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***Original Article***

# Chemical compositions and biological activities of *Scutellaria pinnatifida* A. Hamilt aerial parts

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## *Abstract*

Phytochemical analysis of the methanolic and dichloromethane extracts of the aerial parts of *Scutellaria pinnatifida* led to the isolation of a phenylpropanoid, 1-o-feruloyl-β-D-glucose **(1)**, two known flavonoids including luteolin-7-o-glucoside **(2)** and apigenin-7-o-glucoside **(3)**, three known phenylethanoid glycosides composed of phlomisethanoside **(4),** syringalide A **(5)**, and verbascoside **(6),** and oleic acid **(7)**. Isolation and structural elucidation of compounds were accomplished by HPLC and spectroscopic methods (UV, $^{1}$H-NMR, $^{13}$C-NMR). The extracts were also evaluated for their radical scavenging activity and insecticidal property by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay and contact toxicity method, respectively. Among the extracts, the methanol extract showed the most potent free radical scavenging activity with a $RC_{50}$ value of 0.044 ± 0.350 mg/mL which could be attributed to the presence of the isolated phenolic compounds. In the case of insecticidal activity, the n-hexane extract displayed the most potent activity and caused 10%, 15%, and 40% mortality to *Oryzaephilus mercator* at the concentration of 5, 10, and 15 mg/mL after 4 h of exposure.

***Keywords:*** *Scutellaria pinnatifida*; Phenylpropanoid; Antioxidant activity; Insecticidal activity

## INTRODUCTION

*Scutellaria pinnatifida* (*S. pinnatifida*) A. Hamilt, commonly known as "skullcaps" is one of the 20 Iranian species of the genus *Scutellaria* L. (Lamiaceae family) and also has been extensively used in traditional Chinese medicine (1,2). The root of *S. baicalensis*, is best known candidate of this genus for its anti-inflammatory, antitumor, anticancer, antibacterial, and antioxidant properties, and its flavonoid content (2-4). Other species of this genus have also been used by many cultures for the treatment of hypertension, atherosclerosis, inflammatory diseases, hepatitis, cancer, allergy, and have shown sedative, antioxidant, antithrombotic, antimicrobial, neuroprotective, anxiolytic, insecticidal, and antiviral properties (5-7). Except for the reports on the chemical composition of the volatile oil from the aerial parts of *S. pinnatifida* and some biological activities (7,8), to the best of our knowledge, there is no report available to date on phytochemical investigation of this species. From other *Scutellaria* species, flavonoids especially bioflavonoids (9), iridoids (10), phenylethanoids (11), and diterpenoids (5) have been previously reported. As a part of our continuing studies on the Lamiaceae family and also for having valuable compounds in this genus, we report here the isolation and structure elucidation of a phenylpropanoid, 1-o-feruloyl-β-D-glucose **(1)**, two known flavonoids including luteolin-7-o-glucoside **(2)** and apigenin-7-o-glucoside **(3)**, three known phenylethanoid glycosides composed of phlomisethanoside **(4),** syringalide A **(5)**, and verbascoside **(6),** and oleic acid **(7)** as well as biological activities of different extracts of *S. pinnatifida*.

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## MATERIALS AND METHODS

### *Materials*

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Trolox were purchased from Sigma-Aldrich (United Kingdom). All solvents used were procured from Caledon (Canada).

### *General experimental procedures*

Reversed-phase preparative high pressure liquid chromatography (prep-HPLC) analyses were carried out on a HPLC system (Knauer, Germany), equipped with a Knauer PDA detector 2800 (Heraeus, Germany), using a reversed-phase Reprosil 100 $C_{18}$ column (10 μm particle size, 250 × 20 mm i.d.) (Dr. Maisch, Germany).

The NMR spectroscopic analyses (one-dimensional, $^{13}C$ spectra) were obtained on a Bruker 200 NMR spectrometer (Bruker, Germany) (200 MHz for $^{1}H$, and 50 MHz for $^{13}C$). Chemical shifts (δ, ppm) are reported relative to transcranial magnetic stimulation (TMS) as an internal standard. Sephadex LH20 (Amersham Biosciences, Sweden) used for column chromatography and Sep-pak cartridge (10 g, Waters, Ireland) used for fractionating of methanol (MeOH) extract. Thin layer chromatography (TLC) was performed on silica gel GF-254 plates (Merck, Germany) and spots were detected by ultraviolet (UV) illumination.

