PHYTOCHEMICAL STUDIES OF MELILOTUS OFFICINALIS

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ABSTRACT. GC-MS analysis of the n-hexane extract of Melilotus officinalis seeds revealed twelve compounds with a combined area percentage of 98.33% predominantly, (9Z,12Z)-octadecadienoic acid (20.22%, 366 ppm), 14-methylpentadecanoic acid (19.52%, 353 ppm) and (9E)-octadecenoic acid (15.94%, 289 ppm). Two compounds, namely, cis-coumaric acid-2-O-β-D-glucopyranoside (cis-melilotoside, 1) and 1,2-benzopyrone (coumarin, 2), were isolated from the MeOH extract of the seeds of M. officinalis. The structures of isolated compounds were determined by spectroscopic techniques such as NMR, UV-Vis, and FTIR. The MeOH extract of M. Officinalis was also tested for its antioxidant activity using DPPH assay. The extract showed 29.87% DPPH inhibition at concentration of 100 μg/mL.

KEY WORDS: Melilotus officinalis, Fatty acid methyl esters, Esterification, GC-MS, DPPH radical scavenging assay, Antioxidant activity

INTRODUCTION

Melilotus officinalis belongs to the family Leguminosae (Fabaceae), and is an annual erect often more than 1 m tall with small yellow flowers [1]. It is commonly known as yellow sweet clover or medicinal sweet clover [2]. It is widely distributed around the world and occurs in most parts of Ethiopia, Tanzania, North Asia and India [1]. M. officinalis is used not only as food and forage but also as a medicine. M. officinalis has been used for the treatment of arthritis, brachialgia, bronchitis, hemorrhoids, rheumatism, painful menstruation, palpitations, and kidney stones [3, 4].

This plant showed anti-inflammatory [5], antioxidant [6, 7], hepatoprotective [8], anxiolytic [9] and antiproliferative [10] effects and has also been reported to promote tissue regeneration, prevent skin aging, and reduce fat deposition [11]. Traditionally M. officinalis is used to treat inflammation, vitiligo and arteritis. M. officinalis was reported to contain uric acid, flavonoids and their glycosides, steroids and saponins, fatty acids, triterpenes, oleanane-type triterpeneglucuronides, phenolic acids, and volatile components [2, 4, 1 2-25]. So far there is no detailed analysis made on the chemical composition of the plant seeds. Therefore, we have investigated the chemical compositions of the n-hexane and MeOH extracts of M. officinalis seeds.

EXPERIMENTAL

General experimental procedures

UV-Vis and FTIR spectra were determined using a UV-T60 spectrophotometer and a Perkin-Elmer Spectrum 65 instrument in the range 4000–200 cm⁻¹, respectively. Gas chromatography-mass spectrometry (GC-MS) experiments were conducted on Agilent Technologies 7820A GC system coupled with Agilent Technologies 5977E MSD, USA. ¹H- and ¹³C-NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer at 400.13 and 100.6 MHz, respectively. Column chromatography was performed using Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). Preparative thin-layer chromatography (PTLC) was carried on acid washed silica
gel with 254 nm fluorescent indicator (Sigma-Aldrich, Germany) pre-coated on glass (20 × 20 cm). Thin-layer chromatography (TLC) was performed with Merck Kieselgel 60 F254 plates which were visualized under UV light and by spraying with vanillin, H$_2$SO$_4$ (5%) in MeOH, and cerium molybdate stain (12 g ammonium molybdate, 0.5 g ceric ammonium molybdate, and 15 mL of concentrated H$_2$SO$_4$ in 235 mL of distilled water) followed by heating for a few seconds.

**Plant material**

*M. officinalis* was grown in the garden of Chemistry Department, Addis Ababa University, Addis Ababa, Ethiopia. A voucher specimen (No. Mel-003) was identified and deposited at the National Herbarium, Addis Ababa University, Addis Ababa, Ethiopia.

**Extraction of M. officinalis seeds**

The dried and ground seeds of *M. officinalis* (100 g) was Soxhlet-extracted with n-hexane (300 mL) for 8 h. The n-hexane was removed under reduced pressure to yield a yellow oil (2.12 g). The residue left after n-hexane extraction was further extracted with methanol (300 mL) for another 8 h and the methanol was removed under reduced pressure to yield a brown residue (9.32 g).

