

## Isolation, Structural Characterization and Identification of Major Constituents in *Ephedra foliata* Naturally Growing in Iraq by TLC, GC-MS and UPLC-ESI-MS/MS

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### Abstract

The aerial part of *Ephedra foliata* Family Ephedraceae have long been used in traditional medicine and now *Ephedra* species have medicinal, ecological, and commercial value. The variety of pharmacological actions of this plant is due to its chemical constituents. Ephedrine and related alkaloids; are the new potential medicinal value of Ephedra supplements for weight loss or performance improvement. Other pharmacological actions like antibacterial and antifungal effects of the phenolic acid compounds, the immunosuppressive action of the polysaccharides, and the antitumor action of flavonoids. The genus of this plant widely distributed throughout Asia, America, Europe, and North Africa. The study is aimed at screening the phytochemical constituents due to the importance of pharmacological actions of this plant. That is done by maceration the aerial part of *Ephedra foliata* with 80% ethanol for 9 days and fractionated by n-hexane, chloroform, ethyl acetate, and n-butanol. The n-hexane, chloroform, n-butanol fractions, and isolated compounds were analyzed by gas chromatography-mass spectrometry, thin layer chromatography; ultra-performance liquid chromatography coupled with electrospray ionization mass/ mass spectroscopy. The various chromatographic and spectroscopic results indicate the presence of a different type of phytochemicals like ephedrine, 6-hydroxy kynurenic acid, vicenin 2 and quercetin 3-sophoroside-7-rhamnoside. These active constituents of *Ephedra foliata* have been identified play a crucial role in our life due to its pharmacological actions.

**Keywords:** Ephedra, Gas chromatography, Mass spectrometry, Ultra-performance liquid chromatography electrospray ionization mass/ mass , 6-hydroxy kynurenic acid.

العزل, التوصيف الهيكلي و تشخيص المكونات الرئيسية في نبات العنبد الذي ينمو بشكل طبيعي في العراق من خلال بواسطه كروماتوغرافيا الطبقة الرقيقة و كروماتوغرافيا الغاز و كروماتوغرافيا عالية الاداء السائله

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### الخلاصة

منذ فترة طويلة تستخدم الاجزاء الهوائية لنبات الافيدرا في الطب القديم والان نبات الافيدرا لديها فوائد دوائية وبيئية و تجارية. مجموعة متنوعة من الاستعمالات الدوائية لهذا النبات ويرجع ذلك إلى مكوناته الكيميائية المختلفة. الافيدرين والقلويدات ذات صلة بها؛ لديها القيمة الطبية المحتملة حديثاً لمكملات الإفيدرا لفقدان الوزن أو تحسين الأداء. الاستخدامات الدوائية الأخرى مثل التأثيرات المضادة للبكتيريا والفطريات لمركبات حمض الفينول، المثبط للمناعة من السكريات، والعمل المضاد للورم من مركبات الفلافونويد. جنس هذا النبات الموزع بكثرة في جميع أنحاء آسيا وأمريكا وأوروبا وشمال إفريقيا. تهدف هذه الدراسة إلى فحص المكونات الكيميائية النباتية بسبب أهمية الاستعمالات الدوائية لهذا النبات. يتم ذلك عن طريق تنقية الاجزاء الهوائية من نبات الافيدرا مع الإيثانول بنسبة ٨٠٪ لمدة ٩ أيام ومن ثم تجزئة بواسطة الهكسان، والكلوروفورم، و خلاص الإيثيل، و بوتانول. وقد تم تحليل اجزاء الهكسان، الكلوروفورم، وبيوتانول، والمركبات المعزولة من قيل كروماتوغرافيا الطبقة الرقيقة و كروماتوغرافيا الغاز و كروماتوغرافيا عالية الاداء السائله. تشير النتائج الكروماتوغرافية والطيفية المختلفة إلى وجود أنواع مختلفة من المواد الكيميائية النباتية مثل الإفيدرين، وحمض الكينورينيك ٦-هيدروكسي، والفينيسينين ٢، والكيبورستين ٣-رامنوسايد ٧-سيفروسايد. تم التعرف على هذه المكونات النشطة من نبات الافيدرا التي تلعب دوراً حاسماً في حياتنا بسبب فعاليتها الدوائية. الكلمات المفتاحية: الإفيدرا، كروماتوغرافيا الغاز، كروماتوغرافيا عالية الاداء السائله، حمض الكينورينيك ٦-هيدروكسي.

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For at least five thousand years, ephedra plants have been used in traditional medicines in which dry stems are used for symptoms derived from the common cold, flu, asthma, bronchitis, nasal congestion and hay fever<sup>(1)</sup>. The ephedra plant is also used for the treatment of arthritis, fever, hives, dyspnea, headache, joint and bone pain, wheezing and hypotension<sup>(2)</sup>. *Ephedra* corresponds to a genus of gymnosperms including over 50 species of tropical and subtropical, small, much-branched shrubs found in the dry regions of both hemispheres<sup>(3)</sup>. It is related to the Gnetales division of gymnosperms plants and is related to the conifers<sup>(4)</sup>. The plant species are short, evergreen and virtually leafless shrubs that grow about (60 to 90 cm) tall. The stems are green in color, slender, erect, small ribbed and channeled, about (1.5 mm) in diameter and commonly terminating in a sharp point. Nodes are (4 to 6 cm) apart, and small triangular leaves appear at the stem nodes which are usually reddish brown<sup>(5)</sup>. These species grow in dry weather over wide parts of the Northern hemisphere including North America, Europe, North Africa, and Southwest and Central Asia<sup>(6)</sup>.

#### The chemical constituents and pharmacological actions of *Ephedra* species

The aerial parts of various plant species first of all, ephedrine-type alkaloids, usually have from (0.02% to 3.4%) of six optically active alkaloids as shown in Figure 1, (-)-Ephedrine (EPH) is the major one including 30–90% of the total alkaloids, (+)-Pseudoephedrine (PSE), the diastereomer of (-)-EPH, (-)-N-Methylephedrine, (-)-Norephedrine, (+)-N-Methylpseudoephedrine and (+)-Norpseudoephedrine<sup>(7)</sup>. Secondly, non-ephedrine alkaloids and amino compounds in *Ephedra* species. Ephedroxane<sup>(8)</sup>, Ephedradine A<sup>(9)</sup>, cyclopropyl- $\alpha$ -amino acid<sup>(10)</sup>, maokonine<sup>(11)</sup>, 6-methoxykynurenic acid<sup>(12)</sup>, N-methylbenzylamine<sup>(13)</sup>, Tertmethylpyrazine<sup>(14)</sup>, and 6-hydroxykynurenic acid<sup>(10)</sup>. Thirdly, Miscellaneous Non-alkaloidal Natural Constituents of *Ephedra*: *trans*-cinnamic acid, catechin, syringin, epicatechin, symplocoside, kaempferol 3-*O*-rhamnoside 7-*O*-glucoside, isovitexin 2-*O*-rhamnoside, herbacetin 7-*O*-glucoside, and pollenitin B and herbacetin 7-*O*-neohesperidoside<sup>(15)</sup>. *Ephedra* species have numerous pharmacological actions for instance anti-inflammatory due to the inhibition of prostaglandin E2 biosynthesis<sup>(8)</sup>, antibacterial and antifungal<sup>(16)</sup>, anti-cancer activity<sup>(17)(18)</sup>, CNS stimulant and perhaps thermogenic effects<sup>(19)</sup>, antiviral activity<sup>(20)</sup> and finally antioxidant and hepatoprotective activity<sup>(21)</sup>.

