# Exploring the Phytochemical Profile of *Typha elephantina* Using Tandem Mass Spectrometry for Characterization of Bioactive Compounds

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#### Abstract

Plant bioactivities encompass the range of biological effects due to compounds derived from plants or plant-based products on living organisms. These bioactivities are typically due to the bioactive compounds of plants, such as alkaloids, flavonoids, terpenoids, polyphenols, and other secondary metabolites. These compounds can influence various physiological processes and exhibit medicinal, nutritional, and therapeutic properties. *Typha elephantina* is a medicinally potent plant. The study aimed to qualify the bioactive compounds from the methanolic extract of *Typha elephantina* through the advanced Tandem mass spectrometry technique. After characterization, the data were interpreted based on precursor and product ions from MS, MS<sub>2</sub> and MS<sub>3</sub> Analysis. A total of ten compounds were identified: Isomagnolol, (8E,10E,12E)-octadic-8,10,12-trienoic acid, Linoleic acid, Wogonin, 9E,11E-oxooctadeca-9,11-dienoic acid, 9-hydroxy-10,12-octadecadienoic acid, 18 hydroxy oleic acid, Robustaflavone, 3', 3"'-Binaringenin and 7-*O*-[6'''-*O*-propenyl]-Allosyl(1-2) Glucoside. These bioactive compounds play essential roles in plant defense, ecological interactions, and stress responses in pharmaceutical, agricultural, and food industries, focusing on their antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. Exploring plant secondary metabolites remains a promising avenue for developing novel therapeutic agents and enhancing ecological sustainability.

Keywords: Phytochemicals, Extraction, Fatty acids, Flavonoids, Ms/Ms Analysis

## **1.0. INTRODUCTION**

Plant secondary metabolites (PSMs) are produced as phytochemicals in different parts of the Plant as a natural defence system against various microbial and environmental stress attacks. The role of these compounds is not just to provide protection; they are linked to many biochemical pathways both inside and outside the Plant and have a variety of well-known therapeutic applications [1]. Like nature has been blessed with a variety of life, so for the Plant to grow, it has to produce a wide range of substances throughout its body, including but not limited to carbohydrates, lipids and proteins, all known as primary metabolites [2]. The primary and secondary metabolic pathways combine to produce advanced or above molecules known as secondary metabolites. Further, these secondary metabolites are not believed to be particularly useful for plant development processes.

Nonetheless, biochemistry has proven that secondary metabolites are essential in plants, such as UV radiation protectants, healing agents from viruses, fungi, bacteria, and other plant pathogens, or as repellents to grazers. These secondary metabolites are most relevant in therapeutical interventions and can be classified into three main classes: polyphenols, terpenes and alkaloids [3].Plants are the sources of an enormous diversity of bioactive molecules, the majority of which do not participate in the development of the plants. Traditionally called secondary metabolites, these substances are often differentially distributed among limited taxonomic groups within the plant kingdom [4]. Numerous biological and therapeutic qualities of medicinal plants benefit one's health. Because They can potentially treat a broad spectrum of diseases [5], these natural resources could be a good substitute for synthetic drugs in both therapeutic and preventative settings [6]. Monocotyledonous plants belong to the genus Typha [7]. The Typhaceae family consists of one genus and 10 to 15 species [8] and is popularly referred to as cattails because of the genus' characteristic inflorescence. Around the world, cattails are wetland plants that grow in brackish seas, swamps, and shallow fresh marshes [9]. In Turkish folk medicine, the female flowers of the Typha species are applied externally to treat burns and wounds and control bleeding [10]. The leaves are analgesic, antioxidant, and diuretic, whereas the lower stem is astringent and diuretic [11].