**Preparation of fatty acid methyl esters**

The n-hexane extract of *M. officinalis* seeds (0.5 g) was placed in a 50 mL round bottom flask fitted with a condenser and 2% methanolic KOH (3.0 mL) was added. The mixture was heated at 50 ºC for 30 min on a water bath with continuous shaking. The reaction mixture was cooled to room temperature and transferred to a separatory funnel. Saturated sodium chloride (1 mL) was added, and the mixture was extracted with petroleum ether (15 mL). The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and concentrated to yield the esterified product (150.9 mg).

**Preparation of methyl palmitate standard**

Methyl palmitate was prepared by Fischer esterification [26] of palmitic acid. Thus, in a 50 mL round bottom flask equipped with a reflux condenser, palmitic acid (1 g) was dissolved in MeOH (10 mL) and then concentrated H$_2$SO$_4$ (1 mL) was carefully added. The mixture was heated on a water bath at 50 ºC for 1 h and then cooled to room temperature. Chloroform (30 mL) was added, and the mixture was transferred to a separatory funnel and washed with deionized water (30 mL). The organic layer was then washed with aqueous NaHCO$_3$ (30 mL) and water (30 mL), dried over anhydrous sodium sulfate, filtered, and concentrated to afford methyl palmitate. Standard solutions of methyl palmitate were prepared at concentrations of 1, 10, 25, 50 and 100 ppm and analyzed by GC-MS in triplicates.

**GC-MS analysis**

GC-MS analysis was conducted on an Agilent Technology 7820A GC system coupled with an Agilent Technology 5977E MSD equipped with an autosampler. The chromatographic separation was done on a DB-1701 (14%-cyanopropyl-phenyl-methylpolysiloxane) column (30 m x 0.25 μm) at a pressure of 8 psi and a flow rate of 0.97989 mL/min. Ultra-high pure helium (99.999%) was used as carrier gas at constant flow mode. An Agilent G4567A autosampler was used to inject 1 μL of the sample with a splitless injection mode into the inlet heated to 275 ºC with a total run time of 29.33 min. Oven temperature was programmed with the initial column temperature of 60 ºC and hold-time 2 min. The column temperature was increased to 200 ºC at a rate of 10 ºC/min and then heated again at a rate of 3 ºC/min until the temperature reached 240
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β-C. No mass spectra were collected during the first 4 min of the solvent delay. The transfer line and the ion source temperatures were 280 °C and 230 °C, respectively. The detector voltage was 1600 V and the electron energy was 70 eV. Mass spectral data were collected from 40–650 m/z. The fatty acid methyl esters were identified by matching their mass spectra with those of reference compounds recorded in National Institute of Standards and Technology (NIST) mass spectral library.

**DPPH radical scavenging assay**

DPPH radical scavenging assay is a simple method for quantifying antioxidants by measuring absorbance at 517 nm due to the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical [27]. The DPPH radical scavenging assay of the MeOH extract of the seeds of *M. officinalis* was assessed according to the procedure described by Hoque et al. [28]. The MeOH extract (1 mg) was first dissolved in MeOH (1 mL). Seven different concentrations, 500, 250, 125, 62.50, 31.25, 15.63 and 7.81 µg/mL, of the extract were prepared by diluting the stock solution (1 mg/mL) with MeOH. To 1 mL of each solution, 0.004% DPPH in MeOH (4 mL) was added to make 100, 50.00, 25.00, 12.50, 6.25, 3.13 and 1.56 µg/mL solutions. The mixtures were shaken and left at room temperature for 30 min. The absorbances of the solutions were then recorded at 517 nm using a UV-Vis spectrophotometer. All measurements were performed in triplicates and the same procedure was used to determine the radical scavenging activity of ascorbic acid standards.

**Isolation of compounds from the MeOH extract of *M. officinalis* seeds**

The MeOH extract (2 g) of *M. officinalis* was first dissolved in acetone (100 mL) to obtain the acetone-insoluble fraction (125 mg). The acetone insoluble material (50 mg) was subjected to column chromatography over Sephadex LH-20 using CHCl₃:MeOH (2:1) as eluent to yield compound 1 (12 mg). Another 2 g of the MeOH extract was dissolved in MeOH:H₂O (1:1) (200 mL) and then extracted with ethyl acetate (300 mL). Removal of the solvent under reduced pressure afforded a gummy residue (650 mg). The ethyl acetate-soluble fraction (55 mg) was purified by PTLC using petroleum ether:ethyl acetate (9:1) as eluent to yield compound 2 (15.8 mg) (Figure 2).