This study was designed to investigate the phytochemicals and their proportions of the aerial part of *Ephedra foliata* naturally growing plants in Iraq.

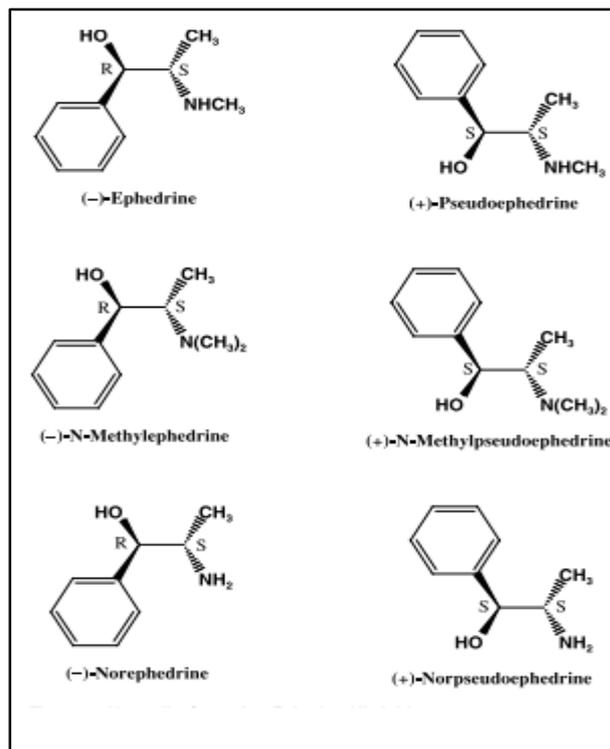


Figure 1. Ephedrine- type alkaloids

#### Material and methods

##### Collection of plant materials:

*Ephedra foliata* was collected during March – June 2018 from Tikrit province, Iraq. This plant was authenticated by Dr. khansaa rasheed / Iraq Natural History Research Center and Museum Plant and Environment Department / University of Baghdad. The stems and aerial parts were, dried in a shed, rendered into a coarse powder.

##### Extraction

Extraction by maceration then fractionation according to active constituents. About 650 grams of aerial part the powdered plant material was soaked in 2500ml (1:7) 80% ethanol, with regular shaking, at room temperature. After 3 days, the ethanol extracts are filtered, repeat the process 3 times for 9 days. The filtrate was evaporated to dryness under vacuum using a rotary evaporator, to get dried extract. The dark greenish residue was suspended in 250ml H<sub>2</sub>O and partitioned successively with n-hexane, chloroform, ethyl acetate, and n-butanol until reaching a clear layer for each fraction. The first three fractions are dried over anhydrous sodium sulfates, filtered, and evaporated to dryness<sup>(22)</sup>.

**Phytochemical investigation of chemical constituents of Iraq *Ephedra foliata*:**

**Preliminary identification by chemical test:**

**1-Test for alkaloids:**

- Mayer's reagent.
- Wagner's reagent

**2-Test for flavonoids**

About 5% alcoholic potassium hydroxide is added and then a few drops of 5% hydrochloric acid are added.

**3-Test for phenols**

Few milligrams of ethanol plant extract are treated with few drops of 1% ferric chloride<sup>(23)</sup>.

**Purification of crude alkaloidal extract:**

The chloroform fraction was acidified by adding hydrochloric acid (5%). This solution was then placed in a separatory funnel and partitioned with equal volume of chloroform (four times). The upper aqueous acidic layer was separated and basified with ammonium hydroxide (25% NH<sub>4</sub>OH) to PH 10 using PH meter. After the basification process, the solution becomes warm and allowed to stand for 2 hours. Then partitioned with an equal volume of chloroform in a separatory funnel (three times). The chloroform layer was separated, dried with anhydrous sodium sulfate, filtered and evaporated under reduced pressure then tested with Dragendorff and Mayer's reagents<sup>(24)</sup>.

**Isolation of some phytochemicals by using preparative TLC**

Thin-layer chromatography was used to determine phytochemical compounds by using different solvent systems like chloroform; methanol (90:1), Chloroform: acetone: formic acid (75:16.5:8.5) and Ethyl acetate: formic acid: acetic acid: water (80:5:6:10) for n-butanol fraction<sup>(25)</sup>. While toluene-chloroform-ethanol-methanol (20:50:30:10), ethyl acetate-isopropanol-ammonia (100:2:1) and cyclohexane-ethanol-diethylamine (80:10:10) for chloroform fraction that were tried for identification to get the best separation and the largest number of spots<sup>(26)</sup>.

- AS1 compound was isolated from n-butanol fraction using readymade preparative TLC silica gel GF254 plates and mobile phase Ethyl acetate: formic acid: acetic acid: water (80:5:6:10)<sup>(25)</sup>. Detection of the AS1 compound was done by examination under UV light with wavelengths, 254 and 366 nm.
- AS2 compound tertiary amine alkaloid was isolated from purified chloroform fraction after basification using readymade [aluminum oxide on TLC-glass plates](#) and mobile phase toluene-chloroform-ethanol-methanol (20:50:30:10)<sup>(26)</sup>. Detection of the AS2 compound was done using Dragendorff's spray reagent is detected as a brown zone.

- The purity of each bands are verified by analytical TLC until a single point are obtained on the TLC plates for identification.

**Identification and structural characterization of isolated compounds and phytochemicals in fractions were done by**

**I-GC-MS analysis**

The conditions used in the GC / MS analysis are compatible with the thermal desorption system (TD-20), GC / MSQP / 2010 Plus (Shimadzu, Japan) composed of an automatic sampler. The mass spectrometer instrument was connected. Column RTX-5MS (30 mm × 0.25 mm × 0.25 μm), operating in electronic printing mode at 70 eV. In this instrument, (99.99%) of helium gas is used as a carrier gas with a movement frequency of (1.2 ml / min). The initial temperature of the column oven is 80 ° C (isothermal for four minutes) with a constant increase from (5 ° C / min to 310 ° C), flow rate of (1.21 ml / min) and column pressure of 81, 7 kPa In the scanning interval of 0.50 s, the mass spectrum is prepared with a mass scan of (40to650) m / z<sup>(27)</sup>.

**II-Ultra performance liquid chromatography-electrospray ionization mass/ mass spectrometry (UPLC-ESI-MS/MS) analysis**

Electrospray ionization mass spectrometry in negative and positive ions acquisition mode is performed in XEVO TQD triple quadruple instruments. Water Corporations, Milford, MA01757 USA UU. The sample solution (100 μg / ml) is prepared using high-performance liquid chromatography (HPLC) analytical grade methanol, the filtrate uses a membrane disk filter (0.2 μm), then subjected to LC / ESI / MS. The sample injection volume (10 μL) is injected into the UPLC instruments Equipped with C-18 reverse phase columns (ACQUITYS UPLC / BEH C18 Particle size of 1.7μm-2.1 ×50mm column). The mobile phase is prepared by filtration using a 0.2μm filter membrane disk and degassed by sonication before injections. The elution of the mobile phase is carried out with a flow rate of 0.2 ml per minute using a mobile gradient phase which includes two eluents: the eluent A is acidified in water with 0.1% of HCOOH and the eluent B is methanol acidified with 0.1% of HCOOH. The elution is performed using the gradient. The parameters for the analysis are performed using the negative ion mode as follow:150° C source temperature, 30eV cone voltage, 3kV capillary voltage, desolvation temperature about (440 ° C, 50L / h) cone gas flow and desolvation gas flow of (900L / h)(28). Mass spectra are detected in electrospray ionization between m / z (100–1000). Peaks and spectra are processed using Maslynx (4.1) software and are tentatively identified by comparing their retention times and masses spectra with the reported data<sup>(27)</sup>.

