Research Article

Cytotoxic Flavonoids from the Aerial Parts of *Stachys lavandulifolia* Vahl
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**Abstract**

*Background:* *Stachys lavandulifolia* Vahl is a herbaceous plant distributed in the west and south-western Asia. Despite of the wide medicinal uses, there are some reports on toxicity potential of this plant. In present study we attempted to evaluate the toxicity and to characterize the cytotoxic principles of *S. lavandulifolia*.

**Methods:** Brine shrimp lethality test (BSLT) was used to evaluate the general toxicity of the extracts and essential oil obtained from the aerial parts of *S. lavandulifolia*. Phytochemical constituents of the active extract were investigated using various chromatographic and spectroscopic methods. GC and GC-MS were also used to analyze the plant essential oil. GC and GC-MS were used to analyze the plant essential oil. Cytotoxic activities of the isolated compounds were also evaluated using MTT assay method.

**Results:** In brine shrimp lethality test (BSLT), chloroform extract and the plant essential oil exhibited the most toxicity against Artemia salina larvae (LD₉₀: 121.8±5.6 and 127.6±14.7 µg ml⁻¹, respectively). GC and GC-MS analyses of essential oil led to the characterization of forty compounds of which α-bisabolol (23.85%) and thymol (17.88%) were identified as the main constituents. Nine flavonoids, Pachypodol (1), chrysosplenetin (2), kumatakenin (3), velutin (4), penduletin (5), viscosine (6), chrysoeriol (7), hydroxygenkwanin (8) and apigenin (9) were isolated from the chloroform extract of *S. lavandulifolia*. Among the isolated compounds, chrysosplenetin (2), a polymethoxylated flavonoid, was found as the most toxic compound toward MDA-MB-23 and HT-29 cells, with IC₅₀ values of 88.23 and 116.50 µg ml⁻¹, respectively. Furthermore, chrysosplenetin (2), kumatakenin (3) and viscosine (6) with selectivity indices of 2.70, 2.59 and 3.33, respectively, showed higher preferential toxicity against MDA-MB-23 cells in comparison with tamoxifen (SI:2.45).

**Conclusion:** This study reports methoxylated flavonoids as compounds which could be involved in toxicity of *S. lavandulifolia*. The results of MTT assay also suggest some of these compounds as appropriate candidates for anti-cancer drug development research.

**Introduction**

The genus *Stachys* L. with about 300 species all over the world is one of the largest genera of Lamiaceae (alt. Labiatae) family.¹ *Stachys lavandulifolia* Vahl from this genus is a perennial herbaceous plant distributed in Iran, Turkey, Iraq, Caucasia and central Asia.² This species has been described under the name of "Marmazad" in Old Persian medicinal literature, useful in the treatment of liver, stomach and uterus diseases and as liver tonic, gastrotonic and emmenagogue agent.³ In different parts of Iran and Anatolia the flowering aerial parts of *S. lavandulifolia* is used as a popular herbal tea for its sedative, gastrotonic and spasmytotropic properties, as well as for the treatment of some gastrointestinal disorders, colds and flu.⁴,⁶

So far different biological and pharmacological effects such as antioxidant,⁷ antimicrobial,⁷,⁸ anxiolytic,⁹,¹⁰ wound healing,¹¹ gastroprotective,¹² analgesic,¹³ anti-inflammatory,¹⁴ anti-tyrosinase⁰ and acetylcholinesterase inhibitory¹⁴ activities have been documented for the various extracts obtained from the aerial parts of *S. lavandulifolia*. The results of clinical trials have also confirmed the therapeutic value of this species in abnormal uterine bleeding caused by polycystic ovary syndrome and as a useful supplement in management of diseases related to oxidative stress.¹⁵,¹⁶ Previous phytochemical investigations on *S. lavandulifolia* have led to the isolation of four phenylethanoid glycosides; lavandulifoliosides A and B, verbascoside and leucosceposide A, three iridoid

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glycosides; monomelittoside, melittoside, 5-allosyloxy- 
auccin, a diterpenoid; stachysolone and a phenolic 
glycoside; arbutin from its aerial parts.\textsuperscript{14,17} Two flavonoid 
derivatives, apigenin and luteolin, have also been 
determined using HPLC in the hydroalcoholic extract of 
\textit{S. lavandulifolia}.\textsuperscript{18} Furthermore, chemical constituents of 
this plant essential oil have been reported from different 
regions of Iran.\textsuperscript{19-23} 