A novel method with numerous benefits for analyzing particular organic molecules in complex mixtures is the serial coupling of mass spectrometers. It is possible to attain sensitivity to picograms of the targeted chemicals with almost immediate response and excellent specificity. The first mass spectrometer selectively ionizes the targeted molecule, separating its distinctive ions from most of the mixture. After the chosen main ions are broken down by collision, the final mass analyzer chooses secondary ions indicative of the compound of interest from the byproducts. Tandem mass spectrometry can complete analyses in significantly less time while achieving specificities and sensitivities comparable to those of techniques like gas chromatography/mass spectrometry and radioimmunoassay [12]. The aquatic Plant *Typha elephantina* (Typhaceae) has impressive therapeutic qualities. Traditionally, almost every portion of *Typha elephantina* 

has been used to cure a variety of illnesses, such as boils, burns, wounds, scab problems, blood coagulation disorders, bacterial infections, leprosy, cystitis, splenic enlargement, and strangury. Additionally, the Plant is said to have a number of medicinal properties, such as the ability to stabilize membranes, thrombolytic, anthelmintic, antioxidant, anxiolytic, wound healing, anti-inflammatory, cytotoxic, and analgesic effects, all of which are supported by pharmacological methods [13]. The study aimed to identify bioactive chemical constituents from Typha elephantina by using Tandem mass spectrometry.

## 2.0. MATERIALS AND METHODS

**2.1.** Fresh *Typha elephantina* (Kondr, Lukhy) was collected from Balochistan from Zhob (daeragi pull, bhatyie). It was identified by the Department of Botany SBK Women's University, Quetta and the Plant Sciences Department, Quaid-i-Azam University, Islamabad.

## 2.2. Extraction

For two months, the *Typha elephantina* aerial parts extract was prepared by maceration of 2100g of plant material in 15L MeOH. The infusions were filtered, and the filtrate was evaporated to dryness using a Heidolph 4000-efficient rotary evaporator. As a result, crude extract in syrupy liquid was obtained and named TE(2)/MeOH.

## **2.3.** Characterization by Tandem Mass Spectrometry

Tandem mass spectrometry (MS/MS) is a highly effective analytical technique used in chemical analysis to identify unknown compounds, quantify known substances, and determine molecular structures. In this method, the sample is first ionized to produce precursor ions, which are then subjected to further fragmentation through subsequent stages of mass spectrometry (e.g., MS<sup>2</sup> or MS<sup>3</sup>), generating fragment ions for detailed analysis.

### **2.4.Instrumental parameters**

The fragmentation experiments by Linear Ion Trap Mass spectrometer were performed using LTQXL (Thermo Electron Scientific, USA) equipped with an electrospray ionization (ESI) source. Methanol was used as a solvent, and the injection mode was the direct insertion method. The flow rate for the sample was 10µL/min. The negative ionization mode was employed for scanning with 4.2kV capillary voltage at 280 °C. Nitrogen was used as thesheath and auxiliary gas at 20 and 5 arbitrary units, respectively. Mass spectral data were acquired over 50-2000 m/z scanning mass range. Helium was employed as a damping and collision gas at a partial pressure of 50 psi. Fragmentation was accomplished using Collision Induced Dissociation (CID). The relative collision energies employed were from 20 to 30 eV. The software used for data acquisition was Xcalibur 2.0.7.

#### Names of compounds **Fragment** ions S.No [M-H]<sup>-</sup> References Xu et al., [14] 1 Isomagnolol 265 247.08, 221.17, 205.00, 175.00, 149.00, 119.17, 103.08, 96.92, 80.00 2 277 262.17, 259.25, 257.25, Levandi et al., [15] (8E,10E,12E)-octadeca-233.25, 217.08, 205.17, 8,10,12-trienoic acid. Mok et al., [16] 179.25, 163.17, 134.08, 121.00, 97.17, 83.17 3 Linoleic acid 279 261.25, 259.25, 235.25, Mok et al., [16] 205.17, 193.17, Grati et al., [17] 168.92, 139.00, 127.00, 117.00, Xia et al.,[18] 96.92, 82.92 4 Wogonin 283 240.08, Zhang et al., [19] 268.00, 255.08, 224.08, 207.17, 183.08, 136.83, 181.08, 155.08, 102.25, 92.92 9E,11E-oxooctadeca-9,11-5 293 275.17, 265.25, 249.25, Mok et al., [16] dienoic acid. 236.17, 221.17, 211.17, Xu et al., [14] 185.17, 171.08, 149.08, 131.08, 113.08, 97.00 9-hydroxy-10,12-6 295 277.25. 263.17. 251.25, Mok et al., [16] octadecadienoic acid. 233.25, 219.25, 195.17, Xu et al., [14],

### **3.0. RESULTS AND DISCUSSION**

The compounds which were identified in the areal parts of *Typha elephantine* are reported in Table (1).