**Results**

**Phytochemical investigation of chemical constituents of *Ephedra foliata*:**

**1-Preliminary identification by chemical test:**

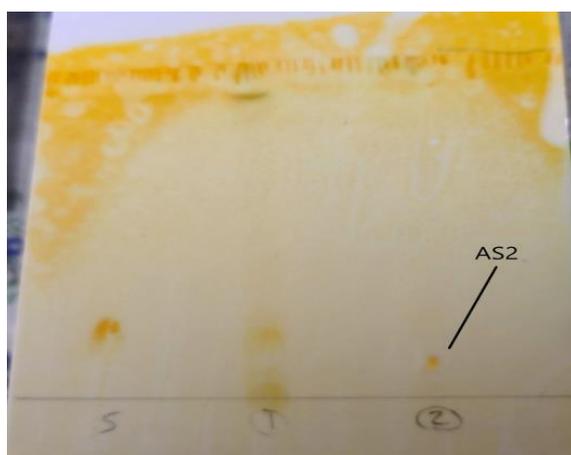
Various qualitative phytochemical screening tests were done to establish the chemical composition of each extract shown in Table1.

**Table1. Phytochemical screening tests for crude extract**

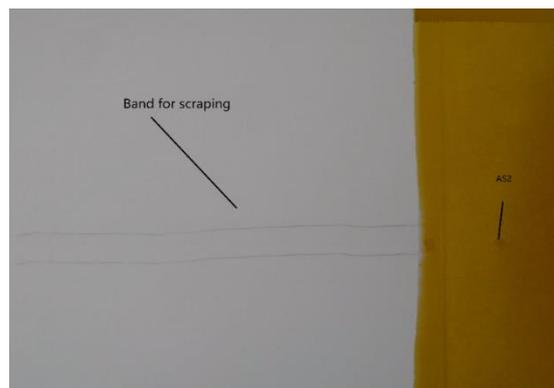
| Phytochemical test | Type of phytochemical | Results |
|--------------------|-----------------------|---------|
| Mayer's            | Alkaloids             | +       |
| Wagner's           | Alkaloids             | +       |
| KOH                | Flavonoids            | +       |
| Fec13              | Phenols               | +       |

**2-Thin layer chromatography TLC (analytical and preparative):**

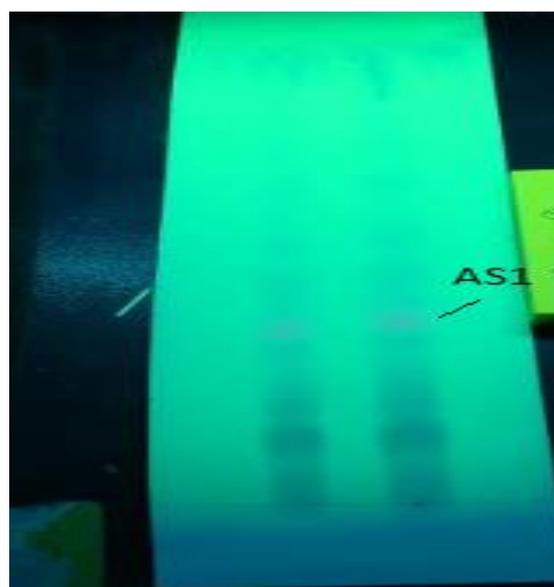
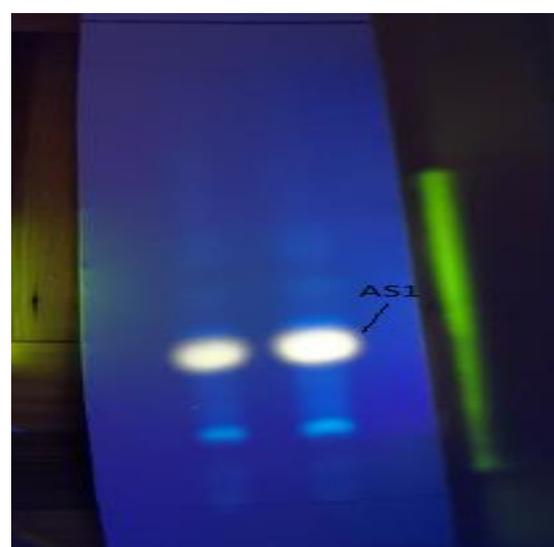
According to TLC results which are shown below A1 and A2 were found the best mobile phases for separation and isolation of AS1 and AS2 respectively as result shown below.



**Figure 2. TLC for chloroform fraction before basify (1), after basify (2) and pseudoephedrine standard (S) developed with A2 solvent system, at 254 nm and after Dragendorff,s spray reagent.**



**Figure 3. preparative TLC for isolated AS2 from chloroform fraction after basify with developed the A2 solvent system with Dragendorff reagent.**



**Figure 4. TLC of n-butanol fraction before hydrolysis with different titration under UV 253nm and 366nm.**

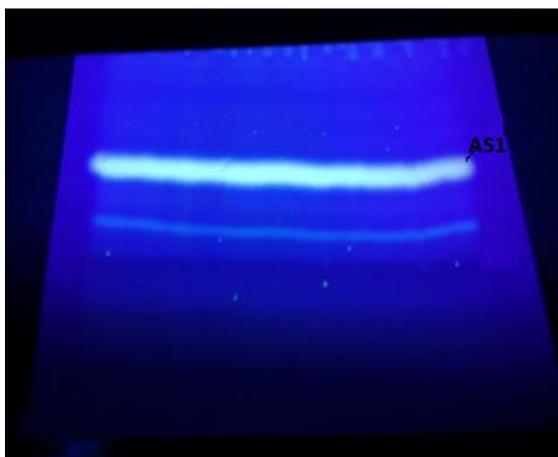


Figure 5. Preparative TLC of n-butanol fraction before hydrolysis with different titration under UV366 nm to isolate AS1.

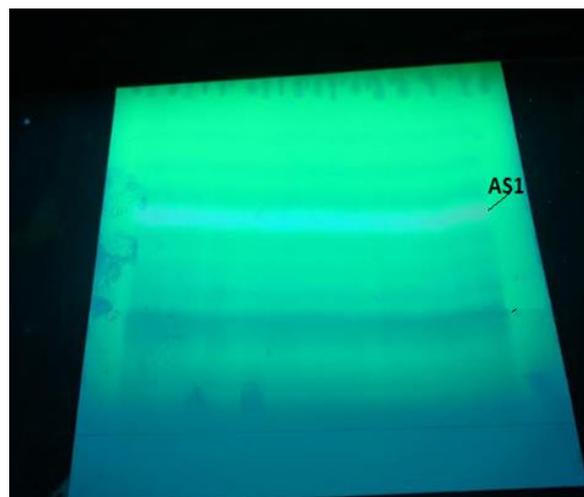


Figure 6. Preparative TLC of n-butanol fraction before hydrolysis with different titration under UV 254 nm to isolate AS1.

**3-Gas chromatography mass spectrometry GC/MS:**

**A.GC/MS of n-hexane fraction:** identification of phytochemical compounds in n-hexane fraction by gas chromatography mass spectrometry.