Despite of medicinal uses and beneficial health effects of 
\textit{S. lavandulifolia}, there are some reports on toxicity 
potential of this plant species.\textsuperscript{24-26} It has been 
demonstrated that peroral administration of 
hydroalcoholic extract of \textit{S. lavandulifolia} with doses 
higher than 140 mg/kg/day could be resulted in significant 
subacute and subchronic toxicity in female mice.\textsuperscript{26} In 
another study, the hydroalcoholic extract of \textit{S. lavandulifolia} aerial 
parts was found hepatotoxic in a dose dependent 
manner, following administration at the doses 
50-200 mg/kg, intraperitoneally for 28 days.\textsuperscript{24} Regarding 
to the lack of information about compounds involved in 
the toxicity properties of \textit{S. lavandulifolia}, the present 
study was designed to assess toxicity and to identify of 
cytotoxic principles of this species using a bioassay 
guided approach.

\section*{Materials and Methods}

\subsection*{Plant material}
The aerial parts of \textit{S. lavandulifolia} Vahl were collected 
during its flowering stage from Ghotour region (Khoy, 
West-Azerbaijan, Iran) in Jun 2014. The plant sample was 
authenticated by botanist Dr. Yousef Ajani and its 
specimen was deposited at the herbarium of Institute of 
Medicinal Plants, ACECR, Karaj, Iran (Voucher no. 2014 
MPIH).

\subsection*{Essential oil preparation}
The essential oil of the plant was extracted by 
hydrodistillation of the air-dried and powdered aerial 
parts (100 g) using a Clevenger-type apparatus for 4 h. 
The obtained essential oil was dried over the anhydrous 
sodium sulfate and stored in 4 °C until analysis.

\subsection*{Extraction}
The air-dried and comminuted plant (1.2 kg) was 
macerated with petroleum ether, chloroform, ethyl acetate 
and methanol:water (8:2), successively (3× 15 L each) at 
the room temperature. The four obtained extracts were 
concentrated using a rotary evaporator under a low 
pressure at 45 °C.

\subsection*{Brine shrimp lethality test}
General toxicity potentials of the plant extracts and 
esential oil were evaluated in brine shrimp lethality test 
(BSLT).\textsuperscript{27} The brine shrimp (\textit{Artemia salina} L.) eggs 
were hatched in sterile artificial seawater (38 g L\textsuperscript{-1}, adjusted 
to pH 9 with NaHCO\textsubscript{3}) under the constant aeration for 48 
hours at 30 °C. the plant samples (50 mg) were dissolved 
in DMSO (200 µl) and tween 80 (~50 µl) and then diluted 
by freshly prepared artificial sea water to obtain the 
solutions with 10, 100, 300, 500, 700 and 1000 µg ml\textsuperscript{-1} 
concentrations in a series of tubes containing about 20 
active nauplii in each. The surviving nauplii in test and 
control tubes were counted following the incubation of 
the tubes at 30 °C for 24 hours under light to achieve the 
LD\textsubscript{50} value, expressing the concentration causing 50% 
lethality. Podophyllotoxin, a natural compound with 
known cytotoxic activity, was applied as positive control. 
The assays were performed in triplicate and the LD\textsubscript{50} 
values were reported as Mean ± SD.

\section*{Phytochemical analyses}
Chloroform extract and essential oil of the plant with the 
highest toxicity effects (Table 1) were subjected to 
phytochemical analyses using various chromatographic 
and spectroscopic methods.

\begin{table}[h]
\centering
\caption{Toxicity of the extracts and essential oil of \textit{S. lavandulifolia} in BSLT.}
\begin{tabular}{ll}
\hline
\textbf{Samples} & \textbf{Brine Shrimp lethality} \\
& \textbf{LD\textsubscript{50} (µg ml\textsuperscript{-1})} \\
\hline
Petroleum ether extract & 685.4 ± 12.2 \\
Chloroform extract & 121.8 ± 5.6 \\
Ethyl acetate extract & 704.4 ± 16.8 \\
Hydroalcoholic extract & 1000 < \\
Essential oil & 127.6 ± 14.7 \\
Podophyllotoxin (Positive control) & 3.1 ± 0.6 \\
\hline
\end{tabular}
\end{table}