### *Plant material*

The aerial parts of *S. pinnatifida* were collected from Payam region near Marand, East Azarbaijan province, Iran. A voucher specimen was identified and preserved (Tbz-Fph-704) in the herbarium of the School of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

### *Extraction and isolation*

Air-dried and ground aerial parts of *S. pinnatifida* (100 g) were Soxhlet-extracted, successively, with n-hexane, dichloromethane (DCM), and MeOH (1.1 L each). All these extracts were separately concentrated using a rotary evaporator at a maximum temperature of 45 °C.

Two gram of the MeOH extract was subjected to solid-phase extraction (SPE) using a $C_{18}$ Sep-pak cartridge, eluting with a step gradient of MeOH-water mixtures (10:90, 20:80, 40:60, 60:40, 80:20, and 100:0).

The SPE fraction eluted with 20% MeOH was analyzed by prep-HPLC using the mobile phase: 0-24 min, linear gradient of 20-40% MeOH in water; 24-36 min, maintained at 40% MeOH in water to isolate compounds **1** (4.7 mg, $t_R$ = 19.25 min), **2** (13.4 mg, $t_R$ = 21.63 min), and **3** (12.7 mg, $t_R$ = 27.31 min). Similar prep-HPLC analyses of the 40% methanolic SPE fraction (mobile phase: 0-24 min, linear gradient of 40-50% MeOH in water; 24-48 min, maintained in 50% MeOH in water) afforded compounds **4** (7.6 mg, $t_R$ = 22.35 min), **5** (4.0 mg, $t_R$ = 27.46 min), and **6** (4.7 mg, $t_R$ = 36.82 min).

In all above prep-HPLC analyses, the flow rate of the mobile phase was 8.0 mL/min. The structures of all compounds **1-6** were elucidated unequivocally by spectroscopic means and comparing with references. 2 g of the DCM extract was fractionated on a Sephadex LH-20 column chromatography eluted with MeOH with the flow rate of 2.0 mL/min and purified the fractions by TLC on silica gel (mobile phase, $CHCl_3$:MeOH 8:2) yielded compound **7** (4.6 mg).

### *Free radical scavenging activity*

The free radical scavenging activities of the extracts were assessed by the DPPH assay used by Takao, *et al* (12) with suitable modifications. DPPH (8 mg) was dissolved in MeOH (100 mL) to obtain a concentration of 80 μg/mL.

The methanolic extract of plants was dissolved in MeOH and the n-hexane and DCM extracts were dissolved in chloroform to obtain a concentration of 1 mg/mL. Serial dilutions were prepared to obtain concentrations of, $2.5 \times 10^{-1}$, $1.25 \times 10^{-1}$, $6.25 \times 10^{-2}$, $3.13 \times 10^{-2}$, $1.56 \times 10^{-2}$, $7.81 \times 10^{-3}$, $3.91 \times 10^{-3}$, and $1.95 \times 10^{-3}$ mg/mL. Diluted solutions (1 mL each) were mixed with DPPH solution (1 mL) and allowed to develop for 30 min. The UV absorbance was measured at 517 nm.

The experiment was performed in triplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive control, quercetin. Percent reduction of the free radical DPPH (R%) was calculated using following equation:

$$R\% = [(AB–AA)/AB] \times 100$$

where, AB is the absorbance of blank and AA is the absorbance of test samples. For calculating the $RC_{50}$ values (concentration providing 50% reduction), the graph of reduction percentage against extract concentrations was utilized.

***Insecticidal assay (contact toxicity method)***

Adults of *Oryzaephilus mercator* (*O. mercator*) were collected from a laboratory culture. *O. mercator* was reared on a mixture of whole wheat and maize flour at the ratio 1:1 in glass containers containing 0.5 kg of the mixture.

All insect species were reared at 27 ± 2 °C, 12% moisture content in continuous darkness for about 3 weeks without exposing to insecticides.

Adults (1-3 week old) were used for contact toxicity insecticidal assay. 1, 5, 10, 15 mg of extracts were dissolved in 1 mL volatile organic solvent n-hexane and these solutions were then coated on the inner surface of 20 mL glass vial (four replicates for each concentration). Each glass vial was rotated by hand until the test solution was distributed on the vial inner wall and floor, and organic solvent was mostly evaporated.

