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Phytochemical study of aerial parts from *Phlomis tuberosa* L

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**Abstract:** Three iridoid glycoside lamiide(I), Ipolamiide(II) and ipolamiide(III) were isolated from N-butanol fractions obtained from the column chromatography of methanol extract from the aerial parts of *Phlomis tuberosa*. In addition, iridoid cyclopenta[c]pyran-4-carboxylic acid, 7-methyl-, methyl ester and phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy were determined from the chloroform fraction of methanol extract of aerial parts from *Phlomis tuberosa*. Isolation and structural elucidation of compounds were accomplished by PTLC, TLC, CC and spectroscopic methods (UV,  $^{13}\text{C}$  and  $^1\text{H}$  NMR and DEPT, GC-MS).

**Keywords:** *Phlomis tuberosa*, iridoid and iridoid glycoside

## INTRODUCTION

The genus of *Phlomis* L belongs to the *Lamiaceae* family and about 100 species widely spread in North Africa, Europe and Asia. It is a popular tea plant which is enjoyed for its taste and aroma. *Phlomis* species are used to treat various conditions such as diabetes, gastric ulcer, hemorrhoids, inflammation and other wounds [1]. The essential oil of *Phlomis* is composed by four dominated chemotypes such as mono-terpenes (alpha-pinene, limonene and linalool), sesquiterpenes (germacrene D and beta-caryophyllene), aliphatic compounds (9, 12, 15-octadecatrienoic acid methyl ester), fatty acids (hexadecanoic acid) and other components (trans-phytol, 9,12,15-octadecatrien-1-ol). Flavonoids, iridoids and phenylethyl alcohol are the main compounds that are isolated from *Phlomis* extracts [2]. The pharmacological activities of some *Phlomis* species have been investigated previously. According to the experiments, they include following biological activities such as antidiabetic, antinociceptive, antiulcerogenic, protection of the vascular system, anti-inflammatory, antiallergic, anticancer, antimicrobial and antioxidant properties. In Asia medicine *Ph. tuberosa* is used as a general roborant, intoxications, tuberculosis, pulmonary and cardiovascular diseases and rheumatoid arthritis [3]. Recent studies on this species from the flora of Bulgaria showed the presence of several iridoid and phenylethanoid glycosides [4 - 6]. In this paper we report the isolation and structure elucidation of three iridoid glucosides, iridoid cyclopenta[c]pyran-4-carboxylic acid, 7-methyl-, methyl ester and a phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy obtained from the aerial parts of *Phlomis tuberosa*.

## EXPERIMENTAL

Column Chromatography (CC) was performed with Silica gel 30-70 (Merck), preparative TLC were carried out on Silica gel 60 PF<sub>254</sub>. Compounds were sprayed with 1% vanillin in H<sub>2</sub>SO<sub>4</sub>, followed by heating at 100°C for 1-2 min. NMR measurements in CD<sub>3</sub>OD at room temperature were measured using a Varian Unity 500 spectrometer operating at 500MHz and 125MHz for  $^1\text{H}$  and  $^{13}\text{C}$  respectively. Gas Chromatography-Mass spectrometry (GC-MS) and well equipped with fused silica capillary column 30mX0.25mmX 0.25  $\mu\text{m}$  were used. Moreover, coated with HP-5 MS phase and coupled with Hewlett Packard 6890/MSD 5793 A E were used. Carrying gas was He at 0.8ml/min flow rate. Program of the GC-MS was as following: temperature 50-300°C at 6°/min, isotherm 0-10min, solvent delay 2.0min, and mass range 50-750. The flame ionization detector was used at T<sub>ini</sub> 260°C, T<sub>aux</sub> 280°C.

**Plant material:** The aerial parts of *Ph. tuberosa* were collected in August 2011 during the full flowering time from mountain of Bayanchandmani soum, Tuv aimag which is central region of Mongolia. A voucher specimen (3020) is deposited in the Herbarium Fund of the Institute of Botany, Mongolian Academy of Sciences (Ulaanbaatar, Mongolia). The plant material was identified by Dr. Ch. Sanchir from the Institute of Botany, Mongolian Academy of Sciences.