\section*{GC and GC-MS analyses}
The essential oil was analysed on a HP 6890 gas 
chromatograph equipped with HP-5MS column (30m 
×0.25 mm id, 0.25µm), connected to HP 5973 mass 
detector (70 eV) under the following conditions; carrier 
gas: helium (1 ml min\textsuperscript{-1}), temperature program: 40 °C to 
250 °C at 3 °C per min, injector temperature: 250 °C, 
injection volume: 1 µl, split ratio: 1:90. The Kovats 
retention indices (KI) of the compounds were calculated 
using a homologous series of \textit{n}-alkanes injected in 
conditions equal to the sample. Identification of the 
compounds was carried out based on computer matching 
with the Wiley7nL library, as well as by comparison of 
KIs and fragmentation pattern of the mass spectra with 
those published for standard compounds.\textsuperscript{28} For 
quantitative purposes, the essential oil was also analysed 
by GC-FID with the same conditions described above for 
GC-MS.

\section*{Isolation and purification of the compounds}
Chloroform extract (12.6 g) was moved on a silica gel 
column (230-400 mesh, Merck, Germany) and eluted by 
CHCl\textsubscript{3}-EtOAc (10:0-5:5) to three main fractions (C\textsubscript{1}-C\textsubscript{3}). 
Silica gel column chromatography of a portion of the 
fraction C\textsubscript{2} (1.5 g) with CHCl\textsubscript{3}-EtOAc (9:1-7:5-2:5) 
yielded nine fractions (C\textsubscript{2A}-C\textsubscript{2I}). Compound I (5 mg) was 
purified using preparative thin layer chromatography 
(PTLC) (Handmade plates, Silica gel 60 GF\textsubscript{254}, Merck, 
Germany) from the fraction C\textsubscript{2B} (18 mg) with CHCl\textsubscript{3}- 
EtOAc (8:2), as a solvent system. Fraction C\textsubscript{2E} (320 mg) 
was chromatographed over the silica gel column and 
eluted with CHCl\textsubscript{3}-EtOAc (7.5:2:5) to get three fractions 
(C\textsubscript{2E1}-C\textsubscript{2E3}). Elution of the fraction C\textsubscript{2E3} (135 mg) over a
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Sephadex LH-20 column (Fluka, Switzerland) with MeOH-EtOAc (8:2) resulted in isolation of compounds 2 (23 mg), 3 (18 mg) and 4 (20 mg). Fraction C3 afforded 175 mg of yellowish powder which was purified on a PTLC plate with CHCl3-EtOAc (8:2) to obtain compound 5 (51 mg). Compound 6 (43 mg) was also obtained from the fraction C3 (160 mg) over a Sephadex LH-20 column, eluted with MeOH. silica gel column chromatography of the fraction C3 (270 mg) with CHCl3-MeOH (9.5:0.5) yielded three fractions (C1a-C3c). Fractions C3b (42) and C3c (28) were individually moved on Sephadex LH-20 columns and eluted with MeOH-EtOAc (8:2) to get a mixture of the compounds 7 and 8 (9 mg) (5.3:4.7), as well as compound 9 (6 mg), respectively. In all steps, chromatography was monitored by thin layer chromatography (TLC) on Pre-coated Silica gel GF254 sheets (Merck, Germany) and the spots were detected under UV (254 and 366 nm) and by spraying anisaldehyde/H2SO4 reagent. 1H-NMR and 13C-NMR spectra of the isolated compounds were obtained on a Bruker Avance DRX 300 and 500 spectrometers. UV spectra were recorded on a CECIL 7250 spectrophotometer in methanol and after the addition of shift reagents (NaOMe, NaOAc and AlCl3). EIMS spectra were also acquired on a HP 5973 system.

