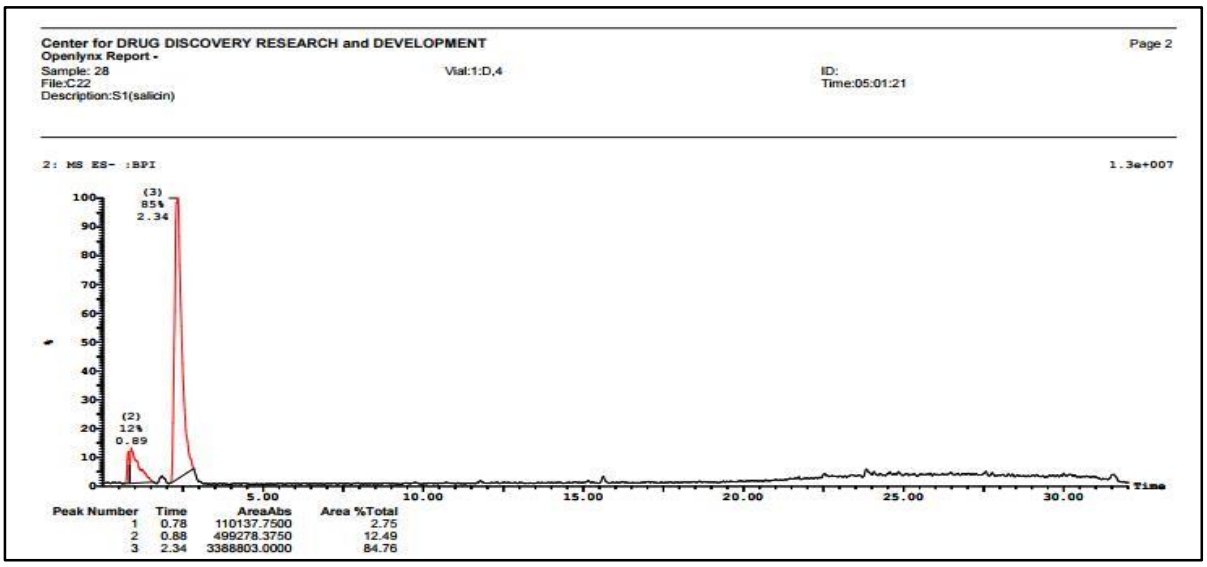


Figure 4 . UPLC chromatogram of isolated S1.

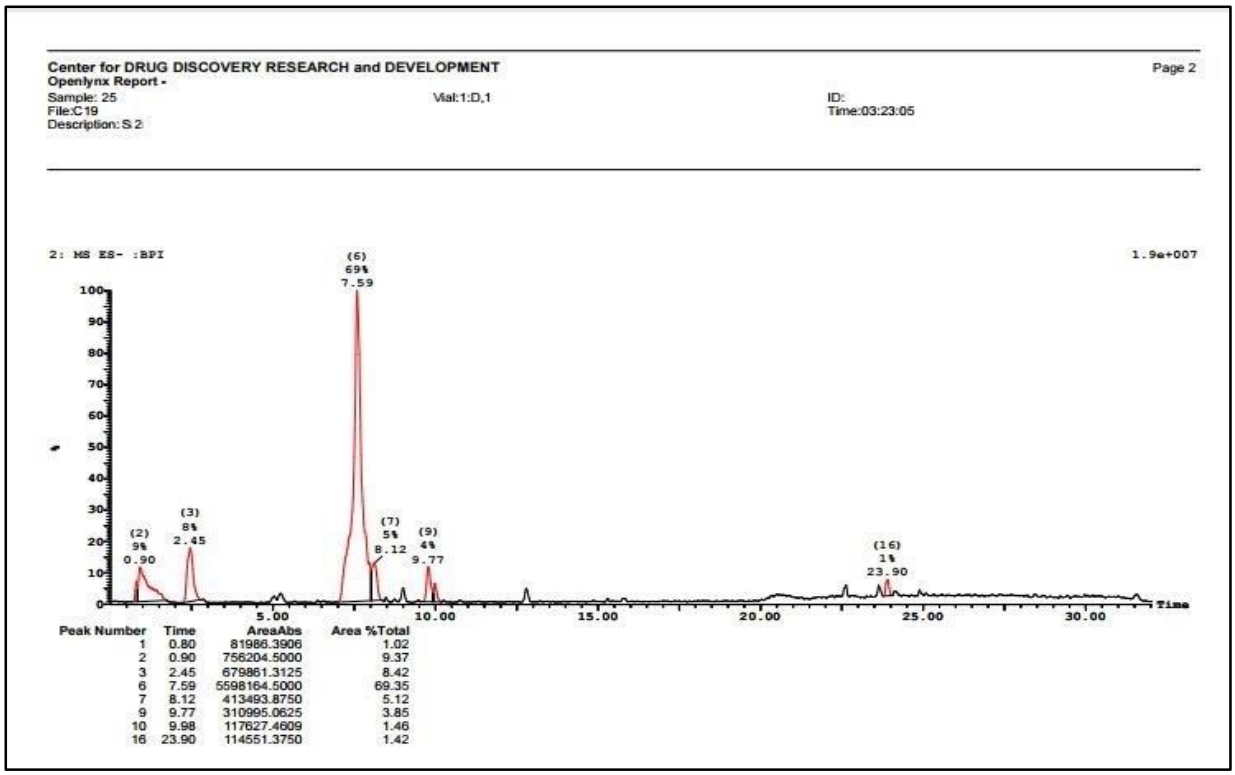


Figure 5. UPLC chromatogram of isolated aq1 compound.

Mass 1 of isolated compounds.