**Extraction and isolation:** The air-dried and powdered aerial parts of *Ph. tuberosa* (600 g) were extracted with MeOH (4 x 3000 ml) at 40°C. Methanol extracts were combined and evaporated to dryness in *vacuo*. Resulting crude extract (125.5 g) was dissolved in H<sub>2</sub>O (400 ml) and isolated by CHCl<sub>3</sub> (5x300 ml) and n-BuOH (6x 300ml). The CHCl<sub>3</sub> layer was then defined by GC-MS method. Furthermore, crude extract of the n-BuOH (50 g) was separated by VLC on neutral alumina employing H<sub>2</sub>O and gradient MeOH-H<sub>2</sub>O mixtures (25-100%).

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These six main yielded fractions were marked as following: Fr. A (33.92 g), Fr. B (3.64 g), Fr. C(826 mg), Fr. D(500 mg), Fr. E(1800 mg), Fr. F(1320 mg). The fraction A was subjected to Silica gel in column chromatography and eluted with CH<sub>2</sub>Cl<sub>2</sub>-MeOH-H<sub>2</sub>O (80:20:1- 80:40:4) to yield seven fractions (fractions A<sub>1</sub>-A<sub>7</sub>). Fraction A<sub>4</sub> (1040 mg) was rechromatographed over silica gel and eluted with CH<sub>2</sub>Cl<sub>2</sub>-MeOH-H<sub>2</sub>O (80:20:1- 80:20:2) to afford six fractions (fractions A<sub>4a</sub>-A<sub>4f</sub>). The fraction A<sub>4e</sub> was pure I (80 mg). Fr. A<sub>4b</sub> (170 mg) was subjected to neutral alumina employing CC, and eluted with H<sub>2</sub>O to separate two fractions (A<sub>4b1</sub>, A<sub>4b2</sub>). The fraction A<sub>4b1</sub> (100mg) was subjected and eluted by CH<sub>2</sub>Cl<sub>2</sub>-MeOH (90:10) and CH<sub>2</sub>Cl<sub>2</sub>-MeOH-H<sub>2</sub>O (90:10:0.5) mixtures and fractionated compound II (40mg). Fraction D+E was subjected to Vacuum liquid chromatography (VLC) using gradient MeOH-H<sub>2</sub>O mixtures (20-50%) and isolated II (70 mg), III (120.8 mg), IV (7.0 mg) and V (84 mg). Fraction F was subjected to VLC. Elution with MeOH-H<sub>2</sub>O mixtures (5-65%) partitioned VI (62.5mg) and additional amounts of VII (32.9 mg).

## RESULTS AND DISCUSSION

Lamiide (I): White amorphous powder, MS C<sub>17</sub>H<sub>26</sub>O<sub>12</sub>, found 423.1163 for (M+H);UV (MeOH) max 232 nm;

<sup>1</sup>H NMR(CD<sub>3</sub>OD, 500 MHz): 6.41 (1H, s, H-1), 7.68 (1H, s, H-3), 2.79 (1H, dd, J= 3.2; 8.8 Hz, H $\alpha$ -6), 2.86 (1H, dd, J= 2.3; 8.8 Hz, H $\beta$ -6), 3.92-3.93 (1H, m, H-7), 3.64 (1H, s, H-9), 1.34 (3H, s, H-10), 3.50 (3H, s, COOMe, H-12), 5.30 (1H, d, J= 4.7 Hz, H-1'), 3.92-3.93 (4H, m, H-2'), 4.20 (1H, m, H-3'), 4.22 (1H, m, H-4'); 3.92-3.93 (1H, m, H-5'), 4.33 (1H, dd, J= 4.8;11.9 Hz H-6'); 4.42 (1H, dd, J= 1.8;11.7 Hz H-6'); (<sup>13</sup>C NMR (CD<sub>3</sub>OD, 125MHz): Table 1.

Lamalbide (II): Compound II was obtained amorphous powder. Its molecular formula was determined as C<sub>17</sub>H<sub>26</sub>O<sub>12</sub>, by MS spectrum displayed an additional signal one mass unit above the [M+H]<sup>+</sup>-peak. UV (MeOH) spectrum of II revealed a maximum at 236 nm, typical for C-4 substituted iridoids.

<sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz): 5.61 (1H, d, J= 1.6 Hz, H-1), 7.40 (1H, s, H-3), 2.92 (1H, dd, H $\alpha$ -5; 10.8; 3.9Hz), 3.94 (1H, dd, 4.4; 3.9 Hz; H $\beta$ -6), 3.54 (1H, d, 4.4 Hz; H $\alpha$ -7), 2.80 (1H, dd, 10.8; 1.6 Hz H-9), 1.20 (3H, s, H-10), 3.72 (3H, s, COOMe, H-12), 4.60 (1H, d, J= 7.9 Hz, H-1'), 3.16(1H,m, H-2'), 3.35 (1H, t, J= 9.0 Hz, H-3'), 3.37 (1H, t, J= 9.0, H-4'), 3.32 (1H, m, H-5'), 3.88 (1H, dd, J= 11.9; 1.7 Hz, H-6'), 3.65 (1H, dd, J= 11.9; 5.7 Hz, H-6'), (<sup>13</sup>C NMR (CD<sub>3</sub>OD, 125MHz): Table 1.

Table1. The <sup>13</sup>C and <sup>1</sup>H NMR spectroscopic data for Lamiide (I), lamalbide (II) and Ipolamiide (III) (CD<sub>3</sub>OD, <sup>13</sup>C; 125MHz; <sup>1</sup>H; 500MHz)

| C/H | Lamiide(I) | Lamalbide(II)                         | Ipolamiide(III) |  |       |  |
|-----|------------|---------------------------------------|-----------------|--|-------|--|
| 1   | 95.31      | 6.41(s)                               | 94.6            | 5.60 d (J=1.6)                                 | 94.1  | 5.80(s)                                |
| 3   | 151.08     | 7.68(s)                               | 152.6           | 7.40 s   | 152.6 | 7.43(s)                                |
| 4   | 115.58     | -                                     | 11.7            | -  | 115.4 | -                                      |
| 5   | 68.75      | -                                     | 37.4            | 2.92 dd (J=10.8, 3.9)                          | 71.6  | -                                      |
|     |            | $\alpha$ :2.79                        |                 |  |       |  |
| 6   | 46.57      | (dd; J= 3.2; 8.8Hz)<br>$\beta$ :2.86  | 78.6            | 3.94 dd (J=4.4, 3.9)                           | 38.8  | $\alpha$ : 2.26(m)<br>$\beta$ :1.92(m) |
|     |            | (dd; J= 2.3; 8.8Hz)                   |                 |  |       |  |
| 7   | 78.61      | 3.92-3.93m                            | 78.7            | 3.54 d (J=4.4)                                 | 61.6  | 2.46(s)                                |
| 8   | 78.27      | -                                     | 78.5            | -  | 78.9  | -                                      |
| 9   | 58.08      | 3.64(s)                               | 49.2            | 2.80 dd (J=10.8, 1.6)                          | 61.6  | 2.48(s)                                |
| 10  | 21.56      | 1.34(s)                               | 22.1            | 1.20 s   | 23.2  | 1.17(s)                                |
| 11  | 166.74     | -                                     | 169.5           | -  | 168.8 | -                                      |
| 12  | 50.53      | 3.50(s)                               | 51.8            | 3.72 s   | 51.7  | 3.72(s)                                |
| 1'  | 100.34     | 5.30(d; J= 4.7Hz)                     | 99.7            | 4.60 d (J=7.9)                                 | 99.5  | 4.57(d; J= 7.9Hz)                      |
| 2'  | 74.22      | 3.92-3.93(m)                          | 74.5            | 3.16 dd (J=7.9, 9.0)                           | 74.3  | 3.2(dd; J= 7.9; 9.5Hz)                 |
| 3'  | 78.27      | 4.20(m)                               | 77.9            | 3.35 t (J=9.0)                                 | 77.3  | 3.46(t; J= 9.2Hz)                      |
| 4'  | 70.89      | 4.22(m)                               | 71.5            | 3.37 t (J=9.0)                                 | 71.4  | 3.42(t; J= 9.0Hz)                      |
| 5'  | 77.09      | 3.92-3.93(m)                          | 78.3            | 3.32 m   | 78.3  | 3.50(m)                                |
|     |            | $\alpha$ :4.33                        |                 |  |       | $\alpha$ :3.90                         |
| 6'  | 62.05      | (dd; J= 4.8; 11.9Hz)<br>$\beta$ :4.42 | 62.8            | 3.88 dd (J=11.9, 1.7)<br>3.65 dd (J=11.9, 5.7) | 62.8  | (dd; J= 12.0; 1.8Hz)<br>$\beta$ :3.71  |
|     |            | (dd; J= 1.8; 11.9Hz)                  |                 |  |       | (dd; J= 12.0; 5.8Hz)                   |