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			179.17, 171.08, 169.08, Grati et al.,[17]
			125.08, 113.08, 98.92
7	18 hydroxy oleic acid	297	279.25, 269.17, 253.17, Gläser et al., [20]
			251.17, 233.17, 197.00, Mok et al., [16]
			183.08, 171.08, 148.00,
			135.83, 107.92, 97.00
8	Robustaflavone .H <sub>2</sub> O	555	537.33, 523.25, 518.92, Yao et al., [21]
			511.42, 483.00, 447.08,
			389.17, 341.08, 317.17,
			299.08, 261.17, 255.25,
			225.00, 207.00, 189.08,
			165.00
9	3', 3"'-Binaringenin .2H <sub>2</sub> O	577	559.33, 545.17, 540.92, Yao et al., [21]
			533.42, 503.42, 485.42,
			451.58, 417.33, 413.25,
			409.33, 383.08, 339.17,
			299.08, 277.25, 255.08,
			225.00, 207.00, 175.17
10	7- <i>O</i> -[6 <sup>'''</sup> - <i>O</i> -propenyl]-	681	649.42, 621.42, 605.42, Petreska et al., [22]
	Allosyl(1-2) Glucoside.		577.50, 537.42, 485.25,
			445.33, 403.08, 385.08,
			343.17, 311.08, 271.08,
			227.00

Table 1. The Compounds Identified from Aerial Parts, Methanolic Extract of Typha elephantina.

The deprotonated molecular ion peak [M-H]<sup>-</sup>for compound (1) appeared at m/z 265 with loss of water molecule[M-H-H<sub>2</sub>O]<sup>-</sup> the product ion m/z 221 generated with loss of 44 Da [C<sub>3</sub>H<sub>8</sub>]<sup>-</sup>. The loss of oxygen radical from m/z 221 gave fragment ion peak at m/z 205. The deprotonated ion yielded m/z 175, m/z 149, m/z 119, m/z 103, m/z 97 and m/z 80 with the loss of 90 Da [M-H-C<sub>7</sub>H<sub>6</sub>]<sup>-</sup>,116 Da, [M-H-C<sub>8</sub>H<sub>4</sub>O]<sup>-</sup>, 146 Da [M-H-C<sub>10</sub>H<sub>10</sub>O]<sup>-</sup>, 162 Da [M-H-C<sub>10</sub>H<sub>10</sub>O<sub>2</sub>]<sup>-</sup>, 168 Da[M-H-C<sub>11</sub>H<sub>4</sub>O<sub>2</sub>]<sup>-</sup>and 185 Da [M-H-C<sub>12</sub>H<sub>9</sub>O<sub>2</sub>]<sup>-</sup>.According to Xu et al.[14]) the deprotonated ion and m/z 265 were the characteristics of isomagnolol.Compound (1) was tentatively considered isomagnolol.

The deprotonated ion  $[M-H]^-$  for compound (2) displayed at m/z 277; the mass of the compound was 277a.m.u. the fragment ion ion m/z 262 appeared due to loss of methyl radical 15da  $[M-H-CH_3]^-$ . The m/z 259, m/z 257 and m/z 233 were obtained by the loss of 18 Da  $[M-H-H_2O]^-$ , 20 Da  $[M-H-H_2O+H_2]^-$ ,44 Da  $[M-H-CO_2]^-$ . The loss of 60 Da  $[M-H-H_2O+H_2]^-$  gave daughter ion peak at m/z 217. The loss of 72 Da  $[M-H-C_3 H 4O_2]^-$ , 98 Da  $[M-H-C_5H_6O_2]^-$ , and 114 Da  $[M-H-C_6H_{10}O_2]^-$ , from deprotonated ion yielded m/z 205, m/z 179 and m/z 163. The fragment ions m/z 134, m/z 121, m/z 97 and m/z 83 appeared with the loss of 143 da  $[M-H-C_8 H_{15}O_2]^-$ , 156 Da  $[M-H-C_9H_{16}O_2]^-$ , 180 Da $[M-H-C_{11}H_{16}O_2]^-$  and 194 Da  $[M-H-C_{12}H_{18}O_2]^-$ From [12] (Mok et al., 2016) it was assumed that the compound belongs to class of fatty acids. The m/z 277 was the characteristic peak the compound (2) was identified as (8E,10E,12E)-octadic-8,10,12-trienoic acid reported by Levandi et al. [15].