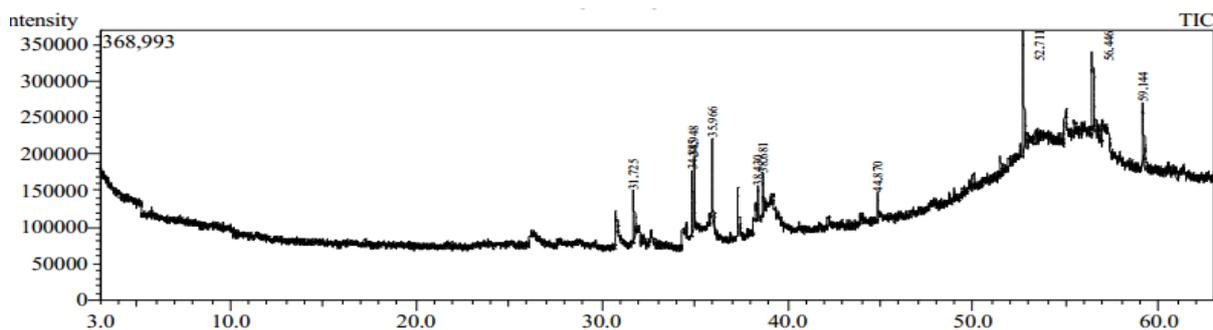


Figure 7. GC/MS of n-hexane fraction.

Table 2. Compounds identified in n-hexane fraction by GC/MS.

| NO. of Peaks | Retention time (R.t) | name  | base peak |
|--------------|----------------------|---|-----------|
| 1            | 31.725               | 1-Octadecyne                                  | 41.00     |
| 2            | 34.885               | n-Heptadecanol-1                              | 43.10     |
| 3            | 34.948               | Hexadecanoic acid, ethyl ester                | 88.05     |
| 4            | 35.966               | Hexadecanoic acid, trimethylsilyl ester       | 73.00     |
| 5            | 38.430               | 1-Methyl-1-(2-tridecyl)oxy-1-silacyclopentane | 143.15    |
| 6            | 38.681               | 1-Octadecene                                  | 43.05     |
| 7            | 44.870               | Di-n-octyl phthalate                          | 149.00    |
| 8            | 52.711               | 17-Pentatriacontene                           | 43.00     |
| 9            | 56.446               | gamma.-Sitosterol                             | 43.05     |
| 10           | 59.144               | Stigmast-4-en-3-one                           | 43.00     |

**B. GC/MS of chloroform fraction:** identification of phytochemical compounds in chloroform fraction

by gas chromatography mass spectrometry.

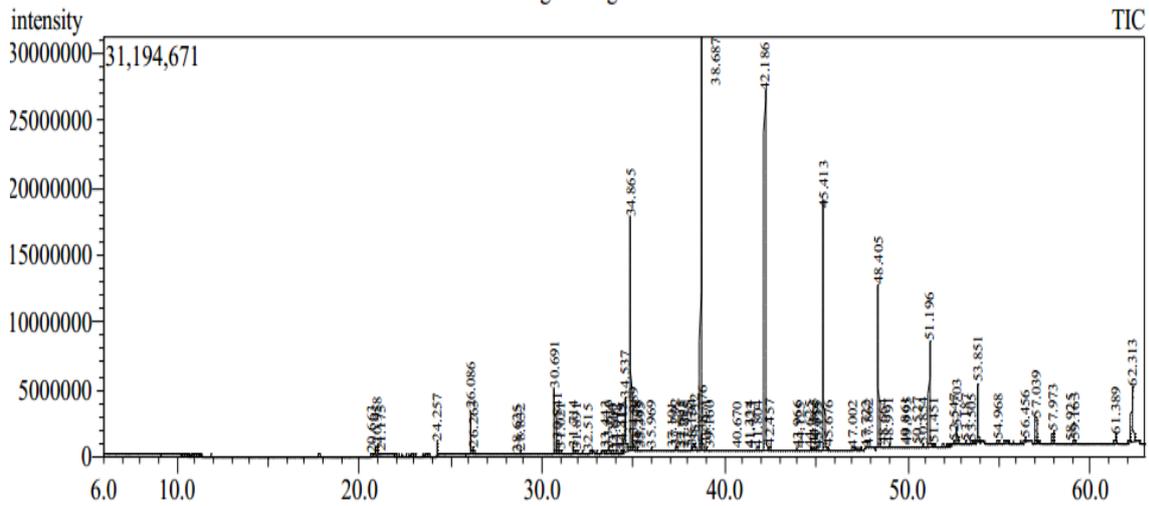


Figure 8. GC/MS for chloroform fraction.

Table 3. Compounds identified in chloroform fraction GC/MS

| No. of peak | Retention time | Name                                     | M.WT | Base peak |
|-------------|----------------|--|------|-----------|
| 1           | 20.663         | 3,4-dimethyl-5-phenyl-2- oxazolidinone   | 191  | 57.05     |
| 2           | 20.958         | 1-Undecene                               | 154  | 41.05     |
| 3           | 21.175         | Ephedrine                                | 165  | 58        |
| 4           | 24.257         | Phenol, 3,5-bis(1,1-dimethylethyl)-      | 206  | 191.05    |
| 21          | 34.537         | Aziridine, 1,2-dimethyl-3-phenyl-, trans | 147  | 146       |
| 33          | 38.194         | Linoleic acid ethyl ester                | 196  | 57.05     |
| 58          | 48.991         | Squalene                                 | 410  | 69        |

C. GC/ MS for isolated AS2: identification of isolated AS2 compound form chloroform fraction by gas chromatography mass spectrometry.

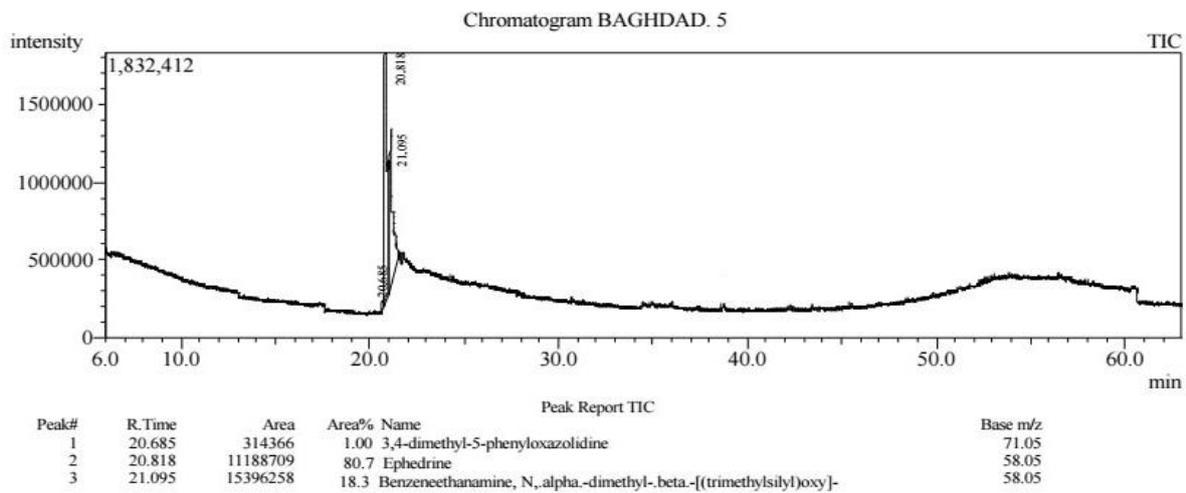


Figure 9. GC MS for isolated AS2 compound from chloroform fraction after basify.