When the solvent was completely evaporated, five *O. mercator* were placed carefully in each vial.

The survival of the insects was assessed after 4, 8, 24, 48 h. Controls consisted of *O. mercator* in vial, treated only with the carrier solvent (13).

## RESULTS

This study was planned to the isolation of 7 compounds from the aerial parts of *S. pinnatifida*, a phenylpropanoid, 1-o-feruloyl-β-D-glucose **(1)**, two known flavonoids: luteolin-7-o-glucoside **(2)** and apigenin-7-o-glucoside **(3)**, three known phenylethanoid glycosides: phlomisethanoside **(4),** syringalide A **(5)**, and verbascoside **(6),** and oleic acid **(7)** (Fig. 1). The chemical structures of all isolated compounds were elucidated unequivocally through UV and NMR and also all spectroscopic data were in agreement with respective published data (14-21). The data of $^{1}$H-NMR and $^{13}$C-NMR of the compounds are given as follows:

1-O-feruloyl-β-D-glucose **(1)**: amorphous solid (4.7 mg), $^{1}$H-NMR (200 MHz, $CD_3OD$): $\delta_H$ 7.74 (1H, d, *J* = 15.8, H-7), 7.20 (1H, d, *J* = 2.0, H-2), 7.09 (1H, dd, *J* = 8.0, 2.0, H-6), 6.84 (1H, d, *J* = 8.0, H-5), 6.44 (1H, d, *J* = 15.8, H-8), 5.60 (1H, d, *J* = 8.0, H-1`), 3.90 (3H, s, $OCH_3$), 3.30-3.90 (6H, overlapped, glucose protons). These data were in agreement with the published data (14).

Luteolin-7-o-β-D-glucoside **(2)**: yellow amorphous solid (13.4 mg), UV (MeOH) $\lambda_{max}$ = 255, 265,351 nm. $^{1}$H-NMR (200 MHz, $CD_3OD$): $\delta_H$ 7.46 (1H, dd, *J* = 8.0, 2.0, H-6`), 7.43 (1H, d, *J* = 2.0, H-2`), 6.92 (1H, d, *J* = 8.0, H-5`), 6.84 (1H, d, *J* = 2.0, H-8), 6.70 (1H, s, H-3), 6.51 (1H, d, *J* = 2.0, H-6), 5.07 (1H, d, *J* =6.0, H-1``), 3.20-3.80 (6H, overlapped, glucose protons). $^{13}$C-NMR (50 MHz, $CD_3OD$): $\delta_C$ 182.4 (C-4), 165.3 (C-7), 163.4 (C-2), 161.6 (C-5), 157.9 (C-9), 150.2 (C-4`), 145.4 (C-3`), 121.4 (C-1`), 120.5 (C-6`), 116.8 (C-5`), 114.1 (C-2`), 104.1 (C-10), 101.7 (C-1``), 101.5 (C-3), 96.0 (C-6), 94.6 (C-8), 77.6 (C-5``), 76.5 (C-3``), 74.5 (C-2``), 71.1 (C-4``), 62.9 (C-6``). These data are in agreement with the published data (15).

Apigenin-7-o-β-D-glucoside **(3)**: yellow amorphous solid (12.7 mg), UV (MeOH) $\lambda_{max}$ = 267, 330, 425 nm. $^{1}$H-NMR (200 MHz, $CD_3OD$): $\delta_H$ 7.92 (2H, *J* = 8.0, H-3`, H-5`), 6.95 (2H, d, *J* = 8.0, H-2`, H-6`), 6.87 (1H, d, *J* = 2.0, H-8), 6.62 (1H, s, H-3), 6.52 (1H, d, *J* = 2.0, H-6), 5.01 (1H, d, *J* = 5.8, H-1``), 3.20-3.80 (6H, overlapped, glucose protons). $^{13}$C-NMR (50 MHz, $CD_3OD$): $\delta_C$ 180.4 (C-4), 166.6 (C-5), 165.3 (C-7), 164.4 (C-2), 159.9 (C-9), 129.7 (C-2`, C-6`), 123.4 (C-1`), 117.0 (C-3`, C-5`), 109.1 (C-10), 101.5 (C-3), 101.5 (C-1``), 96.1 (C-6), 94.8 (C-8), 78.6 (C-5``), 76.6 (C-3``), 74.5 (C-2``), 73.5 (C-4``), 62.6 (C-6``). These data are in agreement with the published data (16).