The compound (3) showed deprotonated ion [M-H]<sup>-</sup> at m/z 279 with an actual mass of 280 *a.m.u.* The precursor ion yielded fragment ions at m/z 261, m/z 259, m/z 235, m/z 205 and m/z 193 by the loss of water molecule 18 Da [M-H-H<sub>2</sub>O]<sup>-</sup>, 20 Da [M-H-H<sub>2</sub>O+ H<sub>2</sub>]<sup>-</sup>, 44 Da [M-H-CO<sub>2</sub>]<sup>-</sup>, 74 Da [M-H-C<sub>3</sub>H<sub>6</sub>O<sub>2</sub>]<sup>-</sup>and 86 Da [M-H-C<sub>4</sub>H<sub>6</sub>O<sub>2</sub>]<sup>-</sup>. The product ions were obtained from deprotonated ion at m/z 169, m/z 139, m/z 127, m/z 117, m/z 97 and m/z 83 upon the loss of 110 Da [M-H-C<sub>8</sub>H<sub>14</sub>]<sup>-</sup>, 140 Da [M-H-C<sub>8</sub>H<sub>12</sub>O<sub>2</sub>]<sup>-</sup>, 152 Da [M-H-C<sub>9</sub>H<sub>12</sub>O<sub>2</sub>]<sup>-</sup>, 162 Da [M-H-C<sub>12</sub>H<sub>18</sub>]<sup>-</sup>, 182 Da[M-H-C<sub>11</sub>H<sub>18</sub>O<sub>2</sub>]<sup>-</sup>and 196 Da [M-H-C<sub>12</sub>H<sub>20</sub>O<sub>2</sub>]<sup>-</sup>. The base peak m/z 261 was the identifying peak as reported earlier by (Mok et al. [16], Grati et al. [17] & Xia et al. [18]) according to the literature, the base peak m/z 261 and m/z 235 were the characteristics of Linoleic acid the compound (3) was considered as Linoleic acid.

The deprotonated molecular ion [M-H]<sup>-</sup>of compound (4) appeared at m/z 283 while the actual mass is 284 *a.m.u.* The base peak at m/z 268 appeared due to the loss of methyl radical. The loss of 28 Da carbon monoxide gave apeak at m/z 255. The product ions m/z 240, m/z 224, m/z 207, m/z 183 were obtained with the loss of 43 Da [C<sub>2</sub>H<sub>3</sub>O]<sup>-</sup>, 59 Da [C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>]<sup>-</sup>, 76 Da [C<sub>6</sub>H<sub>4</sub>]<sup>-</sup>, 100 Da [C<sub>4</sub>H<sub>4</sub>O<sub>3</sub>]<sup>-</sup> the daughter ion peaks with the loss of 102 Da [C<sub>4</sub>H<sub>6</sub>O<sub>3</sub>]<sup>-</sup>, 128 Da [C<sub>9</sub>H<sub>4</sub>O]<sup>-</sup>, 146 Da[C<sub>9</sub>H<sub>6</sub>O<sub>2</sub>]<sup>-</sup>, 181 Da [m/z C<sub>9</sub>H<sub>9</sub>O<sub>4</sub>]<sup>-</sup> and 190 Da [C<sub>10</sub>H<sub>6</sub>O<sub>4</sub>]<sup>-</sup> appeared at m/z 181, m/z 155, m/z 137, m/z 102 and m/z 93. Zhang et al. [19] reported that the m/z 283 and base peak m/z 268 were the characteristics of wogonin. Therefore, compound (4) was assigned to wogonin.