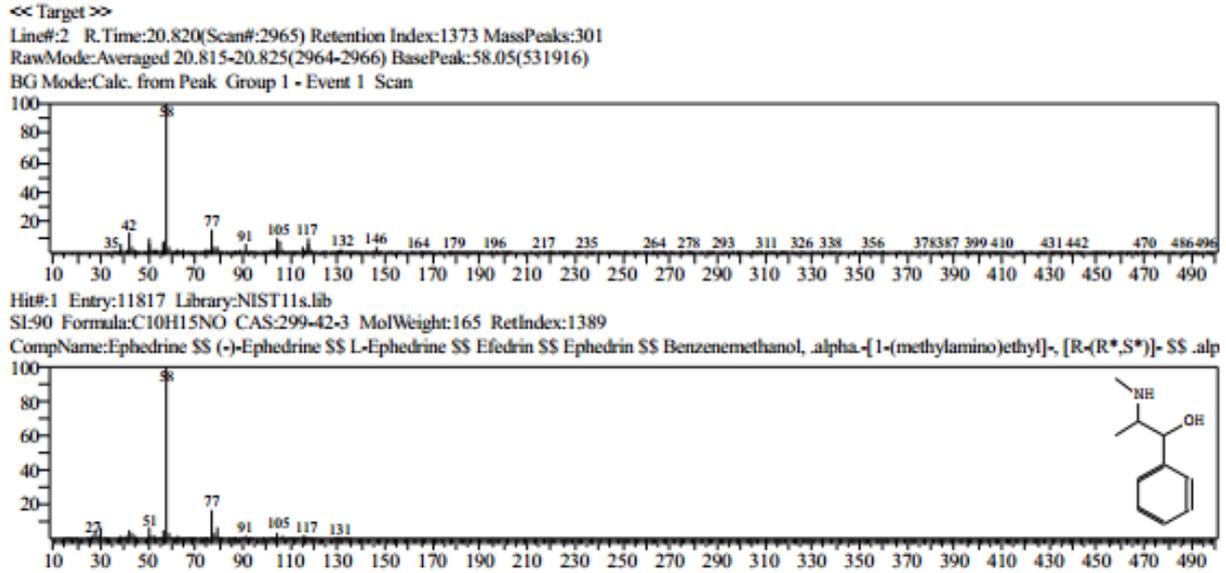


Figure 10. Fragmentation and structure elucidation of isolated AS2 compound by GC/MS.

Table 4. Isolated AS2 compound identified by GC MS<sup>(29-30)</sup>

| No. of peak | Retention time | Name      | Area % | M.WT | Base peak |
|-------------|----------------|-----------|--------|------|-----------|
| 2           | 20.818         | Ephedrine | 80.7   | 165  | 58.05     |

**4- Ultra-performance liquid chromatography electrospray ionization mass/ mass ((UPLC-ESI-MS/MS):**

Identification of the results from UPLC-ESI-MS / MS depended on molecular weight, retention time and mass fragmentation through different sites

specialized in the identification and confirms the result of a search with previous studies.

**A. UPLC for isolated AS2:** identification of isolated AS2 compound form chloroform fraction by ultra-performance liquid chromatography

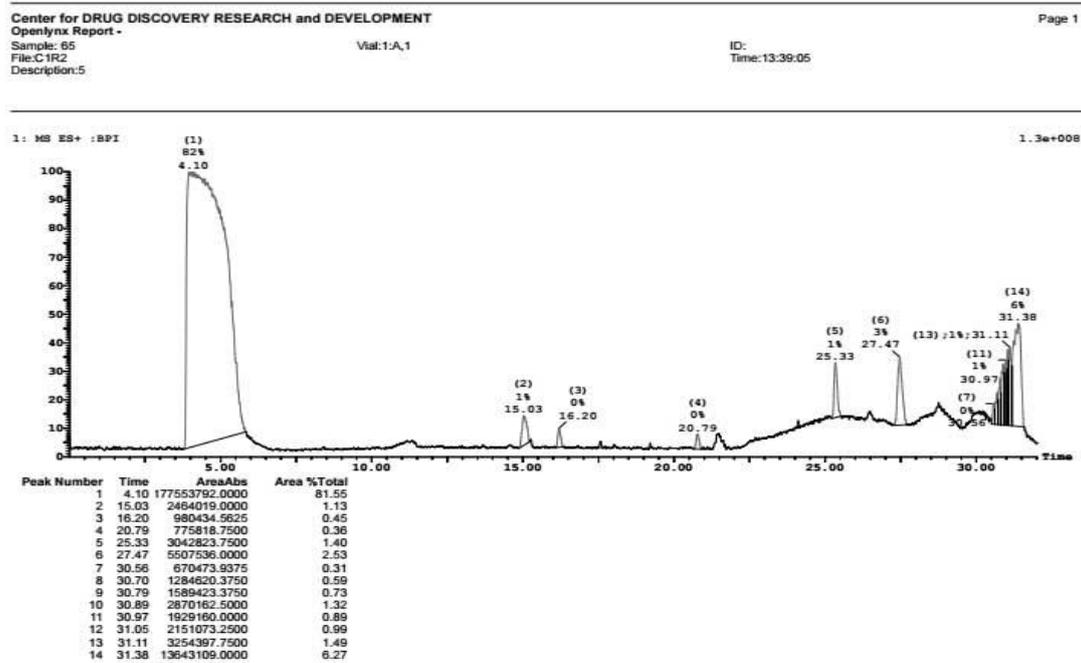


Figure 11. UPLC for isolated AS2 from chloroform fraction after basification.

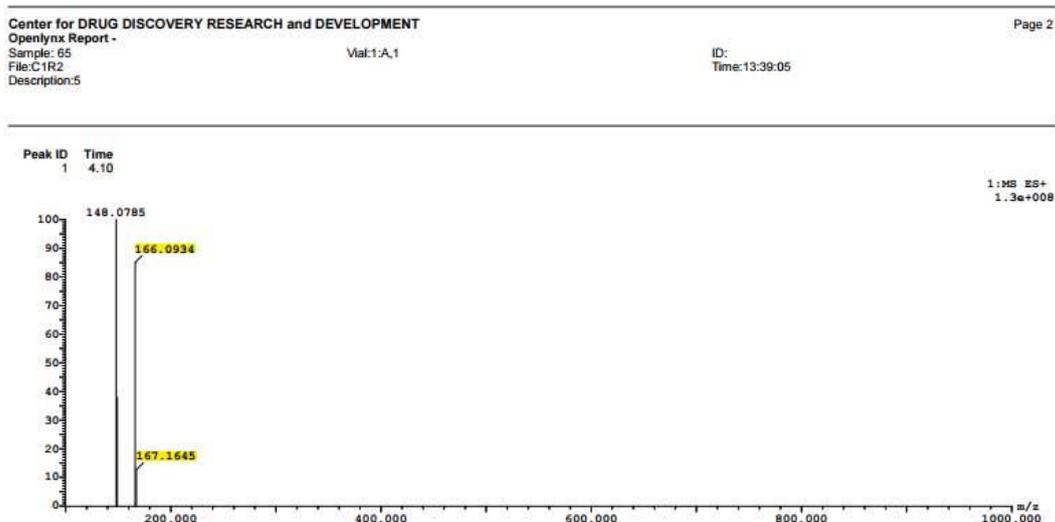


Figure 12. First mass for isolated AS2 peak 1 at retention time 4.1min with major molecular ion  $[M+H]^+$  166.093.