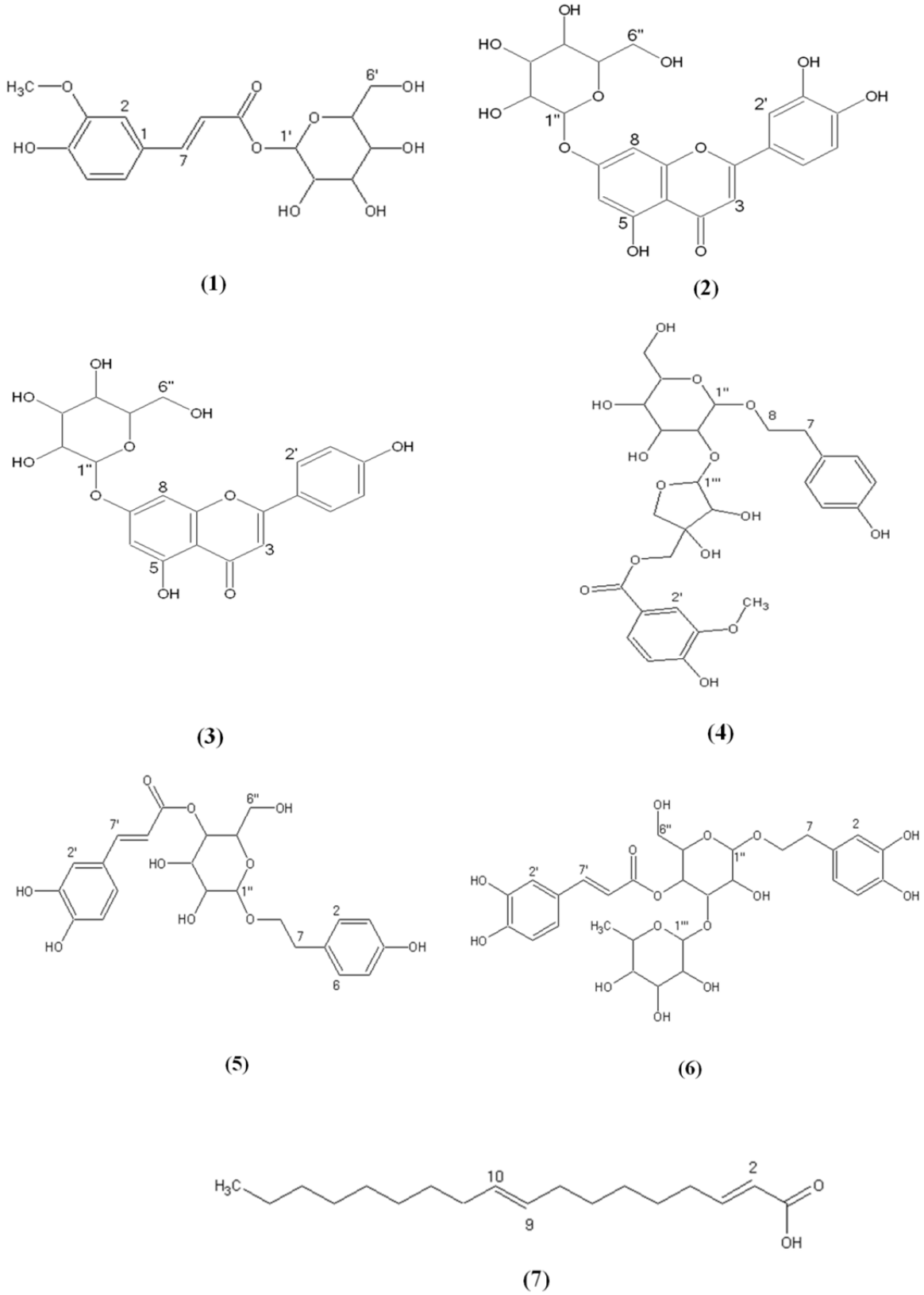


**Fig. 1.** Chemical structures of compounds from *S. pinnatifida*.

Phlomisethanoside **(4)**: pale yellow amorphous solid (7.6 mg), UV (MeOH) $\lambda_{max}$ = 220, 261, 287, 325 nm. $^1$H-NMR (200 MHz, $CD_3OD$): $\delta_H$ 7.64 (1H, dd, *J* = 8.0,2.0, H-6`), 7.56 (1H, d, *J* = 2.0, H-2`), 6.92 (1H, d, *J* = 8.0, H-5`), 6.86 (2H, d, *J* = 8.0, H-2, H-6), 6.64 (2H, d, *J* = 8.0, H-3, H-5), 5.38 (1H, s, H-1```), 4.35, 4.42 (2H, d, *J* = 11.2, 2H-5```), 4.28 (1H, d, *J* = 7.8, H-1``), 4.05 (1H, s, H-2```), 3.78, 4.16 (2H, d, *J* = 9.9, 2H-4```), 3.85 (3H, s, $OCH_3$), 3.61, 3.94 (2H, m, H-8), 3.20-3.90 (6H, overlapped, glucose protons), 2.80 (2H, t, *J* = 7.6, H-7), $^{13}$C-NMR (50 MHz, $CD_3OD$): $\delta_C$ 166.6 (C-7`), 155.1 (C-3`), 154.2 (C-4), 150.2 (C-4`), 129.4 (C-2, C-6), 129.2 (C-1), 124.0 (C-6`), 121.1(C-1`), 114.6 (C-3, C-5), 114.6 (C-5`), 112.4 (C-2`), 108.8 (C-1```), 104.1 (C-1``), 79.3 (C-3```), 78.7 (C-3``), 77.9 (C-5``), 77.8 (C-2``), 77.5 (C-2```), 76.4 (C-4```), 70.3 (C-8), 70.3 (C-4``), 67.1 (C-5```), 61.3 (C-6``), 35.0 (C-7). These data are consistant with the published data (17).

Syringalide A **(5)**: yellow amorphous solid (4.0 mg), UV (MeOH) $\lambda_{max}$ = 210, 230, 265, 325 nm. $^1$H-NMR (200 MHz, $CD_3OD$): $\delta_H$ 7.70 (1H, d, *J* = 15.9, H-7`), 6.95 (1H, d, *J* = 2.0, H-2`), 