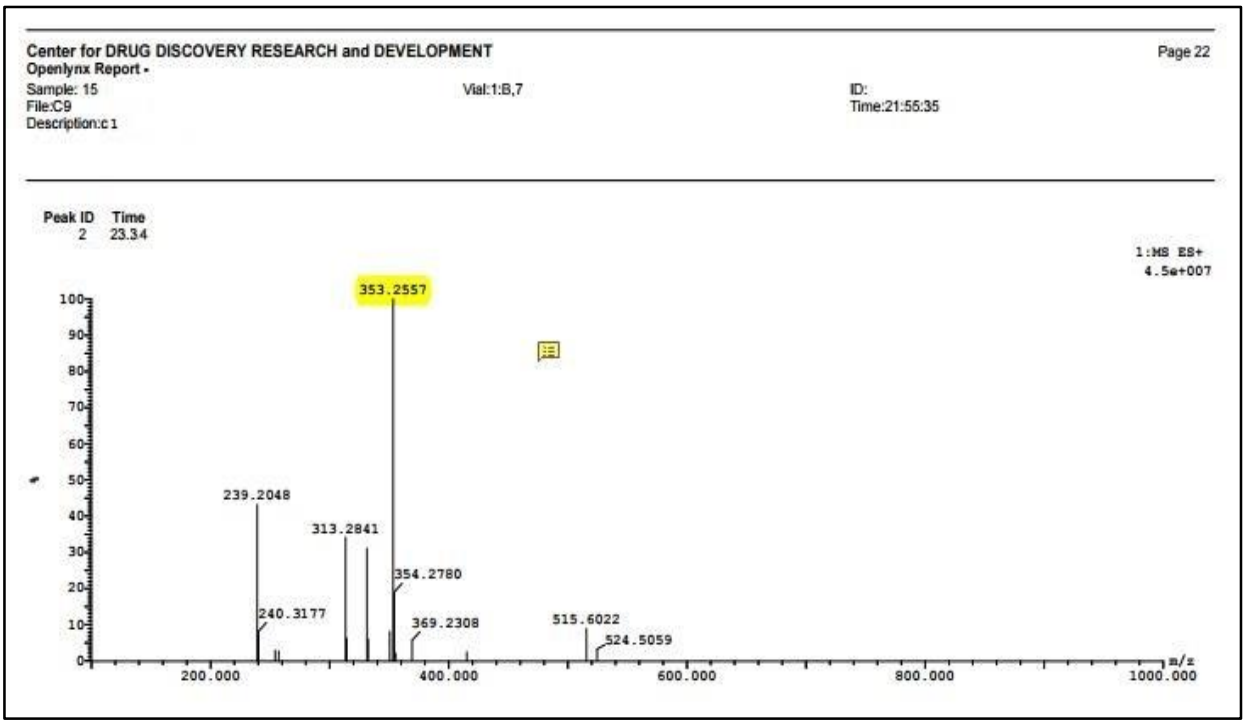


Figure 6. Mass 1 of isolated C1 compound in positive ion mode showing M and M+H

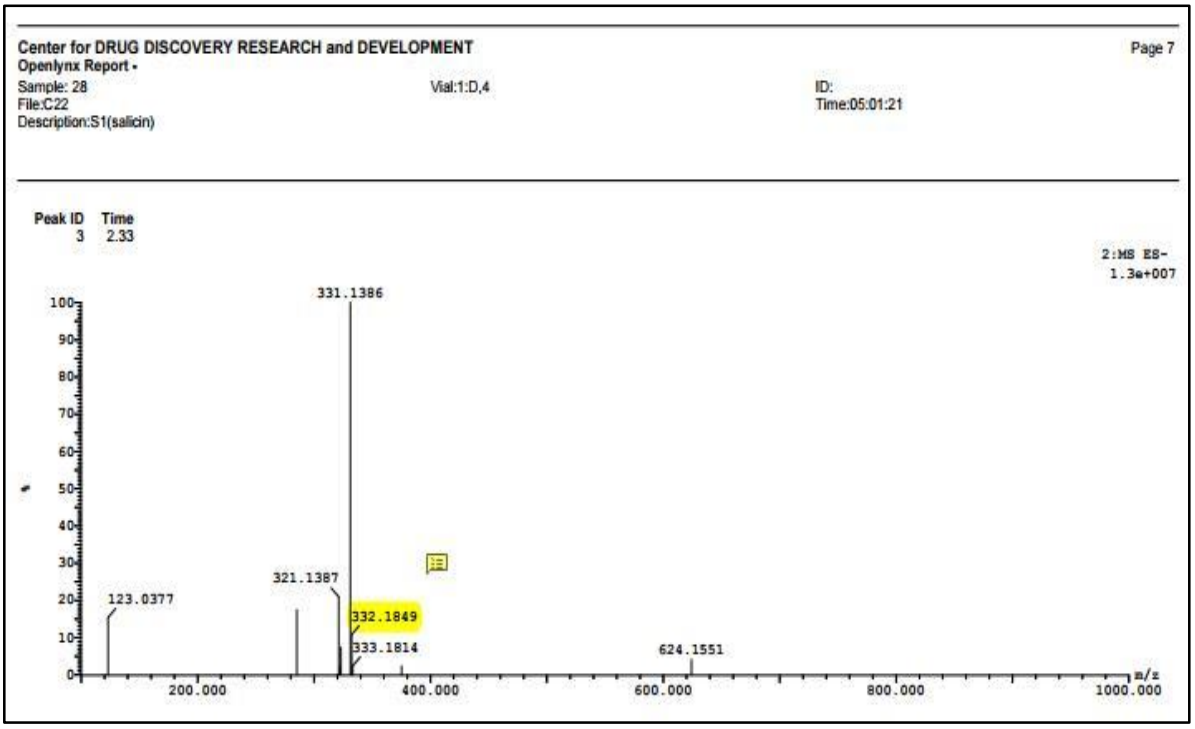


Figure7. Mass 1 of isolated S1 compound in negative ion mode showing M and M-H.

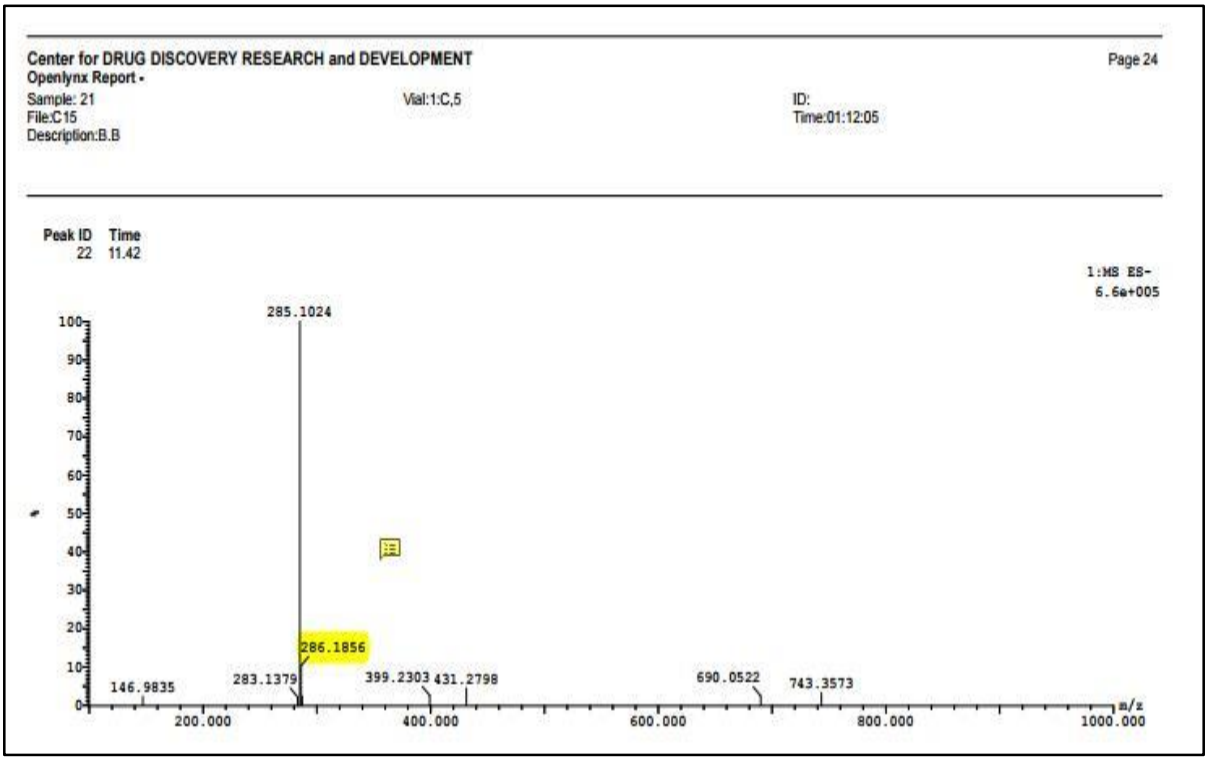


Figure 8. Mass 1 of predicted S1 in B.B fraction in negative ion mode showing M and M-H without adduct formation.