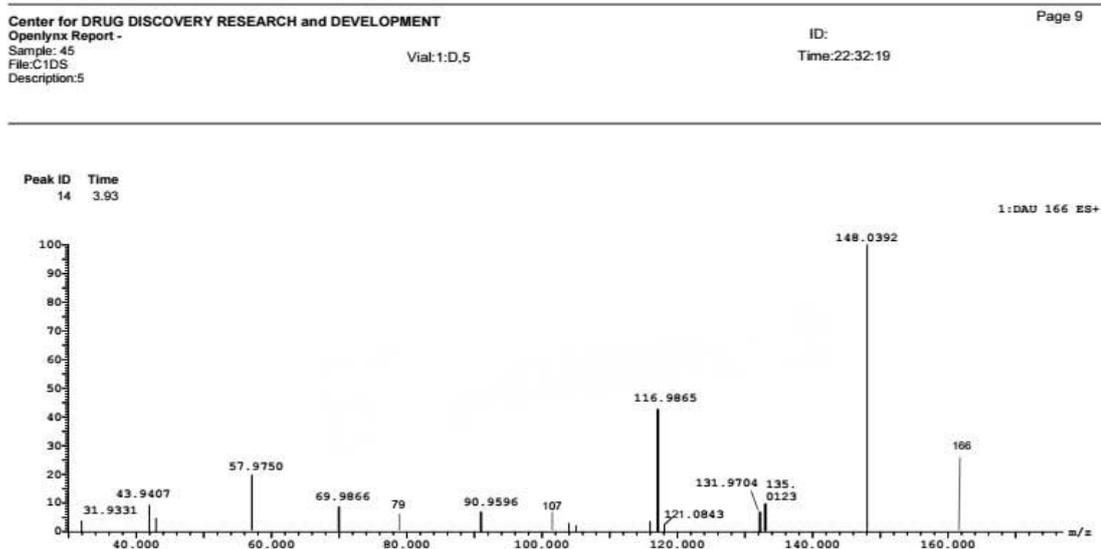


Figure 13. Mass fragmentations for isolated AS2 compound.

Table 5. UPLC ESI MS/MS for isolated AS2 compound

| peak no. of MS <sup>1</sup> | R.T | [M+H] | Peak no. of MS <sup>2</sup> | R.T  | Base peak | Name      | Reference |
|-----------------------------|-----|-------|-----------------------------|------|-----------|-----------|-----------|
| 1                           | 4.1 | 166   | 14                          | 3.93 | 148.0392  | Ephedrine | (31-32)   |

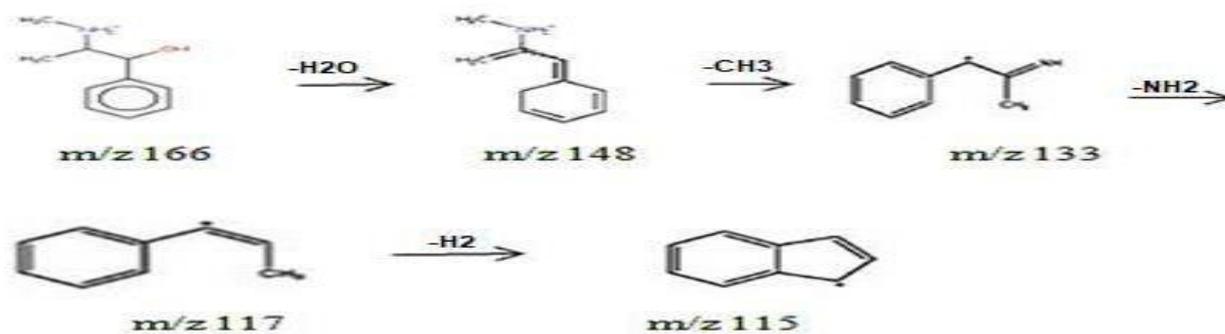


Figure 14. Structural elucidations of AS2 fragmentations <sup>(31)(32)</sup>.

B. UPLC for isolated AS1: identification of isolated AS1 compound form n-butanol fraction by ultra-performance liquid chromatography

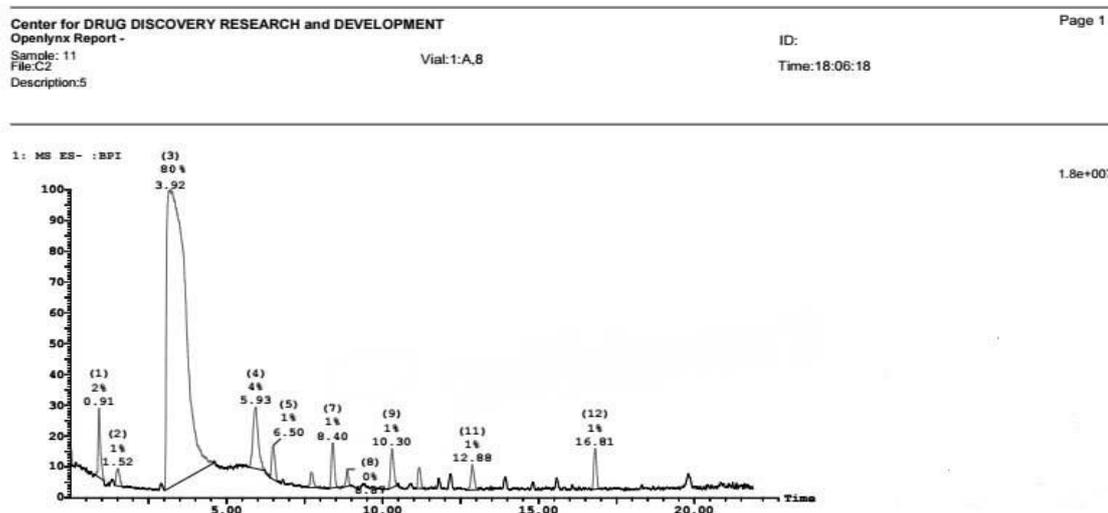


Figure 15. UPLC for isolated AS1 compound from n-butanol fraction before hydrolysis at peak 3.

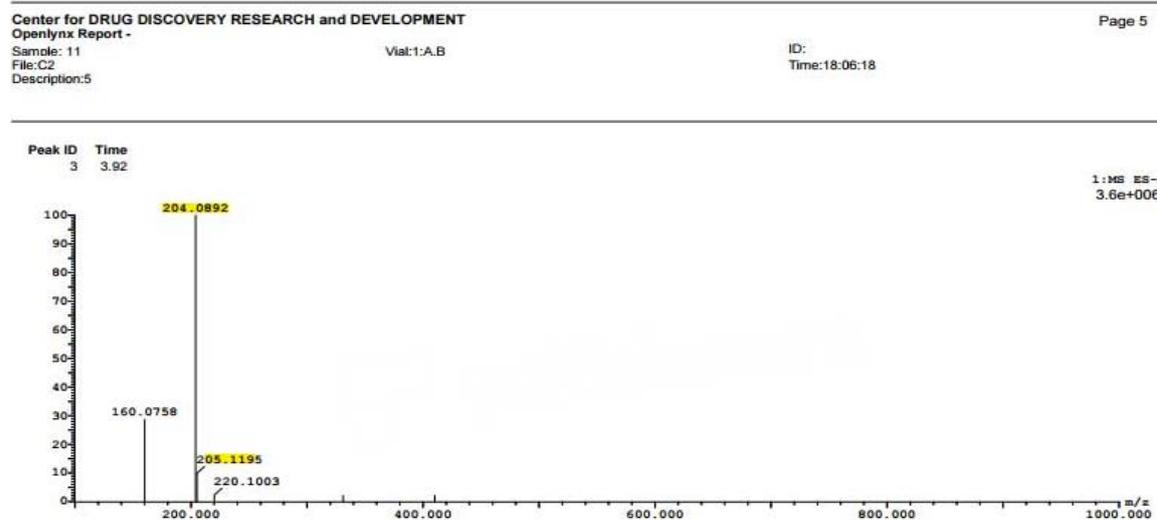


Figure 16. First mass for isolated AS1 compound peak 3 at retention time 3.92 min with major molecular ion [M-H]<sup>-</sup> 204.0892