Ipolamiide (III): Compound III was obtained as a colorless, amorphous compound. MS  $C_{17}H_{26}O_{11}$ , found 407.1193 for (M+H); UV (MeOH) max 229 nm;  $^1H$  NMR ( $CD_3OD$ , 500 MHz): 5.80 (1H, s, H-1), 7.43 (1H, s, H-3), 1.92 (1H, m, H $\alpha$ -6), 2.26 (1H, m, H $\beta$ -6), 2.46 (1H, s, H $\alpha$ -7), 2.48 (1H, s, H-9), 1.17 (1H, s, H-10), 3.72 (3H, s, COOMe, H-12), 4.57 (1H, d, J= 7.9 Hz, H-1'), 3.17 (1H, dd, J= 7.9; 9.5 Hz, H-2'), 3.46 (3H, t, H-3'), 3.42 (1H, t, J= 9.0 Hz, H-4'), 3.50 (1H, m, H-5'); 3.90 (1H, dd, J= 12.0; 1.8 Hz, H-6'), 3.71 (1H, dd, J= 12.0; 5.8 Hz, H-6'),  $^{13}C$  NMR ( $CD_3OD$ , 125MHz): Table 1.

Cyclopenta[c]pyran-4-carboxylic acid, 8-methyl-, methyl ester (V)  $C_{11}H_{10}O_3$  m/z 190M<sup>+</sup>, 189(78.9%) (M<sup>+</sup>-1)<sup>+</sup>, 175(100) (M<sup>+</sup>-CH<sub>3</sub>)<sup>+</sup>, 159(60.5)(M<sup>+</sup>-OCH<sub>3</sub>)<sup>+</sup>, 131(26.9)(M<sup>+</sup>-COOCH<sub>3</sub>)<sup>+</sup> (Fig.1.)

Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy (VI)  $C_{10}H_{12}O_3$  m/z 180M<sup>+</sup>, 179(78.9%)(M<sup>+</sup>-1)<sup>+</sup>, 137(100) (M<sup>+</sup>-C<sub>2</sub>H<sub>3</sub>O)<sup>+</sup>, 124(62.5), 119(26.9), 106(16.4), 91(39.4), 77(11.8), 63(11.18), 51(9.8) (Fig.1).

Compound (I) was obtained as a white amorphous powder and its structure was determined by the results based on  $^1H$ - $^{13}C$ -NMR and DEPT experiments. The  $^1H$  NMR spectrum of compound I showed signals at 7.68 (1H, s) and 5.31 (1H, d) which are characteristic of iridoid glycosides having either carboxy or carbomethoxy group at C-4 and glucose at C-1 respectively. DEPT analysis showed resonance for two different CH<sub>2</sub> which are located at 46.57 (C-6) and 62.05 (C-6') ppm, and there are resonance of four quaternary carbons. Two of them attached to ring and hydroxyl group (68.75; C-5 and 78.27; C-8) ppm,

carbonyl group (C=O, 166.74) ppm and a quaternary double bond (C=CH, 115.58) ppm. By the complete analysis of the NMR data (see table 1) and a comparison with the reported data in the literatures [7], compound I was identified as lamiide (Fig. 2) which has been found in many *Phlomis* species [8 - 13]. Lamiide as an iridoid glycoside has shown anti-inflammatory activity and lipid peroxidation inhibition [14].