6.83 (1H, d, *J* = 8.0, H6`), 6.79 (2H, d, *J* = 8.0, H-2, H-6), 6.62 (1H, dd, *J* = 8.0, 2.0, H-5`), 6.33(1H, d, , *J* = 15.9, H-8`), 6.33 (2H, d, *J* = 8.0, H-3, H-5), 4.40 (1H, d, *J* = 7.2, H-1``), 3.31, 3.71 (2H, m, H-8), 3.20-3.90 (6H, overlapped, glucose protons), 2.82 (2H, t, *J* = 7.5, H-7), $^{13}$C-NMR (50 MHz, $CD_3OD$): $\delta_C$ 162.8 (C-9`), 153.8 (C-4), 148.7 (C-3`), 148.1 (C-4`), 147.7 (C-7`), 130.94 (C-2, C-6), 130.4 (C-1), 121.2 (C-6`), 121.1(C-1`), 116.5 (C-5`), 116.3 (C-3, C-5), 115.1 (C-8`), 110.9 (C-2`), 103.1 (C-1``), 77.8 (C-3``), 76.2 (C-5``), 75.4 (C-2``), 72.3 (C-8), 71.7 (C-4``), 62.7 (C-6``), 36.5 (C-7), These data are in agreement with the published data (18,19).

Verbascoside **(6)**: yellow amorphous solid (4.7 mg), UV (MeOH) $\lambda_{max}$ = 215, 245, 285, 330 nm. $^1$H-NMR (200 MHz, $CD_3OD$): $\delta_H$ 7.44 (1H, d, *J* = 16.1, H-7`), 7.06 (1H, d, *J* = 2.0, H-2`), 6.98 (1H, dd, *J* = 8.0, 2.0, H-6`), 6.80 (1H, d, *J* = 8.0, H-5`), 6.70 (1H, d, *J* = 2.0, H-2), 6.66 (1H, d, *J* = 8.0, H-5), 6.59 (1H, dd, *J* = 8.0, 2.0, H-6), 5.19 (1H, bs, H-1```), 4.41 (1H, d, *J* = 8.0, H-1``), 4.05 ,3.86 (2H, m, *J* = 9.0, H-8), 3.20-4.95 (6H, overlapped, glucose protons), 3.20-3.90 (4H, overlapped, rhamnose protons), 2.80 (2H, t, *J* = 8.3, H-7), 1.11 (3H, d, *J* = 6.1, H-6```). These data are in line with the published data (20).