**Cis-coumaric acid-2-O-β-D-glucopyranoside (cis-melilotoside)** (1). White crystals, m.p. 126–127 °C, Lit. [29] m.p. 128 °C; UV (MeOH, λₘₐₓ, nm): 261; IR, (KBr, cm⁻¹): 3391, 2926, 1691, 1636, 1488, 1455, 1240, 1075; °H- and °C-NMR data (Table 4) [30, 31].

**1,2-Benzopyrone (coumarin)** (2). White powder, m.p. 70–71 °C, Lit. [32] m.p. 70–72 °C; UV (MeOH, λₘₐₓ, nm): 274; IR, (KBr, cm⁻¹): 2959, 2928, 2857, 1729, 1288, 1124; °H- and °C-NMR data (Table 5) [32].

**RESULTS AND DISCUSSION**

**Characterization of seed fatty acids of *M. officinalis***

The fatty acid methyl esters that were obtained by esterification of the n-hexane extract of *M. officinalis* seeds were subjected to GC-MS analysis. Quantifications of components in the n-hexane extract were made by using their relative area percentages. Methyl palmitate external standards were used to determine the concentrations (ppm) of the components. Thus, standard solutions of methyl palmitate were prepared and analyzed by GC-MS in triplicates at concentrations of 1, 10, 25, 50 and 100 ppm (Table 1). Using the mean area of each concentration the calibration curve shown in Figure 1 was constructed and then used to calculate the concentrations (ppm) of the different components.

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Table 1. Methyl palmitate standard solutions and their corresponding areas.

| Concentration (ppm) | Rt (min) | Average area  |
|---------------------|----------|---------------|
| 1                   | 14.946   | 36486415      |
| 10                  | 14.96    | 201524238     |
| 25                  | 14.960   | 367682433     |
| 50                  | 14.986   | 632297811     |
| 100                 | 15.013   | 1157940017    |

Rt = retention time (min), ppm = parts per million.

Figure 1. Calibration curve of methyl palmitate standard.

GC-MS analysis revealed the presence of 12 compounds with a combined area percentage of 98.33% (Table 2) including coumarin (8.40%), 10 fatty acids (87.74%), and 5-dodecyl-dihydrofuran-2(3H)-one (2.19%) with qualities greater than 90% and area percentage greater than 1. For identification of individual components in the extract, NIST 2014 mass spectral library search was used. The names of identified compounds with their respective retention times, area percentages, qualities and concentrations are given in Table 2.

It is apparent from Table 2 that the n-hexane extract of the seeds of *M. Officinalis* is mainly composed of unsaturated fatty acids (UFAs) and polyunsaturated fatty acids (PUFAs). In addition, the extract was found to be rich in (9Z,12Z)-octadecadienoic acid (20.22%, 366 ppm), 14-methylpentadecanoic acid (19.52%, 353 ppm) and (9E)-octadecenoic acid (15.94%, 289 ppm). The presence of unsaturated fatty acids in the seeds of *Melilotus* species, *M. alba* and *M. officinalis*, was previously reported using gas chromatography and flame ionization analysis [23].

**DPPH assay**

Radical scavenging activity was quantified by the decrease in absorbance of seven different concentrations of the MeOH extract of *M. officinalis* seeds in DPPH solution (0.004%) [28]. Antioxidant activity of each concentration was measured in relation to ascorbic acid (a known
antioxidant) standards. All determinations were performed in triplicates and percent DPPH inhibition was calculated as

\[
\text{% DPPH inhibition} = \frac{A_{\text{control}} - A_{\text{extract}}}{A_{\text{control}}} \times 100
\]

where \(A_{\text{control}}\) is the absorbance of DPPH solution without the test sample and \(A_{\text{extract}}\) is the absorbance of the test sample plus DPPH.