Lamalbiide(II) The  $^{13}C$  NMR spectroscopic data indicated the presence of 17 carbons resonances, six of them which were assigned to  $\alpha$ -glucopyranosyl moiety. The  $^1H$  NMR spectrum of II were exhibited the characteristic signals for an iridoid structure, and it showed that existence of a methoxycarbonyl function (H 3.72, s) and a tertiary methyl group (H 1.20, s) respectively. The signal of anomeric proton which is belongs to glucopyranose unit, detected at H 4.60 (d, J = 7.9 Hz). The C-1 position of the iridoid aglycon was glycosidated and shifted to glucopyranose unit. Consequently H-1 signal was observed at (H 5.80, s). The chemical shift values and the splitting patterns of H-3 (H 7.43, s), H-5 (H 2.92, dd) and H-9 (H 2.48, s) were suggestive of C-4, C-8 and C-11 to be substituted. Thus, the methoxycarbonyl group was assigned to be positioned at C-4, due to the high frequency signal of the H-3 proton quaternary carbon resonance detected at C 78.5 (on the other word it attributed to C-8). The overall test of the  $^1H$  and  $^{13}C$  NMR data of II was allowed the assignments of the double signals observed at H 2.92; 3.9 and H 3.54;

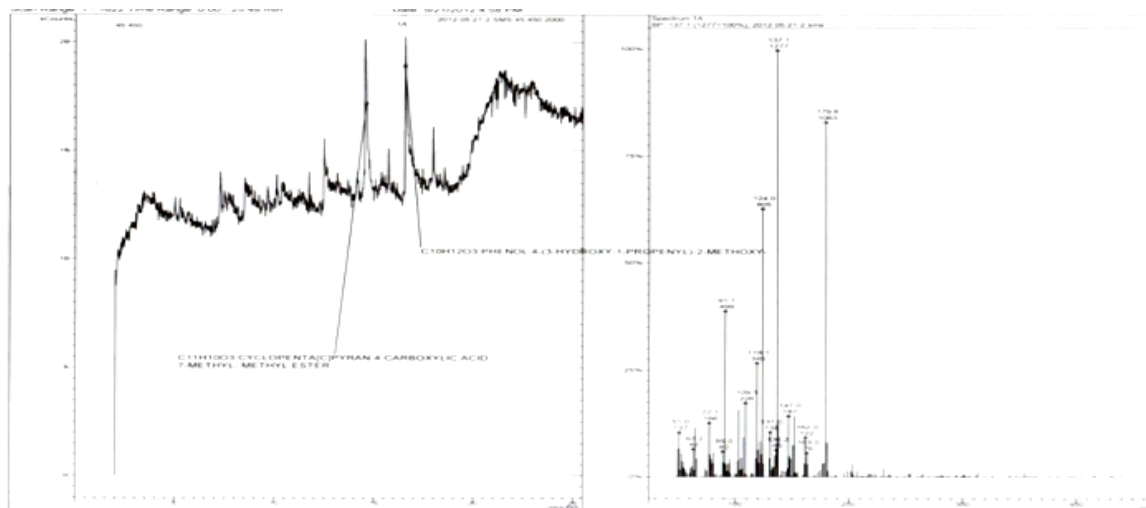


Fig. 1. MS spectrum of Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-

2.80 to the methyne protons at C-5 (C 37.4, t), C-6 (C 78.6, t), C-7 (C 78.7) and C-9 (C 49.2) respectively. However, the chemical shift value of the tertiary methyl group (H 1.20, s) suggested its position at C-8. On the other hand, the chemical shift values of both C-8 (C 78.5, s) and H<sub>3</sub>-10 also indicated the presence

of a tertiary hydroxyl function at C-8 location. According to the complete research of the NMR data of II, and it's compared data given in the literature [6. 15], compound II was defined to be lamalbiide (Fig. 2). Ipolamiide (III) The  $^{13}C$  NMR spectrum of III showed 17 carbon signals, six of them which could be assigned

to  $\alpha$ -glucopyranosyl moiety. The  $^1\text{H}$  NMR spectrum of III exhibited the characteristic signals for an iridoid structure and it showed the existence of a methoxycarbonyl function (H 3.73, s), and a tertiary methyl group (H 1.15, s). In addition, arising of resonances from two methylene groups was observed. The anomeric proton of glucopyranose unit was assigned at signal H 4.58 (d,  $J = 7.9$  Hz). The H-1 signal (H 5.81, s), which was shifted due to glycosidation and it indicated the attachment of the  $\alpha$ -glucopyranose unit at the C-1 position of the iridoid aglycon.