Oleic acid **(7)**: pale yellow oily liquid (4.6 mg). $^1$H-NMR (200 MHz, $CD_3OD$): $\delta_H$ 5.38 (2H, m, Olefinic protons), 2.31 (2H, t, *J* = 8.0, H-2), 2.04 (4H, m, H-8, H-11), 1.64 (2H, m, H-3), 1.20-1.40 (32H, m, H-4, H-5, H-6, H-7, H-12, H-13, H-14, H-15, H-16, H-17), 0.87 (3H, t, H-18). $^{13}$C-NMR (50 MHz, $CD_3OD$): $\delta_C$ 178.0 (C-1), 129.8 (C-10), 129.1 (C-9), 34.9 (C-2), 26.0-31.0 (C-4, C-5, C-6, C-7, C-8, C-11, C-12, C-13, C-14, C-15, C-16), 24.9 (C-3), 22.3 (C-17), 0.86 (C-18). These data are in line with the published data (21).

### *Antioxidant activity of S. pinnatifida extracts*

The results of inhibiting free radicals obtained from extracts of *S. pinnatifida* are given in Table 1.

$RC_{50}$ values of MeOH, DCM, and n-hexane extracts were 0.044 ± 0.350, 0.846 ± 0.610, and 1.323 ± 0.800 mg/mL, respectively in comparison with the $RC_{50}$ values of quercetin as a positive control which was $2.78 \times 10^{-5}$ mg/mL.

### *Insecticidal activity of aerial parts of S. pinnatifida extracts*

The results are shown in Table 2; amongst the extracts, the n-hexane extract displayed the most potent activity and caused 10%, 15%, and 40% mortality to *O. mercator* at the dose of 5, 10, 15 mg/mL after 4 h of exposure.

**Table 1**. Free radical scavenging activity of the extracts of *S. pinnatifida* determined by DPPH assay.

| Test samples | Extracts | $RC_{50}$ values in mg/mL[a] |
|---|---|---|
| *S. pinnatifida* | n-Hexane | 1.323 ± 0.800 |
| | DCM | 0.846 ± 0.610 |
| | MeOH | 0.350 ± 0.044 |
| Quercetin | | $2.78 \times 10^{-5}$ |
| Trolox | | $2.60 \times 10^{-3}$ |

[a]Experiment was performed in triplicate and expressed as Mean ± SD.

**Table 2**. Percent mortality of *Oryzeaphilus mercator* exposed to 1 mL of extracts of *S. pinnatifida* at concentrations of 1, 5, 10, 15 mg/mL after 4, 8, 24 and 48 h.

| **Extracts** | **n-hexane** | | **DCM** | | **MeOH** | |
|---|---|---|---|---|---|---|
| **Hours** | **mg/mL** | **Mortality** | **mg/mL** | **Mortality** | **mg/mL** | **Mortality** |
| 4 | 1 | 0 | 1 | 0 | 1 | 0 |
| | 5 | 10 | 5 | 4 | 5 | 0 |
| | 10 | 15 | 10 | 9 | 10 | 4 |
| | 15 | 40 | 15 | 18 | 15 | 8 |
| 8 | 1 | 0 | 1 | 0 | 1 | 0 |
| | 5 | 12 | 5 | 6 | 5 | 2 |
| | 10 | 18 | 10 | 12 | 10 | 6 |
| | 15 | 44 | 15 | 20 | 15 | 20 |
| 24 | 1 | 2 | 1 | 0 | 1 | 0 |
| | 5 | 16 | 5 | 6 | 5 | 6 |
| | 10 | 18 | 10 | 14 | 10 | 10 |
| | 15 | 46 | 15 | 26 | 15 | 24 |
| 48 | 1 | 4 | 1 | 2 | 1 | 2 |
| | 5 | 16 | 5 | 8 | 5 | 6 |
| | 10 | 20 | 10 | 14 | 10 | 12 |
| | 15 | 48 | 15 | 30 | 15 | 26 |