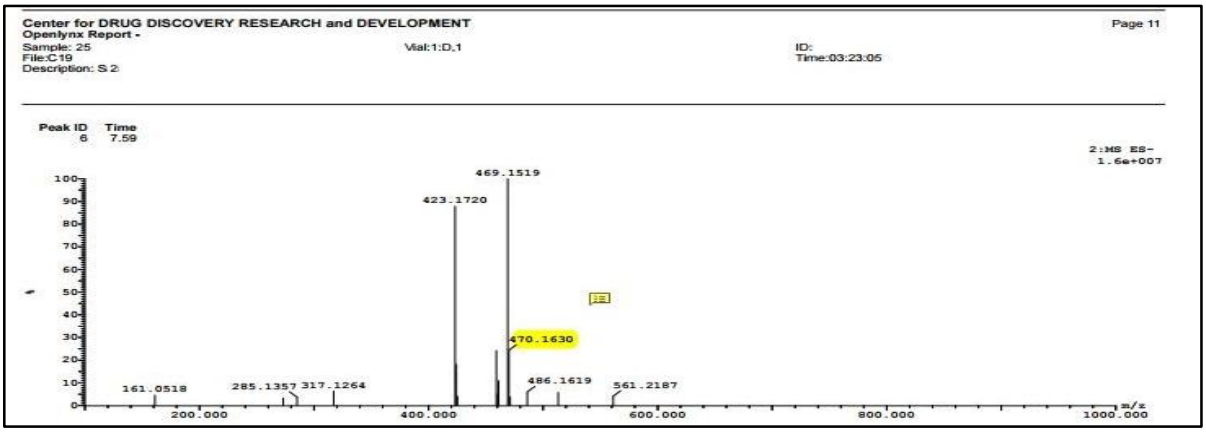


Figure 9. Mass 1 of isolated aq1 compound in negative ion mode showing M and M-H with adduct formation.

Table 3 .UPLC-ESI-MS/MS of the isolated compounds.

Peak no.	Rt	Compound sample	M.W	M+H, [M+FA-H], M-H	Ms/Ms fragments	Ref.
2	23.34	C1	353.1	354	148,163,159,188,190, 275,247,354	(12)(13)
2	2.33	S1	286	331[a]	121,123, 124,207,93,144	(14) (11)
6	7.59	Aq1	424	469[b]	423,317,316,299,285, 155,123,111,121	(11)(16)
2	8.72	N1	610	609	301,300,609,489,283,34 3,257,	(17)(18)

A, b these precursor ions are due to adduct formation presumably arising from combination with the formic acid present in the LC mobile phase which is common. In negative ESI mode especially with phenolic glycoside, the unmodified molecular ion is also present but with small intensity(11).

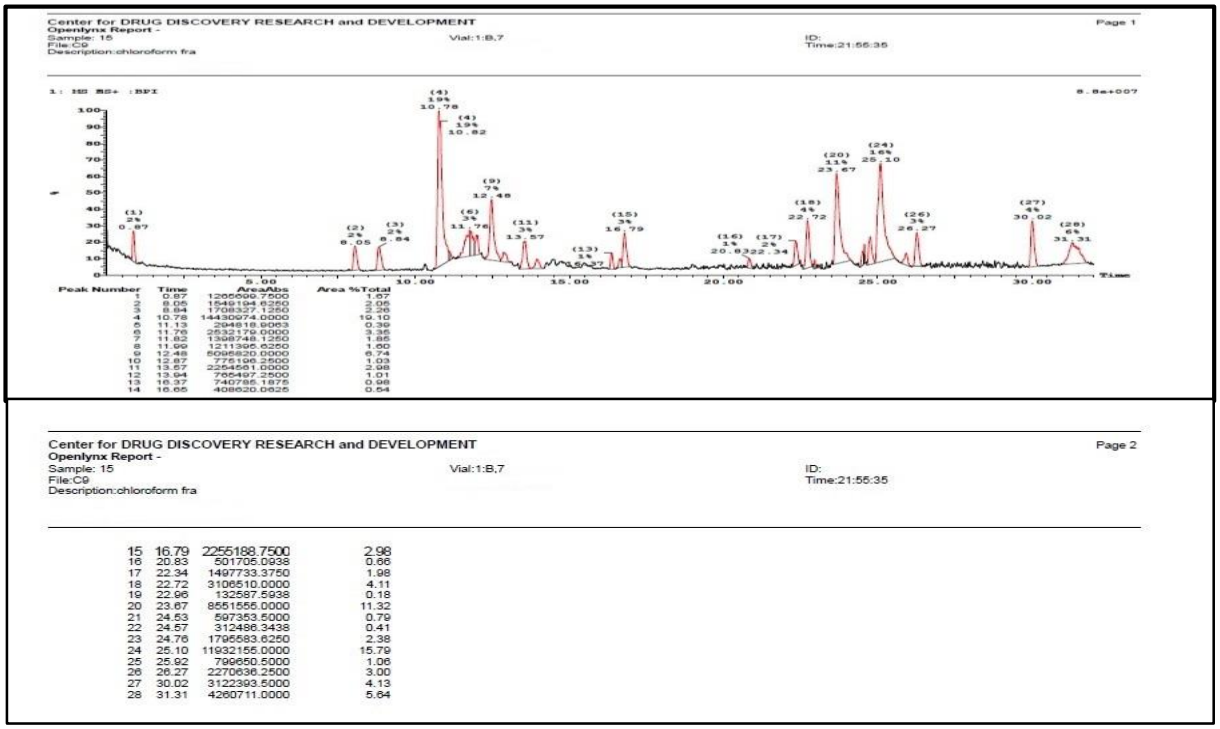
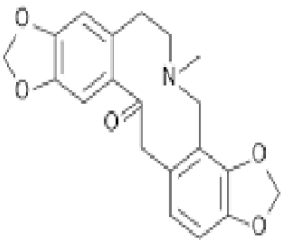
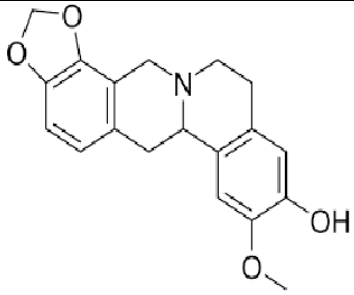
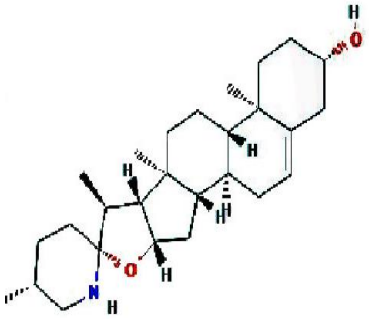
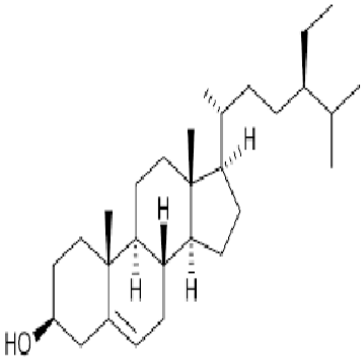


Figure10. UPLC chromatogram of chloroform fraction

Table 4. Identification result by UPLC-ESI-MS/MS fragmentation of chloroform fraction (number in Bold line represent base peak)

Peak no	20	3	4	2
Compound name	Protopine	Cheilanthifoline	solasodine	B-sitosterol
Compound classes	Benzylisoquinoline alkaloid	Benzylisoquinoline alkaloid	glycoalkaloid	Steroids
Rt	23.67	8.84	10.78	8.05
M.W	353.1	325	413	414
M+H	354	326	414	415
MS/MS fragments	**148**,163,159,188,190, 275,247,٢٣٦,354	**148**, 324 , 233,112, 178, 91, 89, 165, 310	**414**,396,271,252,104,157	**119**,295,107,109,57,219,344,91,81
				
reference	(12)(13)(20)	(13)(21)	(22)(23)	(24)(25)

6	di-Caffeoyl-coumaroyl spermidine	Polyamine alkaloids	11.76	615	616	616,**454**,470,308,163,292,147,220,221,204	(26)(27)
24	Zeatin-9-glucoside	Amino purine	25.10	381	382	**220**,219,136,148,202,205,135,	(28)(29)
7	Caffeoyl di_coumaroyl spermidine	Polyamine alkaloids	11.82	599	600	**483**,454,292,147,163,204,318,319,438	(26)(27)