### MTT assay

Two cancerous cell lines, MDA-MB-231 (Human breast cancer) and HT-29 (Human colon adenocarcinoma) and one normal cell line, MRC-5 (Human fetal lung fibroblasts) were obtained from Pasteur Institute, National Cell Bank of Iran Tehran, Iran. All cell lines were cultured for 24 hours in Roswell Park Memorial Institute (RPMI) medium containing different concentrations of test compounds and incubated for 24 h at 37°C. The cells were then treated with fresh medium and maintained in a humidified atmosphere at 37°C in a 5% CO2 incubator.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to evaluate the cytotoxic activity of the isolated flavonoids on two cancer cell lines, MDA-MB-231 and HT-29, and one normal cell line, MRC-5. Cells were seeded in 96-well plates containing RPMI medium and incubated for 24 h at 37°C. The cells were then treated with fresh medium containing different concentrations of test compounds in triplicates. After 24 h the medium was changed by phosphate-buffered saline (PBS) medium containing MTT (10μl, 5mg/ml) and incubated for additional 3 h. Afterwards, the formazan crystals produced from MTT were completely dissolved in DMSO, and their absorbance was recorded at 630nm using microplate reader (Anthons 2020, version 1.8.3, UK). The cell viabilities were calculated by the following formula: 

Relative viability (%) = (Absorbance of the test /Absorbance of the control) ×100. IC50 value was defined as the concentration of the compounds (in µg ml−1) which caused a 50% reduction in the number of viable cells relative to the negative control. Tamoxifen was used as positive control.

### Results

#### General toxicity

The results of general toxicity assay of the plant samples in brine shrimp lethality test have been shown in Table 1. Among the tested samples, chloroform extract and the plant essential oil with LD50 values of 121.8 ± 5.6 and 127.6 ± 14.7 µg ml−1, respectively, exhibited the most toxicity effects against Artemia salina larvae, in comparison with podophyllotoxin (LD50 value; 3.1 ± 0.6 µg ml−1).

#### Essential oil composition

Hydrodistillation of the plant aerial parts led to the extraction of a pale yellowish oil with a yield of 0.3% (V/W). Forty compounds representing 88.15% of the total oil were characterized as a result of GC and GC-MS analyses of the essential oil (Table 2).

#### Isolation and purification of compounds

Phytochemical investigation of the chloroform extract, as the most toxic extract of S. lavandulifolia aerial parts, resulted in the isolation of nine compounds (1-9). The structures of the isolated compounds were identified as pachypodol (1), chrysoespermen (2), kemukatakenin (3), velutin (4), penduletin (5), viscosine (6), chrysoeriol (7), hydroxyegenkwanin (8) and apigenin (9), using 1H-NMR, 13C-NMR, UV and EIMS spectral analyses, as well as by comparison of their spectroscopic data with those published in literature (Figure 1).

#### Chromatographic and spectroscopic data of the isolated compounds

1. **Compound 1**: Pachypodol (Quercetin 3,7,3'-trimethyl ether): Yellow needles; Rf 0.67 (CHCl3-EtOAc, 8:2); 1H-NMR (CDCl3, 500 MHz): δ 12.63 (1H, s, OH-5), 7.70 (1H, br s, H-2'), 7.67 (1H, br d, J=8.5 Hz, H-6'), 7.04 (1H, d, J=8.5 Hz, H-5'), 6.44 (1H, br s, H-8), 6.36 (1H, br s, H-6), 3.98 (3H, s, OCH3), 3.88 (3H, s, OCH3), 3.86 (3H, s, OCH3); 13C-NMR (DMSO-d6, 125 MHz): δ 178.72 (C-4), 165.45 (C-7), 162.08 (C-5), 155.82 (C-2), 157.76 (C-9), 148.37 (C-4'), 138.84 (C-3'), 122.69 (C-6'), 114.58 (C-5'), 110.91 (C-2'), 124.46 (C-1'), 97.84 (C-6), 92.20 (C-8), 60.16 (OCH3), 56.12 (OCH3), 55.81 (OCH3); UV (MeOH) λmax: 270, 355, +NaOMe: 266, 405.

2. **Compound 2**: Chrysoespermen (Quer cetag etin 3,6,7,3'-tetramethyl ether): Yellow needles; Rf 0.62 (CHCl3-EtOAc, 8:2); 1H-NMR (DMSO-d6, 300 MHz): δ 12.62 (1H, s, OH-5), 7.66 (1H, br s, H-2'), 7.62 (1H, br d, J=8.7 Hz, H-6'), 6.96 (1H, d, J= 8.7 Hz, H-5'), 6.89 (1H, s, H-8), 3.91 (3H, s, OCH3), 3.87 (3H, s, OCH3), 3.80 (3H, s, OCH3), 3.72 (3H, s, OCH3); UV (MeOH) λmax: 256, 269 (sh), 355, +NaOMe: 269, 411, +NaOA: 266, 414.