## DISCUSSION

A combination of SPE and reversed-phase prep-HPLC analyses of the methanolic extract and also using Sephadex LH-20 column chromatography of the aerial parts of *S. pinnatifida* led to the characterization of a phenylpropanoid: 1-o-feruloyl-β-D-glucose (**1**) (14), two known flavonoids: luteolin-7-o-glucoside (**2**) (15) and apigenin-7-o-glucoside (**3**) (16), three known phenylethanoid glycosides: phlomisethanoside (**4**) (17), syringalide A (**5**) (18,19), and verbascoside (**6**) (20), and oleic acid (**7**) (21), respectively. The $^{1}$H-NMR spectrum of compound **(1)** displayed signals for three aromatic methine protons at $\delta_H$ 7.20 (1H, d, *J* = 2.0, H-2), 6.84 (1H, d, *J* = 8.0, H-5), 7.09 (1H, dd, *J* = 8.0, 2.0, H-6) suggesting the presence of a 1, 3, 4 tri substituted phenyl moiety, two trans-olefinic protons at $\delta_H$ 7.74 (1H, d, *J* = 15.8, H-7), 6.44 (1H, d, *J* = 15.8, H-8) indicating the presence of an α, β-unsaturated carbonyl functionality, a singlet proton resonance at 3.90 (3H, s) exhibiting the presence of a methoxy group and a doublet proton resonance at 5.60 (1H, d, *J* = 8.0, H-1`) was readily assigned to the anomeric proton of a glucose moiety, indicating the monoglycosidic structure in **(1)**. As a result, the structure of **(1)** was identified as a phenylpropanoid, 1-o-feruloyl-β-D-glucose which was previously isolated as an antioxidant agent from the fruits of *Luffa cylindrica* (L.) (14). But to the best of our knowledge, this is the first report on the isolation of this compound from genus of *Scutellaria*. In the case of compounds **(2)** and **(3)**, UV spectra were identical with flavone monoglucoside (22), which was supported by $^{1}$H-NMR and $^{13}$C-NMR spectrums, showing characteristic signals appeared at $\delta_H$ 6.70 (1H, s, H-3), 6.62 (1H, s, H-3), indicating the chromophore are flavones, luteolin, and apigenin, respectively. A literature survey indicated that luteolin-7-o-β-D-glucoside **(2)** and apigenin-7-o-β-D-glucoside **(3)** have previously been isolated from *Scutellaria* genus (23,24). The UV spectroscopic data of compound **(4)** revealed its phenolic nature. The $^{1}$H-NMR data of **(4)** showed proton resonances ascribed to the acyl group characterized as three signals appearing at $\delta_H$ 7.56 (1H, d, *J* = 2.0, H-2`), 6.92 (1H, d, *J* = 8.0, H-5`), and 7.64 (1H, dd, *J* = 8.0,2.0, H-6`) that suggested the presence of a trisubstituted phenyl moiety in the structure of **(4)**. Additionally, the presence of a methoxyl moiety ($\delta_H$ 3.85, s) and related carbon signals indicated a vanilloyl (4′-hydroxy-3′-methoxy-benzoic acid) moiety as a part of structure. Furthermore, the $^{1}$H-NMR of **(4)** suggested the presence of an aromatic $A_2X_2$ system with two doublets with ortho coupling constants (*J* = 8.0 Hz) at $\delta_H$ 6.86 (2H, d, *J* = 8.0, H-2, H-6) and 6.64 (2H, d, *J* = 8.0, H-3, H-5). According to the chemical shifts and splitting patterns of the