Table 2. Major compounds identified from the n-hexane extract of \(M.\ officinalis\) seeds by GC-MS analysis.

| Pk | Rt  | Area % | Compound names                  | Q  | C (ppm) |
|----|-----|--------|---------------------------------|----|---------|
| 1  | 15.24 | 8.40   | Coumarin                       | 95 | 152     |
| 2  | 18.96 | 19.52  | 14-Methylpentadecanoic acid     | 95 | 353     |
| 3  | 21.12 | 3.41   | Palmitic acid                  | 99 | 61.8    |
| 4  | 21.99 | 15.94  | (9E)-Octadecenoic acid         | 99 | 289     |
| 5  | 22.09 | 20.22  | (9Z,12Z)-Octadecadienoic acid  | 99 | 366     |
| 6  | 22.24 | 8.24   | Octadecanoic acid             | 99 | 149     |
| 7  | 22.38 | 1.95   | Linoleic acid                  | 99 | 35.4    |
| 8  | 24.67 | 4.39   | Oleic acid                     | 99 | 79.5    |
| 9  | 24.80 | 10.50  | Linoleic acid                  | 99 | 190     |
| 10 | 25.05 | 2.19   | 5-Dodecylidihydrofuran-2(3H)-one | 94 | 39.6    |
| 11 | 25.21 | 1.85   | Eicosanoic acid                | 99 | 33.4    |
| 12 | 26.17 | 1.72   | 18-Methylnonadecanoic acid     | 99 | 31.2    |

Pk = peak number, Rt = retention time (min), Q = quality, C = concentration.

The MeOH fraction of the seeds of \(M.\ officinalis\) was able to reduce the stable DPPH radical indicating its potential as a radical scavenger. The extract showed 29.87% DPPH inhibition at concentration of 100 μg/mL (Table 3).

Table 3. Radical scavenging activities of the methanol extract of \(M.\ officinalis\) seeds.

| Concentration (μg/mL) | % DPPH inhibition          |
|-----------------------|---------------------------|
|                       | MeOH extract of \(M.\ officinalis\) | Ascorbic acid standard |
| 100.00                | 29.87 ± 0.49              | 96.09 ± 0.16            |
| 50.00                 | 26.35 ± 0.54              | 96.29 ± 0.06            |
| 25.00                 | 24.82 ± 0.23              | 96.26 ± 0.06            |
| 12.50                 | 24.16 ± 0.16              | 96.06 ± 0.12            |
| 6.25                  | 22.12 ± 0.91              | 91.30 ± 0.31            |
| 3.13                  | 21.62 ± 0.13              | 54.06 ± 1.27            |
| 1.56                  | 21.53 ± 0.12              | 35.00 ± 0.52            |

The results are reported as mean ± SD of three replicates.

Characterization of compounds isolated from MeOH extract of \(M.\ officinalis\) seeds

Compound 1 was obtained as white crystals and had a melting point of 126–127 °C. The UV-Vis spectrum of 1 in MeOH displayed an absorption maximum (\(\lambda_{\text{max}}\)) at 261 nm which is characteristic of a phenolic moiety. The IR spectrum (KBr) indicated the presence of a carbonyl (1691 cm\(^{-1}\)), aromatic (1636, 1240 cm\(^{-1}\)) and hydroxyl (3391 cm\(^{-1}\)) functionalities. The \(^1\)H-NMR data of 1 (Table 4) showed two olefinic hydrogen signals at 7.09 (\(d, J = 12.0\) Hz, 1H, H-7) and 6.05 (\(d, J = 12.0\) Hz, 1H, H-8), which indicated the presence a cis double bond [30]. Besides, the coupling patterns of the aromatic proton signals at 7.12 (\(d, J = 8.0\) Hz, 1H, H-7) and 7.30 (\(d, J = 8.0\) Hz, 1H, H-8), 7.03 (\(t, J = 8.0\) Hz, 1H, H-5) and 7.34 (\(d, J = 8.0\) Hz, 1H, H-6)

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suggested the presence of an ortho-substituted benzene ring. Additionally, the \(^{13}\)C-NMR spectrum (Table 4) indicated the presence of a carbonyl carbon (δ 171.74), six aromatic and two olefinic carbon atoms. The signal at δ 153.81 is due to an oxygenated aromatic carbon atom. The HMBC spectrum of 1 revealed a correlation between the signal at δ 125.53 (C-1) and the proton signals at δ 7.09 (H-7) and 6.05 (H-8). Thus, the olefinic carbon C-7 (δ 137.14) is attached to C-1.