3. **Compound 3**: Kemukatakenin (Kaempferol 3,7-dimethyl ether): Yellow needles; Rf 0.67 (CHCl3-EtOAc, 8:2); 1H-NMR (DMSO-d6, 300 MHz): δ 7.69 (2H, d, J= 8.1 Hz, H-2',6'); 6.94 (2H, d, J= 8.1 Hz, H-3'), 6.70 (1H, br s, H-8), 6.33 (1H, br s, H-6), 3.84 (3H, s, OCH3), 3.78 (3H, s, OCH3); UV (MeOH) λmax: 266, 355, +NaOMe: 259, 395, +NaOA: 266, 368.
Table 2. Essential oil composition of the aerial parts of *S. lavandulifolia* from north-west of Iran.

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<th>No</th>
<th>Compounds</th>
<th>KI</th>
<th>%</th>
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* Identified compounds listed in order of elution from HP-5MS column; ** Kovats retention indices to C₄₋C₃₀ n-alkanes on HP-5MS column.

![Figure 1. Structures of the isolated compounds from the aerial parts of *S. lavandulifolia*.](image-url)
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**Compound 4**: Volutin (Luteolin 7,3'-dimethyl ether); Yellow needles: Rf 0.62 (CHCl3-EtOAc, 8:2); 1H-NMR (DMSO-d6, 300 MHz): δ 12.98 (1H, s, OH-5), 7.60 (1H, br d, J= 8.1 Hz, H-6), 7.59 (1H, br s, H-2'), 6.95 (1H, d, J= 8.1 Hz, H-5'), 6.97 (1H, s, H-3'), 6.81 (1H, br s, H-8), 6.37 (1H, br s, H-6'), 3.90 (3H, s, OCH3), 3.87 (3H, s, OCH3); UV (MeOH) λmax: 250, 264 (sh), 345, +NaOMe: 259, 404, +NaOAc: 256, 355.24

**Compound 5**: Penduletin (5,4'-dihydroxy-3,6,7-trimethoxyflavone); Yellow needles: Rf 0.51 (CHCl3-EtOAc, 8:2); 1H-NMR (DMSO-d6, 500 MHz): δ 7.92 (2H, d, J= 8.6 Hz, H-2,6'), 6.91 (2H, d, J= 8.6 Hz, H-3,5'), 3.89 (3H, s, OCH3), 3.76 (3H, s, OCH3), 3.73 (3H, s, OCH3); 13C-NMR (DMSO-d6, 125 MHz): δ 176.65 (C-4), 158.72 (C-4'), 156.87 (C-7), 154.48 (C-9), 150.35 (C-2), 150.24 (C-5), 128.66 (C-6), 128.34 (C-2',6'), 118.83 (C-1'), 113.96 (C-3',5'), 104.16 (C-10), 89.12 (C-8), 58.44 (OCH3), 57.92 (OCH3), 54.59 (OCH3); UV (MeOH) λmax: 271, 338, +NaOMe: 272, 392, +NaOAc: 272, 344 (sh), 396; EIMS (40 eV) m/z: 344 [M]+, 329 [M-Me]+, 181 [A-Me]+, 121 [B2]+.35

**Compound 6**: Viscosine (5,7,4'-trihydroxy-3,6-dimethoxyflavone); Yellow needles: Rf 0.44 (CHCl3-EtOAc, 8:2); 1H-NMR (DMSO-d6, 500 MHz): δ 7.93 (2H, d, J= 8.2, H-2,6'), 6.94 (2H, d, J= 8.2, H-3,5'), 6.54 (1H, s, H-3), 3.78 (3H, s, OCH3), 3.75 (3H, s, OCH3); 13C-NMR (DMSO-d6, 125 MHz): δ 178.62 (C-4), 160.60 (C-4'), 158.77 (C-2), 156.12 (C-9), 152.84 (C-5), 150.23 (C-7), 137.13 (C-3), 131.60 (C-6), 130.59 (C-2',6'), 121.07 (C-1'), 116.09 (C-3',5'), 105.01 (C-10), 94.43 (C-8), 60.42 (OCH3), 60.13 (OCH3); UV (MeOH) λmax: 268, 338, +NaOMe: 272, 331 (sh), 397, +NaOAc: 275, 350, +AlCl3: 406 (sh), 361, 310 (sh), 272; +HCl-AlCl3: 404 (sh), 362, 306 (sh), 281; EIMS (40 eV) m/z: 330 [M]+, 315 [M-Me]+, 287 [M-MeCO]+, 153 [A]+, 121 [B2]+.35