sugar protons ($\delta_H$ 5.38, s; $\delta_H$ 4.28, d, $J$ = 7.8), the two sugar moieties were indicated as apiose and glucose, respectively. Based on the above evidences and literature survey (17), compound **(4)** was established to be a vanillic acid ester phenylethanoid glycoside, named phlomisethanoside and the spectrum of $^{13}$C-NMR also confirmed this estimated structure. This compound was previously reported from the *Phlomis* species (17), however, it was the first time to be isolated from the genus *Scutellaria*. In the case of compound **(5)**, the $^{1}$H-NMR spectrum indicated the presence of a trisubstituted phenyl moiety that characterized by three signals appearing at $\delta_H$ 6.95 (1H, d, $J$ = 2.0, H-2`), 6.83 (1H, dd, $J$ = 8.0, 2.0, H-6`), 6.62 (1H, d, $J$ = 8.0, H-5`), a *p*-hydroxyphenethyl alcohol moiety with proton resonances at $\delta_H$ 6.79 (2H, d, $J$ = 8.0, H-2, H-6), 6.33 (2H, d, $J$ = 8.0, H-3, H-5), 2.82 (2H, t, $J$ = 7.5, H-7), 3.31, 3.71 (2H, m, H-8) and two olefinic protons ($\delta_H$ 7.70, d, $J$ = 15.9; $\delta_H$ 6.33, d, $J$ = 15.9, AX system) ascribable to H-7` and H-8` of the caffeic acid derivative. Moreover, one anomeric proton signal was observed at $\delta_H$ 4.40 (1H, d, $J$ = 7.2, H-1``) which was consistent with the β- glucopyranose unit. The related anomeric carbon resonances was at $\delta_C$ 103.1. Based on these results, the structure of **(5)** was identified as a phenylethanoid, syringalide A, whose spectral data were consistent with the literature (18,19). Syringalide A was previously isolated from the leaves of *Syringa* species (Oleaceae family) but, to the best of our knowledge, this is the first report on the occurrence of this compound in the genus *Scutellaria*. Compound **(6)** was identified on the basis of its $^{1}$H-NMR and $^{13}$C-NMR data to be a well-known phenylethanoid, verbascoside (acteoside). A comparison of the spectroscopic data with previous published data (20) confirmed the estimated structure. This compound was previously reported from *Scutellaria* genus (25,26), however, it is the first time to be obtained from this species. The structure of compound **(7)** was identified by NMR comparison with published data (21). As a wide-spread fatty acid, oleic acid was previously reported from *Scutellaria* species (27). Furthermore, free radical scavenging activity of the corresponding extracts was evaluated *in vitro* by the DPPH assay. The $RC_{50}$ values of all extracts are presented in Table 1. The DPPH-scavenging capacity of the extracts was compared with known antioxidants, quercetin, and trolox as positive controls. Among the extracts, the methanolic extract showed the most potent free-radical-scavenging activity with a $RC_{50}$ value of 0.044 ± 0.350 mg/mL which could be attributed to the presence of the isolated phenolics. Phenylpropanoids, phenylethanoids, and flavones exhibited potent antioxidant activities in various studies (5,28). Both DCM and n-hexane extracts showed low potency in this assay which may be explained by deficiency of hydrogen donating components. The insecticidal property of the extracts of *S. pinnatifida* has been evaluated by the assay described by Freedman B (1982) (13). Among the extracts, n-hexane extract displayed the most potent activity and caused 10%, 15%, 40% mortality to *O. mercator* at the dose of 5, 10, 15 mg/mL after 4 h of exposure. Over 48% mortality at 2 days after treatment was achieved at the dose of 15 mg/mL of n-hexane extract. Responses varied according to the concentration of extracts and exposure time and it was concentration dependent. Previous researches demonstrated that most of the lipophilic compounds can penetrate into insects' membranes rapidly and disturb their vital physiological functions (29). Therefore, the possibility of the higher insecticidal activity exhibited by n-hexane extract reported here would be due to the presence of these types of lipophilic compounds.

## CONCLUSION

In summary, the results of the present study revealed the isolation of six compounds from the methanolic extract of *S. pinnatifida* including 1-o-feruloyl-β-D-glucose, phlomisethanoside, syringalide A and verbascoside, luteolin-7-o-glucoside and apigenin-7-o-glucoside of which 1-o-feruloyl-β-D-glucose, phlomisethanoside and syringalide A have not been reported previously in *Scutellaria* genus. Free radical scavenging activity of methanolic extract is attributed to presence of phenolic compound

identified in this study and indicates good medicinal potentials of this which requires further biological and pharmacological studies. Furthermore, the pure compounds of the n-hexane extract which exhibited high insecticidal activity could be isolated and evaluated for their biological activities.

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