The compound (5) yielded deprotonated ion  $[M-H]^-$  at m/z 293 with an actual mass of 294 *a.m.u.* The daughter ion peaks appeared at m/z 275, m/z 265, m/z 249 and m/z 236 by the loss of 18 Da  $[M-H-H_2O]^-$ , 28 Da  $[M-H-CO]^-$ ,44 Da  $[M-H-CO_2]^-$ , and 57 Da  $[M-H-C_2HO_2]^-$ . from deprotonated ion the loss of 72 Da  $[M-H-C_3H_4O_2]^-$ , 82 Da  $[M-H-C_4H_2O_2]^-$ ,108 Da  $[M-H-C_7H_8O]^-$  and 122 Da  $[M-H-C_8H_{10}O]^-$  gave fragment ions at m/z 221, m/z 211, m/z 185 and m/z 171. The m/z 149, m/z 131, m/z 113 and m/z 97 resulted in the loss of 144 Da  $[M-H-C_8H_{16}O_2]^-$ , 162 Da  $[M-H-C_{11}H_{14}O]^-$ , 180 Da  $[M-H-C_{12}H_{20}O_2]^-$  and 196 Da  $[M-H-C_{12}H_{20}O_2]^-$ . From m/z 97, the loss of 2 Da gave the last fragment ion peak at 95. From the literature, it was observed [12, 14], it was observed that the m/z 293 and m/z 275 were characteristic of 9E,11E-oxooctadeca-9,11-dienoic acid so the compound (5) was considered as 9E,11E-oxooctadeca-9,11-dienoic acid.

The deprotonated ion [M-H]<sup>-</sup> for compound (6) appeared at m/z 295 with an actual mass of compound 296. The precursor ion yielded product ions m/z 277, m/z 263, m/z 251 and m/z 233 with the loss of 18 Da [M-H-H<sub>2</sub>O]<sup>-</sup>, water molecule 32 Da [M-H-O<sub>2</sub>]<sup>-</sup> oxygen molecule 44 Da [M-H-CO<sub>2</sub>]<sup>-</sup> carbon dioxide molecule and 62 Da [M-H-C<sub>2</sub>H<sub>6</sub>O<sub>2</sub>]<sup>-</sup>. The product ions m/z 219, m/z 195, m/z 179 and m/z 171 were generated by the loss of 76 Da [M-H-C<sub>3</sub>H<sub>8</sub>O<sub>2</sub>]<sup>-</sup>, 100 Da [M-H-C<sub>5</sub>H<sub>8</sub>O<sub>2</sub>]<sup>-</sup>, 116 Da [M-H-C<sub>6</sub>H<sub>12</sub>O<sub>2</sub>]<sup>-</sup> and 124 Da [M-H-C<sub>7</sub>H<sub>8</sub>O<sub>2</sub>]<sup>-</sup>. The loss of 126 Da [M-H-C<sub>7</sub>H<sub>10</sub>O<sub>2</sub>]<sup>-</sup>, 170 Da [M-H-C<sub>9</sub>H<sub>14</sub>O<sub>3</sub>]<sup>-</sup>, 182 Da [M-H-C<sub>10</sub>H<sub>14</sub>O<sub>3</sub>]<sup>-</sup> and 196 Da [M-H-C<sub>11</sub>H<sub>16</sub>O<sub>3</sub>]<sup>-</sup> from precursor ion yielded m/z 169, m/z 125, m/z 113 and m/z 99. From literature (Mok et al., [16]; Xia et al., [18];Grati et al., [17] reported that m/z 295 and m/z 277 base peaks were the characteristics of 9-hydroxy-10,12-octadecadienoic acid so the compound (6) was considered as 9-hydroxy-10,12-octadecadienoic acid.

The deprotonated ion [M-H]<sup>-</sup> for compound (7) appeared at m/z 297 with an actual mass of 298. The fragment ions m/z 279, m/z 269 and m/z 253 were generated with the loss of 18 Da [M-H- H<sub>2</sub>O]<sup>-</sup>, 28 Da [M-H- CO]<sup>-</sup> and 44 Da [M-H-CO<sub>2</sub>]<sup>-</sup> water molecule, carbon mono oxide and carbon dioxide. From m/z 253,2 Da (H<sub>2</sub>) loss gave fragment ion at m/z 251. The m/z 233, m/z 197, m/z 183 and m/z 171 resulted from deprotonated ion by the loss of 64 Da [M-H-CO<sub>2</sub>+H<sub>2</sub>O+H<sub>2</sub>]<sup>-</sup>, 100 Da [M-H-C<sub>6</sub>H<sub>12</sub>O]<sup>-</sup>,114 Da [M-H-C<sub>6</sub>H<sub>10</sub>O<sub>2</sub>]<sup>-</sup> and 126 Da [M-H-C<sub>8</sub>H<sub>14</sub>O]<sup>-</sup>. Product ions m/z 148, m/z 136, m/z 108 and m/z 97 were obtained by the loss of 149 Da [M-H-C<sub>7</sub>H<sub>17</sub>O<sub>3</sub>]<sup>-</sup>, 161 Da [M-H-C<sub>10</sub>H<sub>25</sub>O]<sup>-</sup>, 189 Da [M-H-C<sub>12</sub>H<sub>29</sub>O]<sup>-</sup>, and 200 Da [M-H-C<sub>13</sub>H<sub>28</sub>O]<sup>-</sup>. According to the literature Gläser et al., [20] andMok et al. [16] showed that the compound (7) was identified as 18 hydroxy oleic acid.