**Compound 7**: Chrysoeriol (Luteolin 3'-methyl ether); Yellow solid: Rf 0.40 (CHCl3-EtOAc, 8:2); 1H-NMR (DMSO-d6, 300 MHz): δ 12.96 (1H, s, OH-5), 7.89 (1H, br d, J= 8.0 Hz, H-6), 7.52 (1H, br s, H-2'), 6.90 (1H, s, H-3), 6.89 (1H, d, J= 8.0 Hz, H-5), 6.56 (1H, br s, H-8), 6.18 (1H, br s, H-6'), 3.74 (3H, s, OCH3).40

**Compound 8**: Hydroxygerkenwakin (Luteolin 7-Methyl ether); Yellow solid: Rf 0.40 (CHCl3-EtOAc, 8:2); 1H-NMR (DMSO-d6, 300 MHz): δ 13.06 (1H, s, OH-5), 7.89 (1H, br d, J= 8.0 Hz, H-6), 7.52 (1H, br s, H-2'), 6.90 (1H, s, OH-5), 6.89 (1H, d, J= 8.0 Hz, H-5), 6.48 (1H, br s, H-6), 3.88 (3H, s, OCH3).37

**Compound 9**: Apigenin (5,7,4'-trihydroxyflavone); Yellow solid: Rf 0.30 (CHCl3-EtOAc, 8:2); 1H-NMR (DMSO-d6, 300 MHz): δ 12.95 (1H, s, OH-5), 7.91 (2H, d, J= 8.0 Hz, H-2,6'), 6.91 (2H, d, J= 8.0 Hz, H-3,5'), 6.77 (1H, s, H-3), 6.46 (1H, br s, H-8), 6.17 (1H, br s, H-6'); UV (MeOH) λmax: 266, 335, +NaOMe: 273, 325 (sh), +NaOAc: 272, 384.38

**Cytotoxic activity**

The results of cytotoxic activity of isolated compounds on MDA-MB-23, HT-29 and MRC-5 cell lines by MTT assay have been summarized in Table 3.

**Table 3.** Cytotoxic activity of the flavonoids from *S. lavandulifolia* on different cell lines.

<table>
<thead>
<tr>
<th>Samples</th>
<th>MDA-MB-23 IC50 (µg ml⁻¹)</th>
<th>MDA-MB-23 SI</th>
<th>HT-29 IC50 (µg ml⁻¹)</th>
<th>HT-29 SI</th>
<th>MRC-5 IC50 (µg ml⁻¹)</th>
<th>MRC-5 SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pachypodol (1)</td>
<td>165.21</td>
<td>1.74</td>
<td>235.76</td>
<td>1.21</td>
<td>287.16</td>
<td></td>
</tr>
<tr>
<td>Chrysoerolenin (2)</td>
<td>88.23</td>
<td>2.70</td>
<td>116.50</td>
<td>2.04</td>
<td>238.04</td>
<td></td>
</tr>
<tr>
<td>Kumatakenin (3)</td>
<td>130.93</td>
<td>2.59</td>
<td>370.15</td>
<td>0.91</td>
<td>335.07</td>
<td></td>
</tr>
<tr>
<td>Volutin (4)</td>
<td>289.64</td>
<td>0.83</td>
<td>205.27</td>
<td>1.17</td>
<td>240.98</td>
<td></td>
</tr>
<tr>
<td>Penduletin (5)</td>
<td>156.14</td>
<td>2.04</td>
<td>163.63</td>
<td>1.95</td>
<td>319.20</td>
<td></td>
</tr>
<tr>
<td>Viscosine (6)</td>
<td>148.15</td>
<td>3.33</td>
<td>150.01</td>
<td>3.29</td>
<td>493.17</td>
<td></td>
</tr>
<tr>
<td>Chrysoeriol (7) &amp; Hydroxygerkenwakin (8)</td>
<td>112.89</td>
<td>0.72</td>
<td>145.20</td>
<td>0.56</td>
<td>80.92</td>
<td></td>
</tr>
<tr>
<td>Apigenin (9)</td>
<td>141.99</td>
<td>0.25</td>
<td>168.70</td>
<td>0.21</td>
<td>35.67</td>
<td></td>
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<tr>
<td>Tamoxifen</td>
<td>4.61</td>
<td>2.45</td>
<td>2.5</td>
<td>4.52</td>
<td>11.31</td>
<td></td>
</tr>
</tbody>
</table>