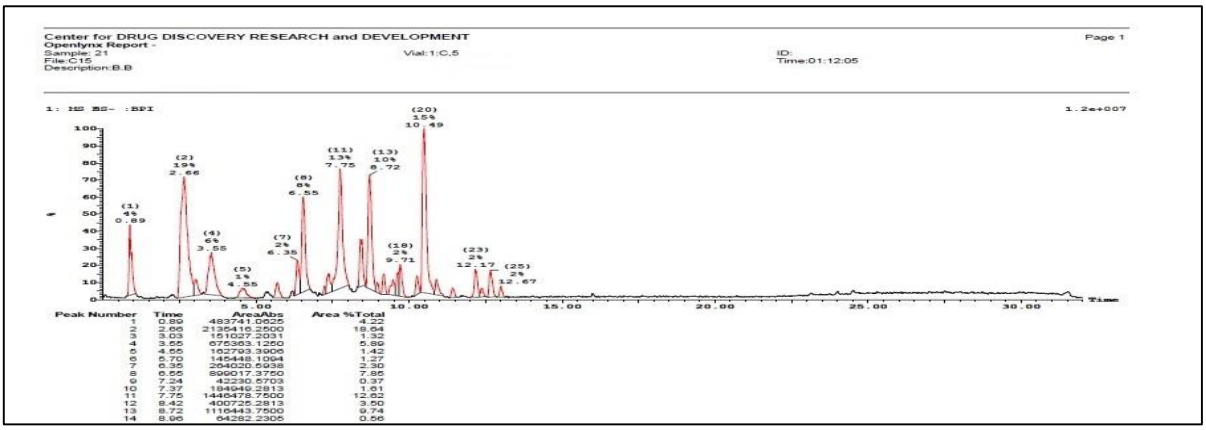
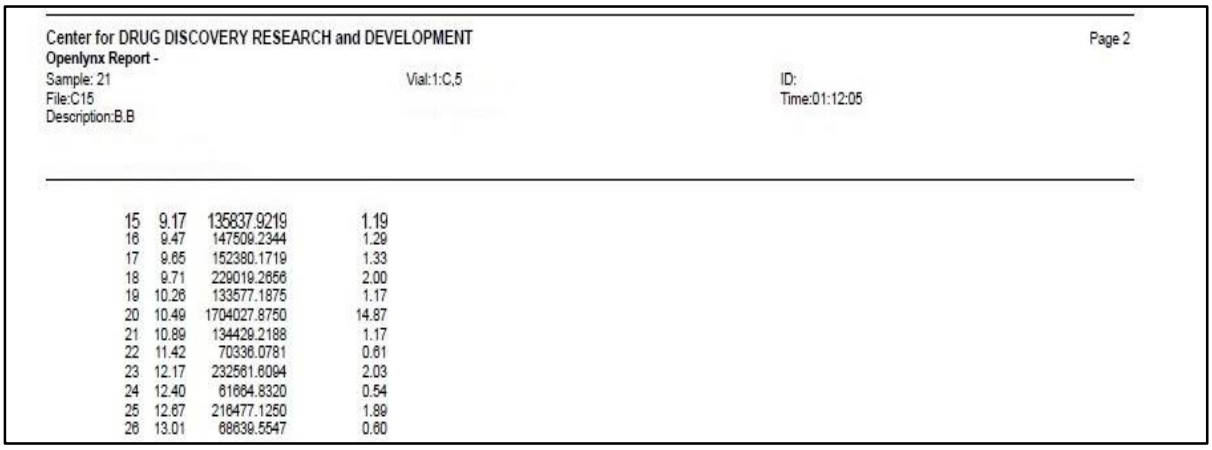


Figure 11. UPLC chromatogram of B.B fraction

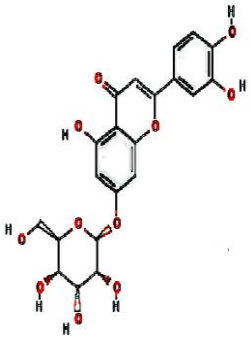
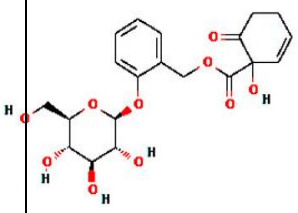

Center for DRUG DISCOVERY RESEARCH and DEVELOPMENT
Openlynx Report -
Sample: 21
File:C15
Description:B.B
Vial:1:C,5
ID:
Time:01:12:05
Page 2

15	9.17	135837.9219	1.19
16	9.47	147509.2344	1.29
17	9.65	152380.1719	1.33
18	9.71	229019.2656	2.00
19	10.26	133577.1875	1.17
20	10.49	1704027.8750	14.87
21	10.89	134429.2188	1.17
22	11.42	70336.0781	0.61
23	12.17	232561.6094	2.03
24	12.40	61864.8320	0.54
25	12.67	216477.1250	1.89
26	13.01	68639.5547	0.60

Continue to Figure11. UPLC chromatogram of B.B fraction .

Table 5 .UPLC-ESI-MS/MS of B.B fraction.

Table 5 .UPLC-ESI-MS/MS of B.B fraction.

Peak no.	Rt	Compound name	Compound class	M.W	M-H& [M+FA-H]	MS/MS fragments	Structure	Reference
12	8.42	Luteolin 7-O-beta-D-glucoside	Flavonoid glycosides	448.38	447	**284.9**,240.9, 266.9, 256.9, 242.9, 216.9, 198.9, 174.8, 150.8, 132.9		(30)
11	7.75	Salicortin	Phenolic glycosides	470 [M+FA- H]-	469[a]	423,317,316,299,285,**155**,123,111, 121		

13	2	18	20	5
8.72	2.66	9.71	10.49	5.44
Neohesperidin	Salicin	Isorhamnetin-3-glucoside	Phloretin-2'-O-glucoside	scopolin
Flavonoid glycosides	Phenolic glycosides	Flavonoid glycoside	Flavonoid O-glycosides	Coumarin glycosides
610	286	478	436	354
609	331[b]	477	435	353
301,300,609,489,283,343, 257	267,121,**123**, 124,207,93, 144	477,**315**,271,299,357,285, 243	**273**,274,229,167,435,123,125	**191**,191,176,179,133,337,325,148,255
				(36)(37)

A,b these precursor ions are due to adduct formation presumably arising from combination with the formic acid present in the LC mobile phase which is common in negative ESI mode especially with phenolic glycoside, the unmodified molecular ion is also present but with small intensity [11].