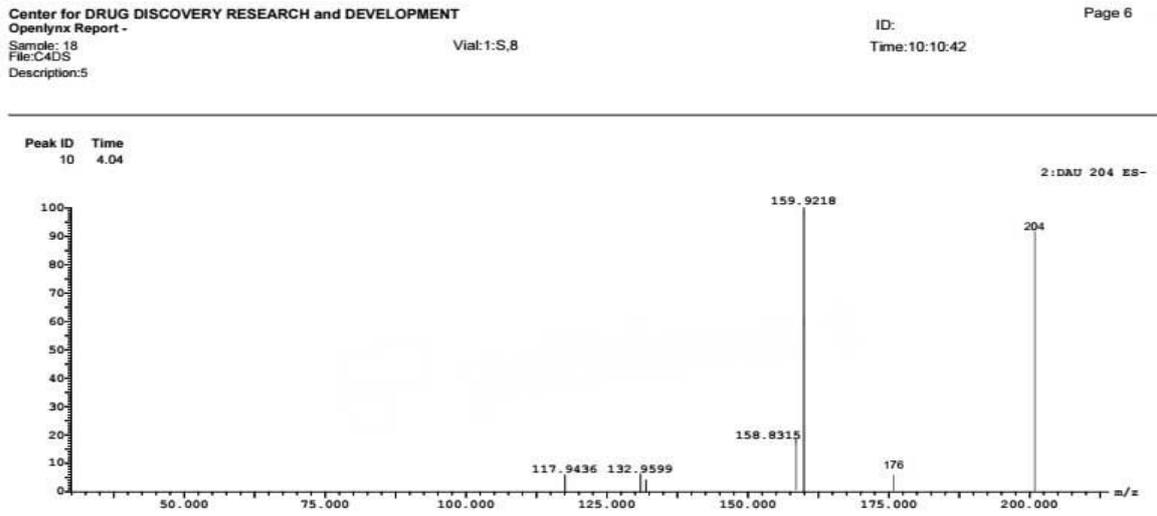


Figure 17. Mass fragmentation for isolated AS1 compound.

Table 6. UPLC ESI MS/MS for isolated AS1 compound

| peak no. of MS <sup>1</sup> | R.T <sup>1</sup> | [M-H]    | Peak no. of MS <sup>2</sup> | R.T <sup>2</sup> | Base peak | Name                    | Reference     |
|-----------------------------|------------------|----------|-----------------------------|------------------|-----------|-------------------------|---------------|
| 3                           | 3.92             | 204.0892 | 10                          | 4.04             | 159.9218  | 6-hydroxykynurenic acid | (33-10-34-35) |

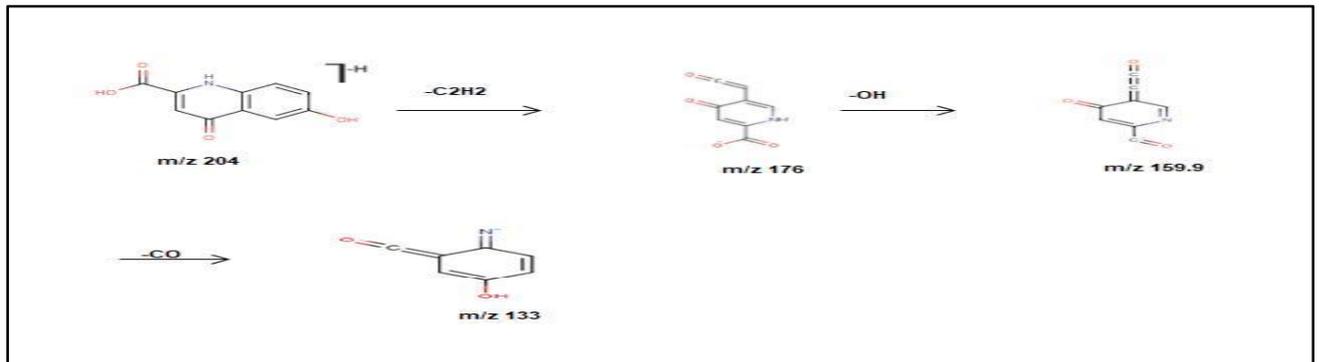


Figure 18. Structural elucidations of AS2 fragmentations<sup>(33)</sup>.

C. UPLC n-butanol fraction: identification of phytochemical compounds in n-butanol fraction by ultra-performance liquid chromatography

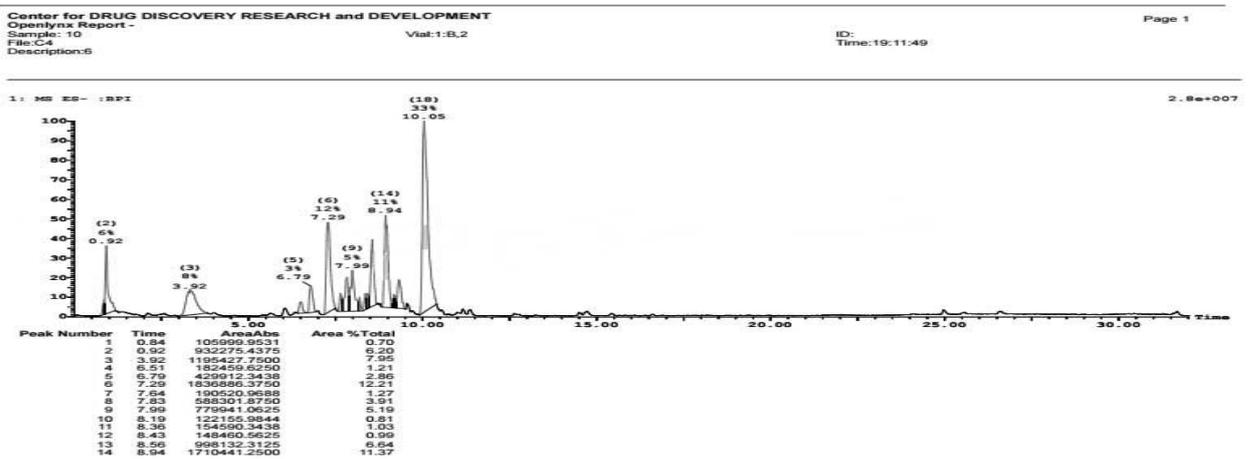


Figure 19. UPLC for n-butanol fraction.