The presence of a glucopyranosyl ring in the structure of 1 was indicated by the analysis of its \(^{1}H\)-NMR spectrum together with its \(^{13}\)C-NMR spectrum. The glucose moiety, which can be assigned a β-configuration on the basis of the coupling constant of the anomeric proton at δ 5.97 (d, J = 7.4 Hz, 1H) [33] is attached to the C-2 (δ 153.81) position. Based on the analysis of the spectroscopic data and literature data, it can be concluded that compound 1 is cis-coumaric acid-2-O-β-D-glucopyranoside (cis-melilotoside, Figure 2) [30, 31]. This is the first report on the isolation of cis-melilotoside (1) from \textit{M. officinalis}. cis-Melilotoside has previously been reported from several other plants such as \textit{Ajuga chamaecistus} ssp. \textit{Tomentella} and \textit{Mikania laevigata} [30, 34].

\begin{table}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{Position} & \textbf{DEPT-135} & \textbf{\(^{1}H\)-NMR (D$_{2}$O, 400 MHz)} & \textbf{\(^{13}\)C-NMR (D$_{2}$O, 100 MHz)} & \textbf{\(^{1}H\)-NMR (Methanol-d$_{4}$, 500 MHz)} & \textbf{\(^{13}\)C-NMR (Methanol-d$_{4}$, 125 MHz)} \\
\hline
1 & C & 125.53 & – & 126.9 & – \\
2 & C & 153.81 & – & 156.7 & – \\
3 & CH & 115.36 & 7.32 (d, J = 8.0 Hz, 1H) & 116.6 & 7.19 (d, J = 7.9 Hz, 1H) \\
4 & CH & 130.56 & 7.30 (d, J = 8.0 Hz, 1H) & 131.5 & 7.30 (d, J = 7.9 Hz, 1H) \\
5 & CH & 122.85 & 7.03 (t, J = 8.0 Hz, 1H) & 122.9 & 6.97 (t, J = 7.9 Hz, 1H) \\
6 & CH & 130.09 & 7.34 (d, J = 8.0 Hz, 1H) & 131.7 & 7.53 (d, J = 7.9 Hz, 1H) \\
7 & CH & 137.4 & 7.09 (d, J = 12.0 Hz, 1H) & 139.7 & 7.31 (d, J = 12.5 Hz, 1H) \\
8 & CH & 122.02 & 6.05 (d, J = 12.0 Hz, 1H) & 121.4 & 5.97 (d, J = 12.5 Hz, 1H) \\
9 & C & 171.74 & – & 170.4 & – \\
1' & CH & 100.41 & 5.05 (d, J = 7.2 Hz, 1H) & 102.7 & 4.94 (d, J = 7.4 Hz, 1H) \\
2' & CH & 72.93 & 3.53 (m, 1H) & 75.0 & 3.49 (m, 1H) \\
3' & CH & 76.12 & 3.53 (m, 1H) & 78.2 & 3.46 (m, 1H) \\
4' & CH & 69.44 & 3.42 (m, 1H) & 71.4 & 3.40 (m, 1H) \\
5' & CH & 75.71 & 3.53 (m, 1H) & 78.3 & 3.43 (m, 1H) \\
6' & CH & 60.58 & 3.84 (dd, J = 12.4, 2.3 Hz, 1H) & 3.61 (dd, J = 12.4, 5.4 Hz, 1H) & 62.6 & 3.87 (m, 1H) & 3.70 (m, 1H) \\
\hline
\end{tabular}
\end{table}

Compound 2 was isolated from the MeOH extract of the seeds of \textit{M. officinalis} as a white powder and has a melting point of 70–71 °C. The UV-Vis spectrum of 2 in MeOH revealed an absorption maximum (\(\lambda_{\text{max}}\)) at 274 and the IR spectrum in KBr showed absorption bands at 1729, 1288, 1124 cm\(^{-1}\) due to the presence of an aromatic ring and an α,β-unsaturated carbonyl carbon.