The pseudomolecular deprotonated compound (8) ion was displayed at m/z 555 with an actual mass of 556 *a.m.u.* The loss of water molecule 18 Da [M-H-H<sub>2</sub>O]<sup>-</sup>from deprotonated ion gave fragment ion at m/z 537. The m/z 555 was considered as pseudomolecular ion derivatized, so further losses were made from m/z 537. From m/z 537 the fragment ions m/z 523, m/z519, m/z 511 and m/z 483 were obtained with the loss of 14 Da [CH<sub>2</sub>]<sup>-</sup>, 18 Da [M-H-H<sub>2</sub>O]<sup>-</sup>, 26 Da [C<sub>2</sub>H<sub>2</sub>]<sup>-</sup> and 54 Da [C<sub>3</sub>H<sub>2</sub>O]<sup>-</sup>. The m/z 447, m/z 389, m/z 341 and m/z 317 were generated upon the loss of 90 Da [C<sub>6</sub>H<sub>2</sub>O]<sup>-</sup>, 148 Da [C<sub>9</sub>H<sub>8</sub>O<sub>2</sub>]<sup>-</sup>, 196 Da [C<sub>12</sub>H<sub>4</sub>O<sub>3</sub>]<sup>-</sup> and 220 Da [C<sub>12</sub>H<sub>12</sub>O<sub>4</sub>]<sup>-</sup>. The m/z 299, m/z 261, m/z 255 and m/z 225 were generated with the loss of 238 Da [C<sub>14</sub>H<sub>6</sub>O<sub>4</sub>]<sup>-</sup>, 276 Da [C<sub>15</sub>H<sub>16</sub>O<sub>5</sub>]<sup>-</sup>, 282 Da [C<sub>16</sub>H<sub>10</sub>O<sub>5</sub>]<sup>-</sup> and 312 [C<sub>16</sub>H<sub>8</sub>O<sub>7</sub>]<sup>-</sup>. The fragment ion m/z 207 appeared with 18 Da [H2O] loss from m/z 225. The last 2 fragment ions,m/z 189 and m/z 165, resulted in the loss of 348 Da[C<sub>19</sub>H<sub>8</sub>O<sub>7</sub>]<sup>-</sup> and 372 [C<sub>22</sub>H<sub>12</sub>O<sub>6</sub>]<sup>-</sup>. From the literature, Yao et al., [21] and the fragmentation scheme proved that the compound (8) was considered a monohydrate of Robustaflavone.

The deprotonated ion for compound (9) displayed at m/z 577 yielded two fragment ions, 559 and m/z 545, upon the loss of 18 Da [M-H-H<sub>2</sub>O]<sup>-</sup> and 32 Da [M-H-O<sub>2</sub>]<sup>-</sup> water molecule and oxygen molecule. The loss of 36 Da [M-H-2H<sub>2</sub>O]<sup>-</sup> from deprotonated ion gave m/z 541. The other losses were done from m/z 541 because the deprotonated ion is derivatized. the 533, m/z 503, m/z 485, m/z 452 and m/z 417 were generated from m/z 541 with the loss of 8 Da [4H<sub>2</sub>]<sup>-</sup> 38 Da[2H<sub>2</sub>O+H<sub>2</sub>]<sup>-</sup>, 56 Da [C<sub>3</sub>H<sub>4</sub>O]<sup>-</sup>, 89 Da [C<sub>4</sub>H<sub>9</sub>O<sub>2</sub>]<sup>-</sup> and 124 Da [C<sub>6</sub>H<sub>4</sub>O<sub>3</sub>]<sup>-</sup>. The m/z 413 was appeared by the loss of 4 Da [2H<sub>2</sub>]<sup>-</sup> from m/z 417. The loss of 4 Da [2H<sub>2</sub>]<sup>-</sup> from m/z 417 gave fragment ion at m/z 409. The m/z 383, m/z 339, m/z 299, m/z 277, m/z 255 and m/z 225 were resulted by the loss of 158 Da [C<sub>7</sub>H<sub>10</sub>O<sub>4</sub>]<sup>-</sup>, 202 Da [C<sub>11</sub>H<sub>6</sub>O<sub>4</sub>]<sup>-</sup>, 242 Da [C<sub>13</sub>H<sub>6</sub>O<sub>5</sub>]<sup>-</sup>, 264 Da [C<sub>14</sub>H<sub>16</sub>O<sub>5</sub>]<sup>-</sup>,