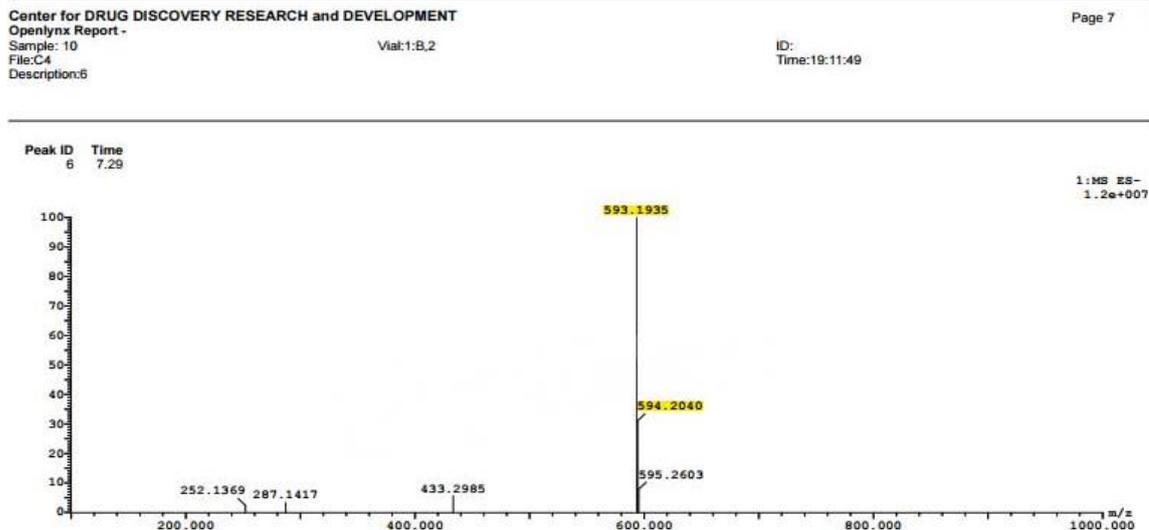


Figure 20. First mass for peak 6 at retention time 7.29 min with molecular ion [M-H] 593.1935 from n-butanol.

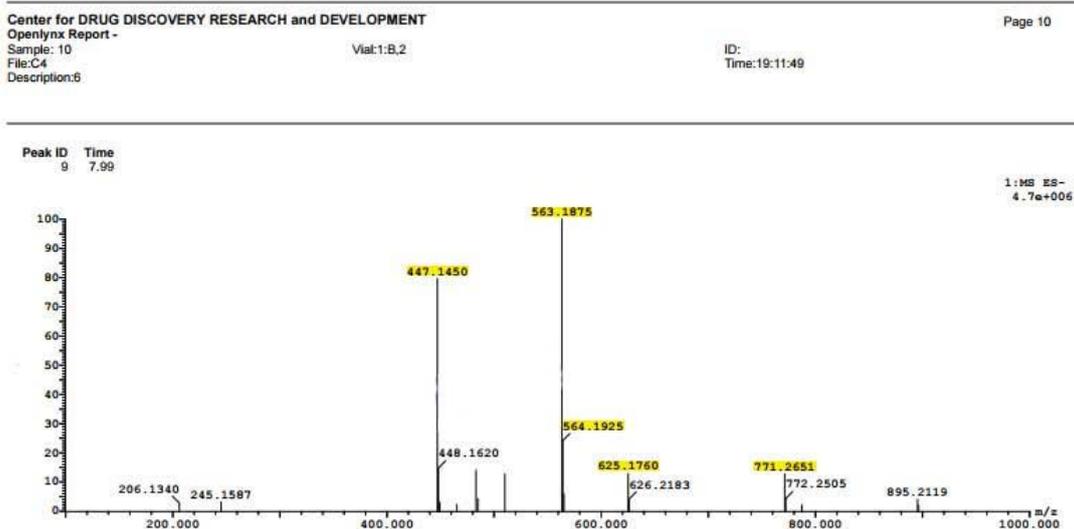


Figure 21. First mass for peak 9 at retention time 7.99 min with molecular ion [M-H] 771.2651 from n-butanol fraction.

Table 7. Identified compounds by UPLC-ESI-MS/MS fragmentation of n-butanol fraction:

| Peak no. | Compound name                        | Class                       | Rt. <sup>1</sup> | M.W | MS <sup>1</sup><br>M-H | Rt. <sup>2</sup> | MS/MS  | References    |
|----------|--------------------------------------|-----------------------------|------------------|-----|------------------------|------------------|--|---------------|
| 6        | vicenin 2                            | Flavonoid glycosides        | 7.29             | 594 | 593.19<br>31           | 7.58             | 593,575, 565,533,<br>503,475,459,445,431,38<br>2,353,<br>311,105,87,73   | (36-37-38)    |
| 9        | Quercetin 3-sophoroside-7-rhamnoside | Flavonoid glycosides        | 7.99             | 772 | 771.26<br>51           | 7.3              | 771,505,461,447,341,30<br>1,299,271,253,179,161,1<br>47,133,103,73,59,43 | (39-40-41)    |
| 3        | 6-hydroxy kynurenic acid             | quinoline-2-carboxylic acid | 3.32             | 205 | 204.08<br>92           | 3.8              | 204,176,159.9,<br>158,132.9,117.9  | (33-10-34-35) |

## Discussion

Natural products have always been a preferred choice of all as it plays a great role in discovering new medicines. The Hexane fraction of the plant was investigated by GC-MS which revealed the presence of gamma-Sitosterol and Stigmast-4-en-3-one, the chromatogram showed peaks with retention times (56.446 and 59.114) respectively and corresponding to the molecular ion peaks in comparison with NIST database as shown in (Figure 7, Table 2). The chloroform fraction of the plant was investigated by TLC, GC MS and UPLS-ESI- MS/MS which showed the presence of a different type of secondary metabolites like alkaloids and triterpene. AS2 compound isolated from chloroform fraction after basify by alumina TLC Plates investigated as ephedrine due to its results. First of all, analytical TLC gives a brown zone with Dragendorff's spray reagent as shown in (Figure 2-3). Furthermore, the GC MS result showed the presence of ephedrine in chloroform fraction at peak 3 (Figure 8 and Table 3) also, isolated AS2 investigate by GC MS as ephedrine according to its retention time, molecular weight [165] and base peak [58] depending on NIST database (Figure 9,10, Table 4). Finally, UPLS-ESI- MS/MS characterized AS2 compound as ephedrine according to its retention time, Molecular ion peak at  $m/z$  166  $[M+H]^+$  and mass fragmentation show loss of water  $[M+H-H_2O]$  to give 148 (base peak), then  $[M+H-CH_3]$  yield  $m/z$  132 and loss of methyl group from nitrogen atom yield  $m/z$  117 (Figure 11-12-13-14, Table 5)<sup>(31)</sup>. The n-butanol fraction was investigated by TLC and UPLS-ESI- MS/MS which showed the presence of flavonoid glycosides which play important anticancer activity (Figure 19-20-21, Table 7). AS1 compound was isolated from n-butanol fraction by preparative TLC recognized as 6-hydroxykynurenic acid since it is given under ultraviolet light at 254 nm reddish-white fluorescence and 366 nm strong fluorescence and a very small amount could be detected<sup>(42)</sup>. Besides UPLS-ESI- MS/MS results of AS1 compound and n-butanol fraction predicted 6-hydroxykynurenic acid depending on its retention time, molecular ion peak at  $m/z$  204.0892  $[M-H]^-$  and mass fragmentation suggesting the loss of 44 Da  $[M - H -44]^-$  to give  $m/z$  159.9 (base peak) in comparison with mass bank database. Beside, 6-hydroxykynurenic acid was previously isolated from *Ephedra foliata* (Figure 15-16-17-18, Table 6)<sup>(33-10-34-35)</sup>.

## Conclusion

The results of the current study showed isolate ephedrine from chloroform fractions after purification. While 6-hydroxykynurenic acid presence in n-butanol fraction due to its acidity. The active components of *E. foliata* have been identified play a crucial role in our life due to its pharmacological actions.

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