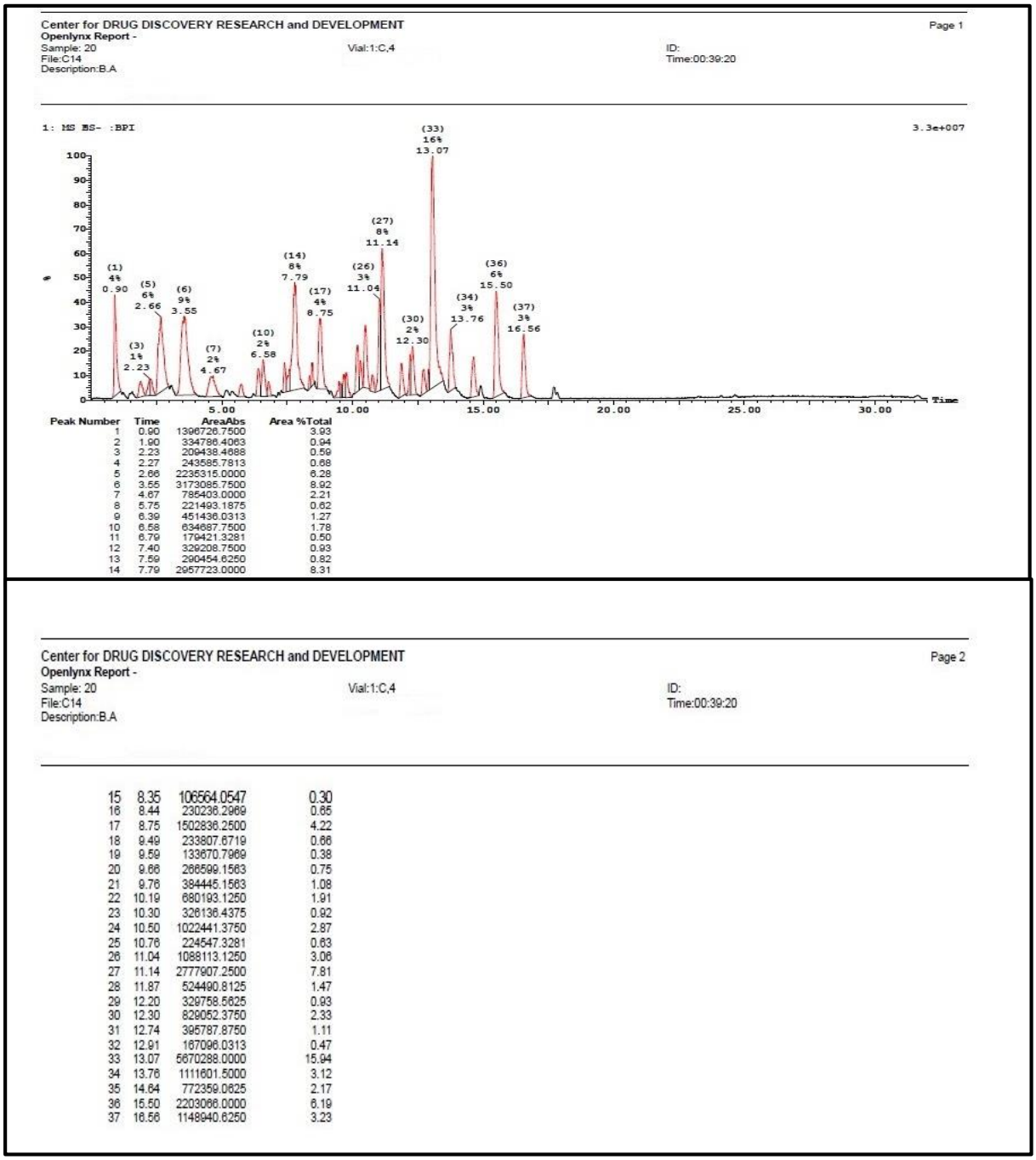


Figure12. UPLC chromatogram of B.A fraction

Table 6. UPLC-ESI-MS/MS of B.A fraction.

Peak no.	3	33	10	30
Rt	2.23	13.07	6.58	12.50
Compound name	Chlorogenic Acid	Salicyloylsalicin	(5-*p*-coumaroylquinic acid)	tremulacin
Compound class	Phenolic acid	Phenolic glycoside	Phenolic acid	Phenolic glycosides
M.W	354	406	338	528
M-H	353	405	337	573[c]
MS/MS fragments	190.9,**178.8**,126.8,84.9 ,172.9,110.8 ,92.9, 108.8	137,299,**243**,138,123,93373,85	**191**,162.9,173,127,171,85	405,**155**,527,137,121
Structure				
reference	(30)(38)	(39)(11)	(30) (40)	(41)(39) (11)

36	15.5	NCGC00347678-02!7-(3,4-dihydroxyphenyl)-1-(4-hydroxyphenyl)-5-[(2S,3R,4S,5R)-3,4,5-trihydroxyoxan-2-yl]oxyheptan-3-one	Phenylpropanoids and polyketides	462	508[d]	**311**,461,205,189,109,443,163	(42)(43)
1	0.90	Scopoletin	Coumarins	192	191	133,122,137, **176**,148	(44)(45)

Identification of isolated compounds

Compound C1: is isolated from fraction ChA and give positive results with alkaloidal identification results such as Dragendorff´s, Mayer´s, and Hager´s. So it is alkaloid. the UPLC-ESI-MS/MS method is powerful method for identification of phytochemicals it revealed that the molecular weight of this compound is 354 da and fragmentation pattern as in figure 13 during search in various mass libraries such as RESPECT, MONA, GNPS. the only alkaloid that matches both the molecular weight and fragmentation pattern is protopine alkaloid and to get 100% identification conformity protopine standard is compared with the isolated compound in HPTLC so both gave the same rf 0.75 value as in figure 2[12,13].

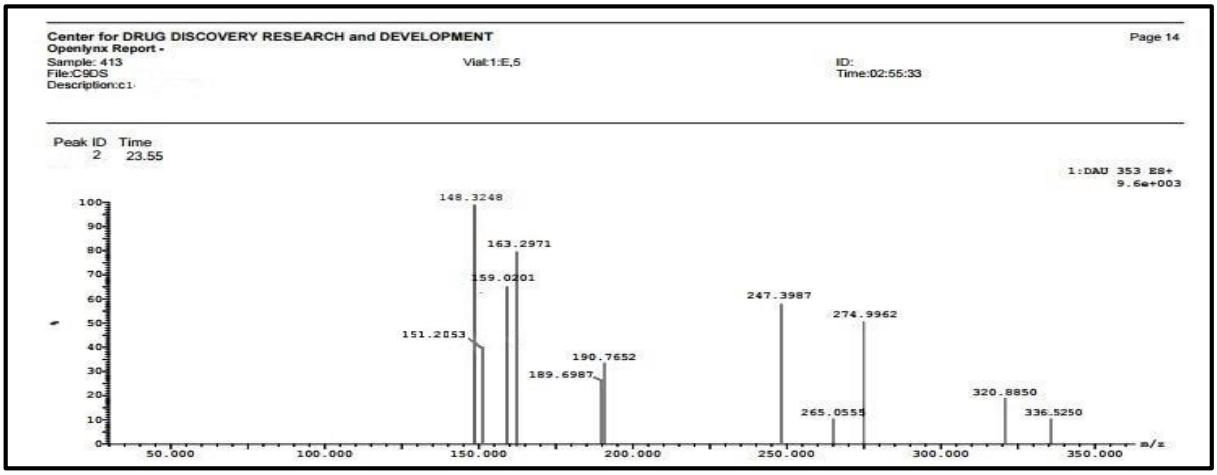


Figure13. Mass 2 fragmentation of protopine .

The protopine alkaloids can fragment by the RDA reaction, and, it also can undergo another characteristic fragmentation pathway. Selecting protopine as an example, fragment ion at m/z 206.0823 and 149.0603 in the MS/MS spectrum is generated by RDA C ring-opening (Figure 14) but given the presence of hydroxyl groups, the product ion at m/z 336.1209, m/z 188.0721 are probably formed by loss of H2O from the molecular ion and from the m/z 206.0823 ion [27].