286 Da  $[C_{16}H_{14}O_5]^-$  and 316 Da  $[C_{15}H_8O_8]^-$  from m/z 541. From m/z 225 the loss of 18 Da  $[H_2O]^-$  and 50 Da  $[C_4H_2]^-$  gave two fragment ions m/z 207 and m/z 175. Yao et al., [21] observed that m/z 541 was the characteristic of biflavonoid. Therefore, compound (9) was considered a dihydrate of 3', 3'''-binaringenin.

The deprotonated ion for compound (10) appeared at m/z 681 with an actual mass of 682 *a.m.u.* fragment ions m/z 649, m/z 621, m/z 605, m/z 578 and m/z 537 were generated by the loss of 32 Da [M-H-O<sub>2</sub>]<sup>-</sup>, 60 Da [M-H-C<sub>2</sub>H<sub>4</sub>O<sub>2</sub>]<sup>-</sup>, 76 Da [M-H-C<sub>4</sub>H<sub>12</sub>O]<sup>-</sup>, 103 Da [M-H-C<sub>4</sub>H<sub>7</sub>O<sub>3</sub>]<sup>-</sup> and 144 Da [M-H-C<sub>7</sub>H<sub>12</sub>O<sub>3</sub>]<sup>-</sup>. m/z 485, m/z 445, m/z 403 and m/z 385 were obtained with the loss of 196 Da [M-H-C<sub>8</sub>H<sub>20</sub>O<sub>5</sub>]<sup>-</sup>, 236 Da [M-H-C<sub>9</sub>H<sub>16</sub>O<sub>7</sub>]<sup>-</sup>, 278 Da [M-H-C<sub>11</sub>H<sub>18</sub>O<sub>8</sub>]<sup>-</sup> and 296 Da [M-H-C<sub>11</sub>H<sub>20</sub>O<sub>9</sub>]<sup>-</sup>. The loss of 338 Da [M-H-C<sub>13</sub>H<sub>22</sub>O<sub>10</sub>]<sup>-</sup>, 370 Da [M-H-C<sub>15</sub>H<sub>30</sub>O<sub>10</sub>]<sup>-</sup> and 410 Da [M-H-C<sub>16</sub>H<sub>26</sub>O<sub>12</sub>]<sup>-</sup> from deprotonated ion gave fragment ions m/z 343, m/z 311 and m/z 271. From m/z 271 the loss of 44 Da [CO<sub>2</sub>]<sup>-</sup> gave fragment ion m/z 227. Petreska et al., [22] reported that the compound (10) was assigned as 5'-hydroxy-Hypolaetin 7-*O*-[6'''-*O*-propenyl]-allosyl(1-2) glucoside.

## 4. Conclusion

Identifying plant secondary metabolites is pivotal in advancing our knowledge of plant chemistry and its practical applications. It deepens our understanding of plant biology and opens doors for developing new natural products, contributing to drug discovery, sustainable agriculture, and bioengineering. The study planned to make the methanolic extract of aerial parts for identifying bioactive compounds by using tandem mass spectrometry. A total of ten compounds were identified. One Isomagnolol, 5 fatty acids, one flavonoid, two biflavonoids and one flavonoid glycoside. According to the literature, all these compounds are bioactive and have great antioxidant potential. In the future, these compounds will be used in drugs to treat different diseases. Further research and refinement of identification techniques will continue to unlock the vast potential of Plant secondary metabolites in improving human health and the environment.

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