The chemical shift values and the splitting patterns of H-3 (H 7.44, s) and H-9 (H 2.48, s) were suggestive of C-4, C-5 and C-8 to be substituted. Thus, the methoxycarbonyl group was assigned to be positioned at C-4, due to the high frequency signal of the H-3 proton, and the quaternary carbon resonance at C 71.6 was attributed to C-5. The complete analysis of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of II was allowed the assignments of the multiplicity signals observed at H 2.26; 1.92 and H 2

.10; 1.59 ppm to the methylene protons at C-6 (C 38.8, t) and C-7 (C 40.3, t), respectively. The multiplicity of H-9 was also indicated that it totally substituted C-8. However, the chemical shift value of the tertiary methyl group (H 1.15, s) suggested its attachment at C-8. On the other hand, the chemical shift values of both C-8 (C 78.9, s) and H<sub>3</sub>-10 also indicated the presence of a tertiary hydroxyl function at C-8 position. Both the complete analysis of the NMR data of III, and the data given in the literature [16, 17, 18], confirms that the compound III was determined to be ipolamiide (Fig. 2). Ipolamiide showed anti-inflammatory activity [19].

The  $\text{CHCl}_3$  layer was defined by GC-MS method and identified Cyclopenta[c]pyran-4-carboxylic acid, 8-methyl-, methyl ester and phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy (Fig.1).

Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy- showed various activities such as antioxidant, antimicrobial, anti-inflammatory [20] and so on.

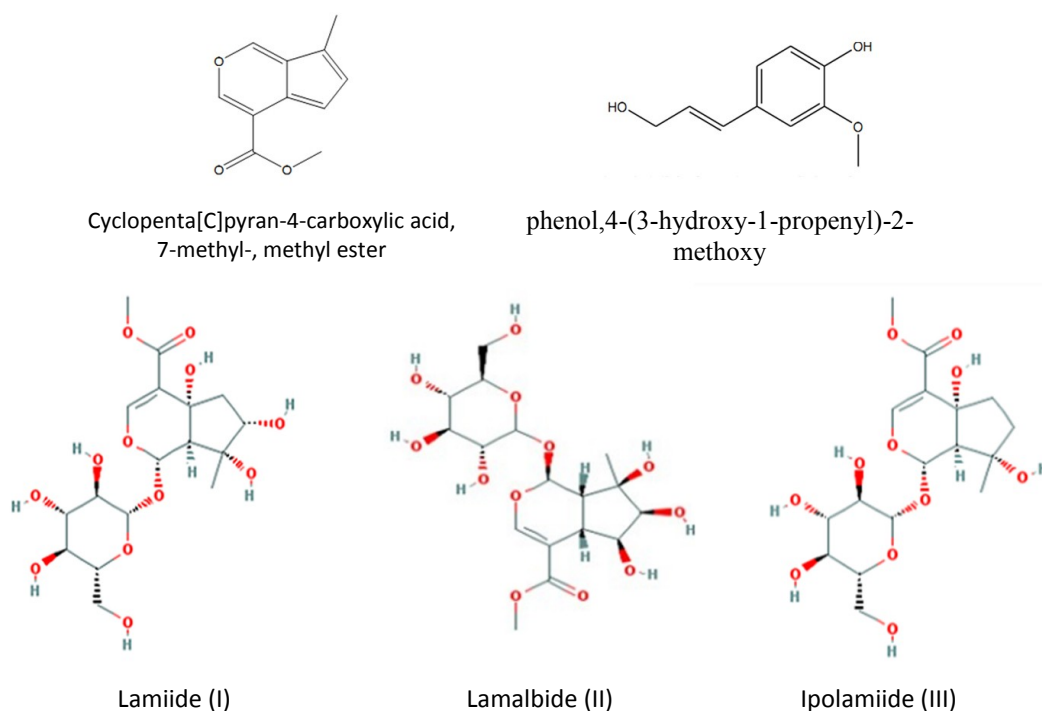


Fig. 2. Secondary metabolites of *Ph. tuberosa*

## CONCLUSIONS

In this investigation, from the aerial parts of *Phlomis tuberosa* three iridoid glucosides lamiide I, lamalbiide II and ipolamiide III, iridoid cyclopenta[c]pyran-4-carboxylic acid, 7-methyl-, methyl ester and phenolic compound Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy- from *Ph. tuberosa* were identified. The Ipolamiide, Cyclopenta[c]pyran-4-carboxylic acid, 7-methyl-, methyl ester and Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy- from *Ph. tuberosa* have been reported for the first time.

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