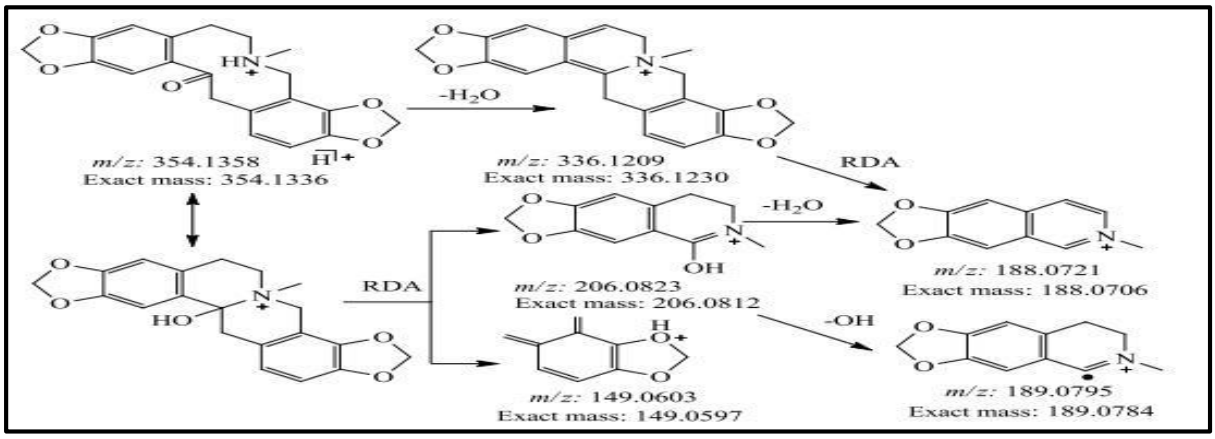


Figure 14 . MS/MS fragmentation pathway of protopine. [27] **.**

Compound S1: is isolated from butanol before hydrolysis fraction this compound gave positive results with KOH so it is glycoside and also gave gray violet color with Vanillin –glacial acetic acid reagent (VGA) this reagent gave gray violet color with salicin and its derivatives [46] the isolated S1 gave the same rf value 0.56 with standard in HPTLC analysis as in figure 2, in UPLC-ESI-MS/MS it gave molecular ion 331 instead of 285 of salicin, this precursor ion (331) is due to adduct formation presumably arising from combination with the formic acid present in the LC mobile phase which is common In negative ESI mode especially with phenolic glycoside [M+FA-H] [11]. Compound S1 has displayed the fragment ions at m/z 267, 123 (base peak), 121 and 93 in accordance with the loss of [M–H-18]−, [M–H-162]−, and [M–H-164]−, [M–H-192]−. A comparison of these MS/MS ions with the literature, Moreover, this compound has previously been reported in this plant, according to these data S1 is salicin [14] [11] . as in fragmentation pattern (Figures 15 and 16).

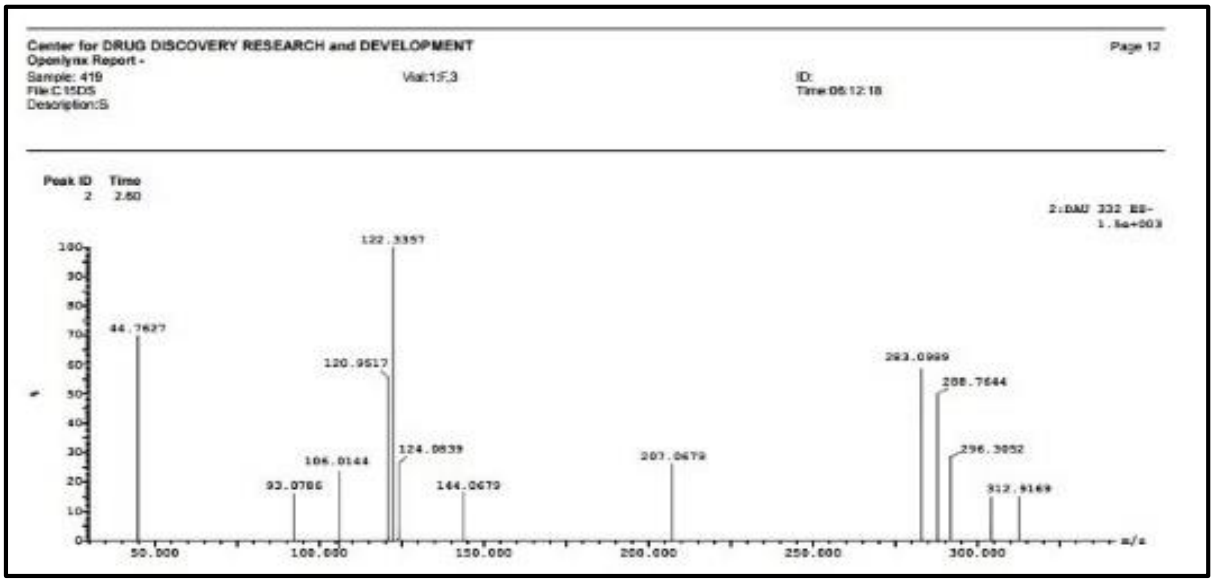


Figure 15 .Mass fragmentation of salicin

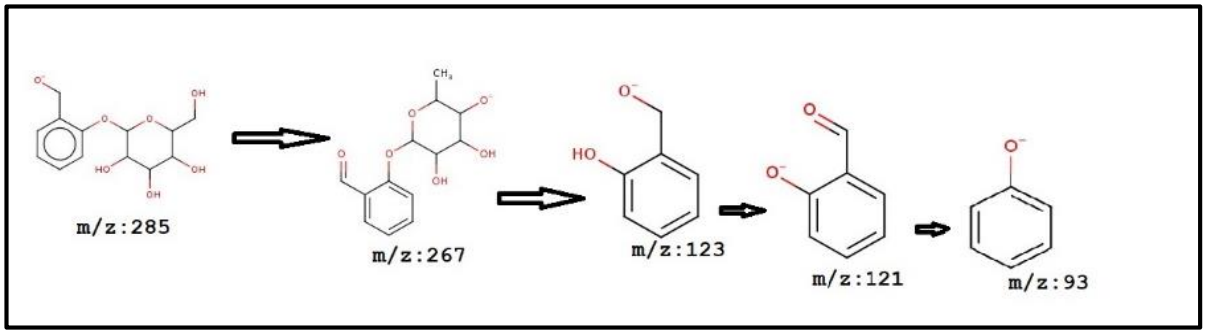


Figure 16. Salicin fragmentation pathway.

Compound aq1 : it is isolated from aqueous fraction (aqf) it gives positive result with KOH, it is also gave positive result to VGA reagent which confirm that it is from salicin derivatives, HPLC-ESI-MS/MS gave precursor ion 469 and mass fragmentation as that of salicortin and again due to adduct formation with formic acid salicortin (M.W 424) had result this molecular ion [M+FA-H] m/z 469 and mass fragment as in figure17[11,15] .

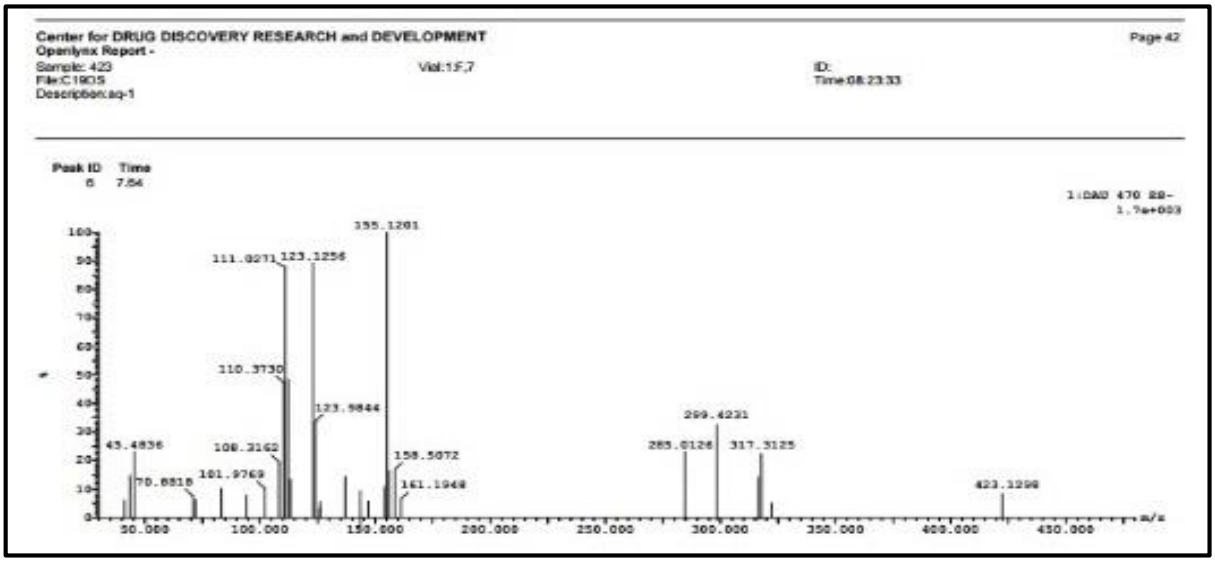


Figure17 .Mass fragmentation pattern of salicortin.