*Selectivity index*
Chen et al. demonstrated cytotoxic activity of α-bisabolol on PC-3, Hela, ECA-109 and HepG2 cell lines. Moreover, they showed α-bisabolol induces apoptosis in HepG2 cells in a dose- and time-dependently manner through both Fas- and mitochondrial-related pathway with involvement of p53 and NFκB either. It has also been reported the exposure of permanent colonic cell line, Caco-2, to thymol and its mixture with carvacrol could be led to the mitochondrial damage, lipid degeneration, chromatin condensation and finally cell death through apoptosis and necrosis. This study reports the isolation and identification of compounds 1-8 from the aerial parts of S. lavandulifolia for the first time. Furthermore, our literature review indicates this is the first report on the isolation of Pachypodol (1), kumatakenin (3), velutin (4), viscosine (6) and hydroxygenkwanin (8) from the genus Stachys. The isolated flavonoids (1-9) exhibited a moderate cytotoxic activity on MDA-MB-23, HT-29 and MRC-5 cell lines, of which chrysosplenetin, was found as the most toxic compounds toward MDA-MB-23 and HT-29 cells, with IC50 values of 88.23 and 116.50 μg ml-1, respectively. Furthermore, most of the examined methoxylated flavonoids inhibited the growth of mentioned cancerous cell lines with higher selectivity in comparison with MRC-5 normal cells (Table 3). Among the tested compounds, chrysosplenetin, kumatakenin and viscosine with selectivity indices of 2.70, 2.59 and 3.33, respectively, showed higher preferential toxicity against MDA-MB-23 cells than tamoxifen (SI: 2.45).

Previous studies demonstrated that methylation of hydroxyl residue increases the cytotoxic potential of flavonoids and their selective activity against tumor cells, either. The results of recent investigation on cytotoxicity of dietary flavonoids on different human cancer types have been reviewed by Sak. In agreement with our findings, Kawai and colleagues found that higher degrees of methylation of the A-ring and also the presence of a 3'-methoxyl residue are associated with enhanced antiproliferative activity of some synthesized polymethoxylated flavonoids against the leukemic HL60 cells.

A mechanistic study on HepG2 and MCF-7 cells treated with two polymethoxylated flavonoid derivatives isolated from the aerial parts of *Euryops arabicus* indicated that antiproliferative activity of tested compounds was attributed to S-phase cell cycle arrest. In another study, Saito et al. demonstrated nobiletin, a citrus polymethoxylated flavonoid, potentiated the cytolytic activity of KHYG-1 cells (a natural killer leukemia cell line) by induction of the expression of granzyme B gene, a serine protease which acts as a cytotoxic effector. Polymethoxylated flavonoids have also received great attention because of their antiangiogenic activity. Potent antiangiogenic activity of xanthomicrol and calycoperin from the aerial parts of *Dracocephalum kotschyi* and its relation with inhibition of VEGF (vascular endothelial growth factor) expression has been shown during previous investigations. Beside cytotoxic activity, viscosine has been previously reported for its anxiolytic and anticonvulsant effects via GABA A receptor modulation. Therefore, this major flavonoid isolated from the chloroform extract of *S. lavandulifolia*, could be considered as an active compound involved in anxiolytic activity of *S. lavandulifolia* extracts documented by Rabbani et al.

Some hormone induced effects followed by administration of the aerial parts of *S. lavandulifolia* may be associated with the presence of penduletin and apigenin, flavonoids with confirmed estrogen receptor-β (ERβ) selective activity.

**Conclusion**

In conclusion, flavonoids (1-9) identified in the chloroform extract of *S. lavandulifolia* could be assumed as toxic principles of this species. However, future in vivo studies need to confirm this finding. Furthermore, this study suggests some of the isolated methoxylated flavonoids such as chrysosplenetin (2), kumatakenin (3) and viscosine (6) as appropriate candidates for anti-cancer drug development research.

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**Conflict of interests**

The authors claim that there is no conflict of interest.

**References**