The \(^{13}\)C-NMR spectrum of 2 (Table 5) revealed the presence of 9 carbon atoms. The signal at δ 160.75 is due to the carbonyl of a δ-lactone. Besides, the two olefinic carbon signals at δ 116.94 and 143.38, and the six aromatic carbon signals at δ 116.76, 118.86, 124.41, 127.84, 131.83 and 154.11 indicated the presence of an aromatic ring and a double bond. In the \(^{1}H\)-NMR spectrum of 2 (Table 5) the two olefinic proton doublets at δ 6.46 (d, J = 9.6 Hz, 1H) and 7.74 (d, J = 9.6 Hz, 1H) are attributable to H-3 and H-4 of the lactone nucleus, respectively. The signals at δ 7.34 (m, 2H, H-5 and H-7) and 7.54 (m, 2H, H-6 and H-8) revealed the presence of an ortho-substituted benzene ring. Compound 2 is then identified as 1,2-benzoypyrone (coumarin, Figure 2) by comparison of its spectral data with data in the literature [32]. Coumarin has...
Previously been isolated from the MeOH extract of the aerial parts of *M. Officinalis* [4]. It was also found in many plants of the genus *Melilotus*.

Table 5. $^1$H- and $^{13}$C-NMR data of compound 2 and from the literature.

| Position | DEPT-135 | $^1$H-NMR (Chloroform-$d$, 400 MHz) | $^{13}$C-NMR (Chloroform-$d$, 100 MHz) | Literature [32]  
|-----------|-----------|-------------------------------------|----------------------------------------|------------------|
| 2         | C         | 160.75 (d) $\delta$ 159.84          |                                        |                   |
| 3         | CH        | 116.94 (d) $\delta$ 116.18 (d)      | 6.46 (d, $J$ = 9.6 Hz, 1H)             | 6.47 (d, $J$ = 9.6 Hz, 1H) |
| 4         | CH        | 143.38 (d) $\delta$ 144.10 (d)      | 7.74 (d, $J$ = 9.6 Hz, 1H)             | 8.03 (d, $J$ = 9.9 Hz, 1H) |
| 5         | CH        | 127.84 (m) $\delta$ 128.35 (m)      | 7.34 (m, 1H)                          | 7.29 (m, 1H)      |
| 6         | CH        | 124.41 (m) $\delta$ 124.38 (m)      | 7.54 (m, 1H)                          | 7.58 (m, 1H)      |
| 7         | CH        | 131.83 (m) $\delta$ 131.84 (m)      | 7.34 (m, 1H)                          | 7.29 (m, 1H)      |
| 8         | CH        | 116.76 (m) $\delta$ 116.11 (m)      | 7.54 (m, 1H)                          | 7.58 (m, 1H)      |
| 9         | C         | 154.11 (m) $\delta$ 153.43 (m)      |                                        |                   |
| 10        | C         | 118.86 (m) $\delta$ 118.6 (m)       |                                        |                   |

It was reported from $^{14}$C feeding experiments that cis-melilotoside (1) is a biosynthetic precursor of coumarin (2) (Figure 2) [36, 37]. In the biosynthesis of coumarin (2), the trans isomer of $\beta$-glucoside-O-coumaric acid (3) undergoes isomerization to its cis isomer, cis-melilotoside (1), which could then hydrolyze to coumarinic acid (4) which in turn lactonizes spontaneously to form coumarin (2).

![Figure 2. Proposed biosynthetic pathway of coumarin (2) [36, 37].](image)

- **CONCLUSION**

In this work, the fatty acid composition of the n-hexane extract of the seeds of *M. officinalis* was determined using GC-MS. Quantifications of components in the n-hexane extract were made by using their relative area percentages and methyl palmitate external standards. Coumarin (2),

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which has been grouped among compounds that show hepatotoxicity [38], was detected in significant amount in the seed oil. Therefore, it may not be suitable to use the oil for cooking purposes just as it is. In order to use the oil for cooking purposes, the amount of coumarin (2) needs to be limited to tolerable daily intake (TDI) set by European Food Safety Authority (EFSA) which is 0.1 mg/kg body weight [39]. Phytochemical investigation of Melilotus officinalis seeds resulted in the isolation of cis-melilotoside (1) and coumarin (2) by column chromatography over Sephadex LH-20 and preparative TLC techniques. The isolated compounds were then characterized by their NMR, UV chromatography and FTIR spectra. Antioxidant activity of the MeOH extract of Melilotus officinalis seeds was also evaluated which exhibited a 29.87% DPPH inhibition at concentration of 100 μg/mL.

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