Compound aq1 has displayed fragmentation ion m/z 423 in the mass1 as in figure9 due to loss of format adduct [M-H-HCOOH] [469-46=m/z 423] the fragment ions at m/z 285,155,137,123 and 120 in accord with the loss of [M–H(m/z423) -138]−, [M–H-268]−, and [M–H-286]−, [M–H-300]−, [M–H-303]−, A comparison of these MS/MS ions with the literature, Moreover, this compound has previously been reported in this plant, according to these data compound S2 has been identified as salicortin [47] .

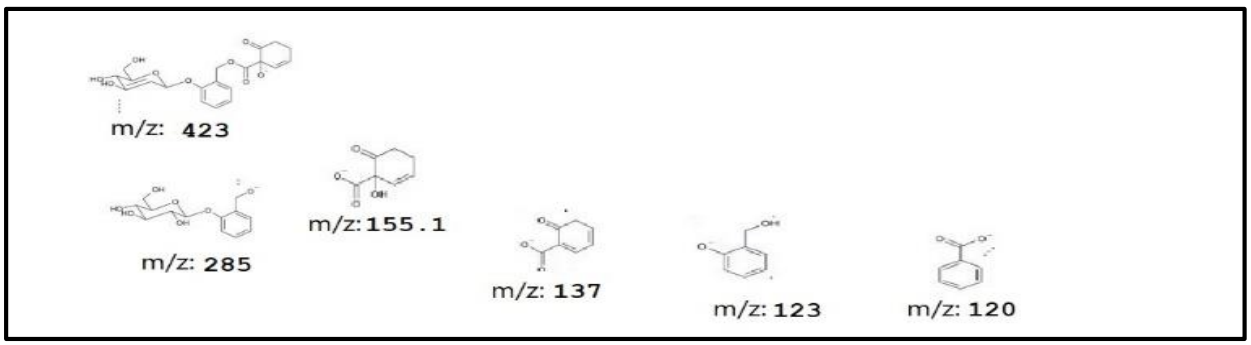
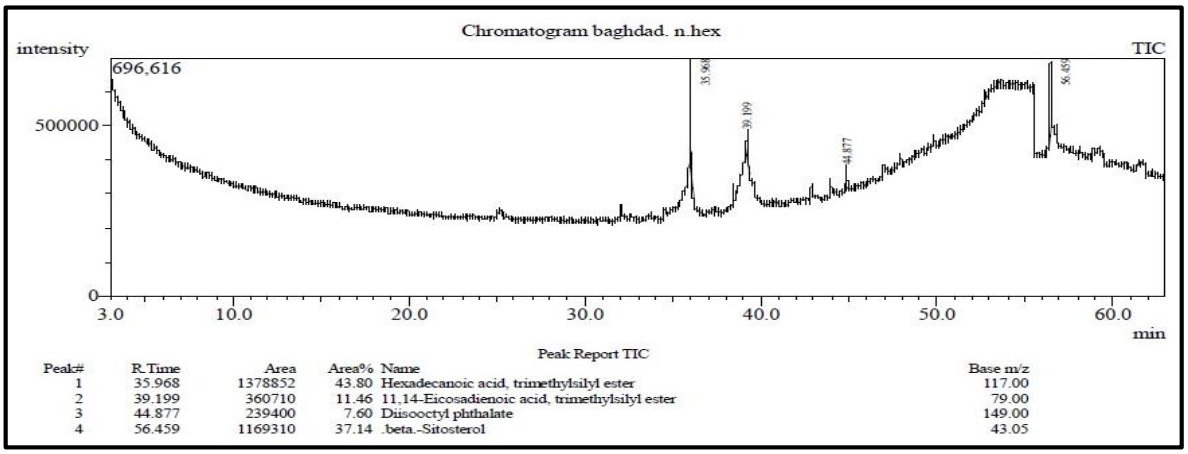


Figure 18. Fragmentation pathway of salicortin

Phytochemicals identified in hexane fraction of Populus euphratica leaves.

The n-hexane fraction was analyzed using GC-MAS spectroscopy and revealed the following compound as shown below**.**



Peak Report TIC

Peak#	R.Time	Area	Area%	Name	Base m/z
1	35.968	1378852	43.80	Hexadecanoic acid, trimethylsilyl ester	117.00
2	39.199	360710	11.46	11,14-Eicosadienoic acid, trimethylsilyl ester	79.00
3	44.877	239400	7.60	Diisooctyl phthalate	149.00
4	56.459	1169310	37.14	.beta.-Sitosterol	43.05

Figure 19.GC-MS of n-hexane fraction of *Populus euphratica* leaves

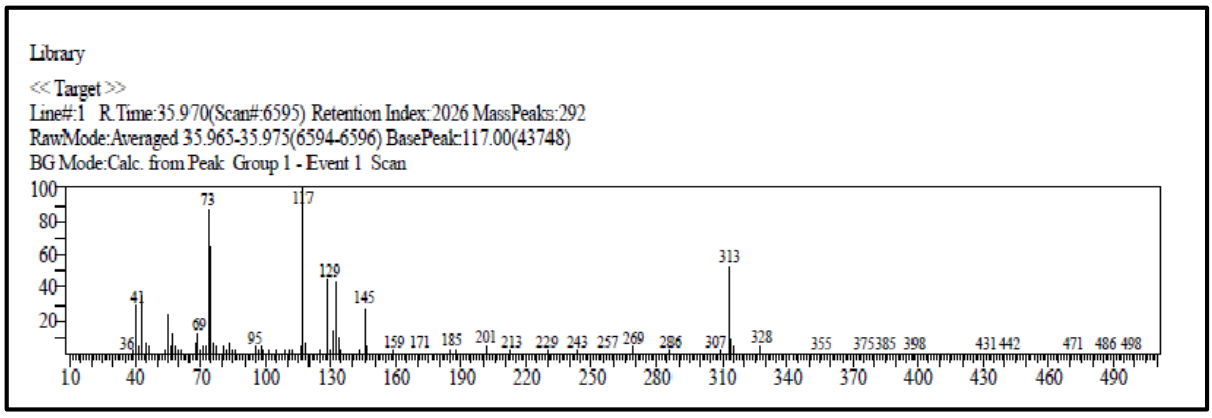


Figure20. (A1) Mass spec. of peak 1

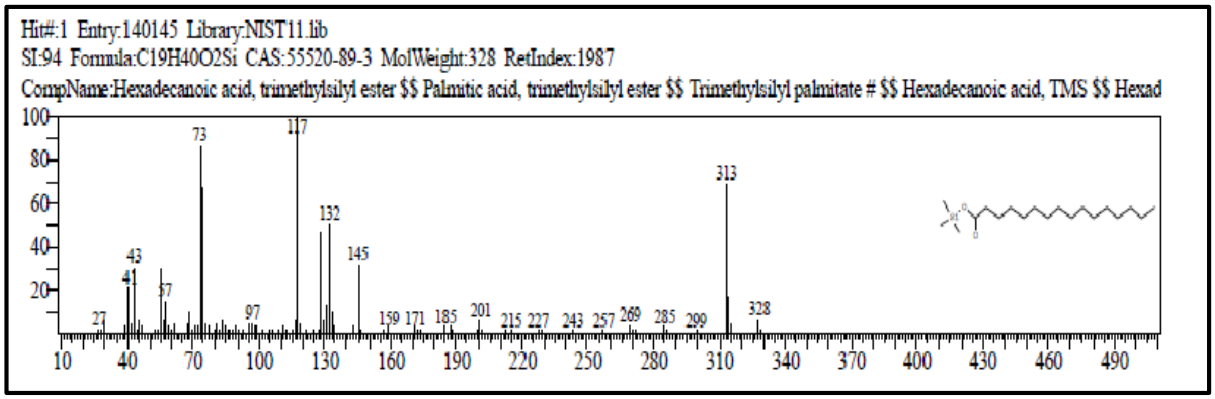


Figure 20. (B1) Mass spec. of Heptadecanoic acid trimethylsilyl ester (palmitic acid trimethylsilyl ester) from NIST Library

As shown in the figure 20complete match of mass spec. between A1 and B1 it indicates the peak 1 is palmitic acid trimethylsilyl ester.

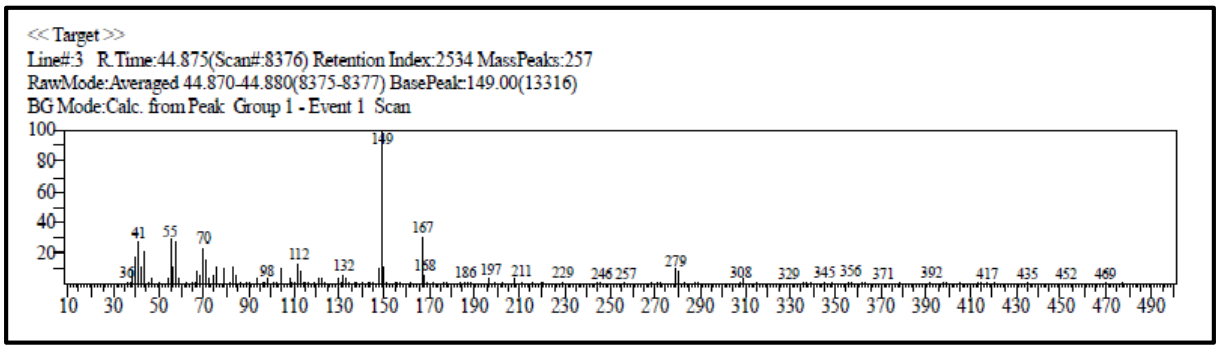


Figure 21 . (A2) Mass spec. of peak 3

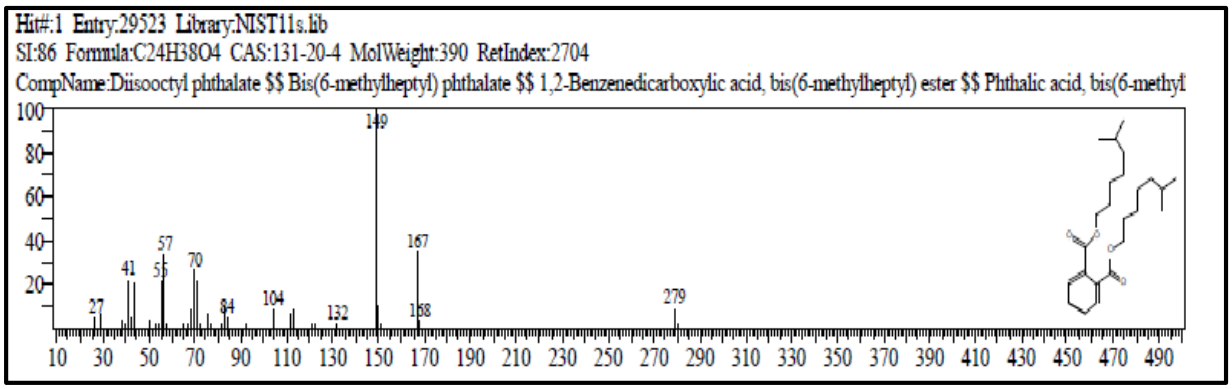


Figure21 . (B2) Mass spec. of Diisooctyl phthalate from NIST library

From mass spec. of the figure 21 complete match of mass data between A2 and B2 indicate that peak 3 is Diisooctyl phthalate.

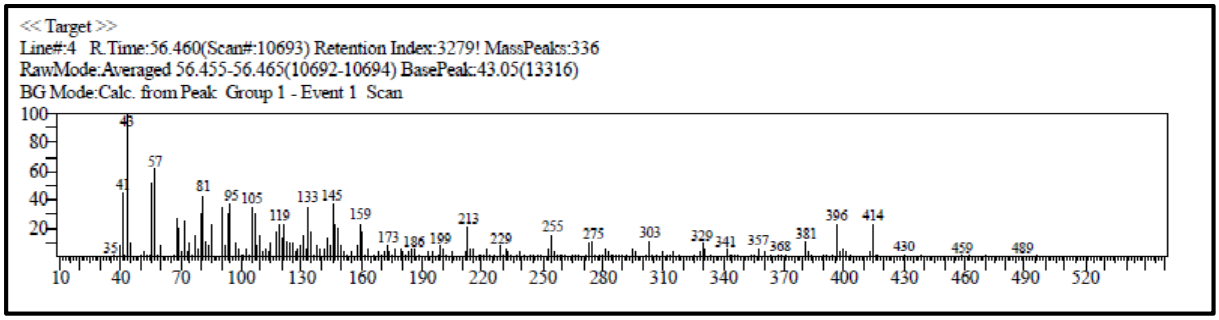


Figure 22. (A3) Mass spec. of peak 4

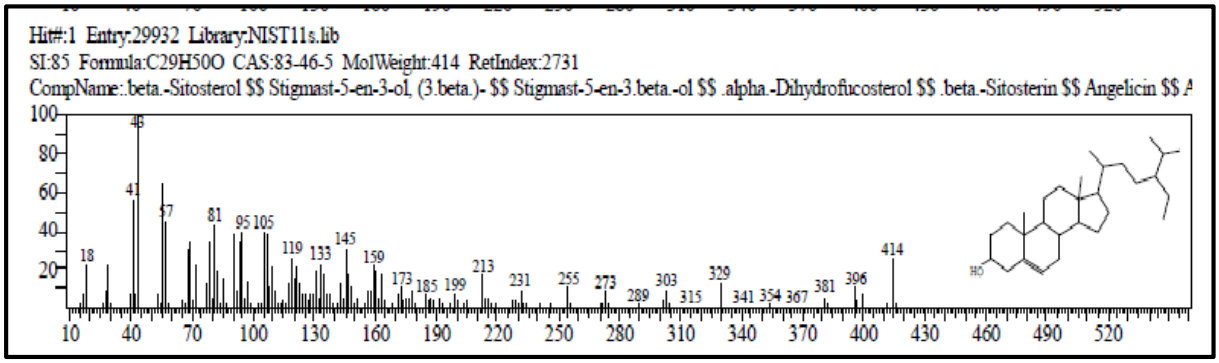


Figure 22. (B3) Mass spec. of beta-Sitosterol

Peak 5 is beta-sitosterol due to the exact mass pattern between A3 and B3mass charts.

Conclusion

Based on the result the following point may be concluded

1. UPLC-ESI-MS/MS is a powerful method for identification of compounds in mixture base on their molecular weight, retention time and MS/MS fragmentation
2. Twenty compounds have been tentatively identified with this method as in tables 3.8,3.9 and 3.10
3. protopine alkaloid is identified for the first time in *Populus euphratica* and genus populs.
4. salicinoids are major compounds in Populus species 4 of them have been identified, salicin, salicortin, salisaoylsalicin, and trmulacin.
5. salicin, neohesperidin, protopine, and salicortin have been isolated from different fractions and identified by various methods.
6. Hexane fraction of leaves of *Populus euphratica* is rich in beta-sitosterol with 37.14% of total hexane fraction, this may be the cause that extracts of this plant is used traditionally by farmers for various skin conditions like dermatitis and eczema, so the leaves of this tree may be exploited as a rich source for beta-sitosterol
7. spermidine derivatives, zeatin-9-glucoside, and solasodine have been identified for the first time in this plant.

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