Flavone Glycosides from the Aerial Parts of Stachys lavandulifolia Vahl

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Abstract
Background: Stachys lavandulifolia Vahl is an herbaceous perennial plant which its flowering aerial parts are used traditionally as gastrotonic, spasmylytic, sedative and for the treatment of gastrointestinal disorders. In the present study the aerial parts of this medicinal plant was investigated for its flavonoid glycosides content.

Methods: n-butanol fraction derived from hydroalcoholic extract of S. lavandulifolia was subjected to phytochemical analysis using chromatography on Sephadex LH-20 and RP-18 silica gel columns. The structures of isolated compounds were identified using 1H-NMR, 13C-NMR and UV spectral analysis.

Results: Four flavone glycosides, chrysoeriol-7-O-β-D-glucopyranoside (1), apigenin-7-O-(6″-O-acetyl)-β-D-glucopyranoside (2), luteolin-7-O-β-D-glucopyranoside (3), apigenin-7-O-β-D-glucopyranoside (4), along with apigenin (5) and chlorogenic acid (6) were isolated from S. lavandulifolia aerial parts.

Conclusion: Identification of these phenolic compounds with some known biological activities in S. lavandulifolia explains some medicinal properties reported for this species and make scientific rationale for its traditional uses.

Introduction
Stachys lavandulifolia Vahl from Lamiaceae family is an herbaceous perennial plant distributed in Iran, Turkey, Caucasus, Iraq and central Asia. In Iranian folk medicine, the infusion of flowering aerial parts of this plant is known as “Chaye-e-Koohi” and used as gastrotonic, spasmylytic, sedative and for the treatment of gastrointestinal disorders. S. lavandulifolia has been mentioned as “Marv-azad” or “Marmazad” in Old Persian medicinal literature, useful in some liver and uterus diseases and as liver tonic, gastrotonic and emmenagogue. This species is also used by Turkish people as a sedative and antipyretic, as well as in insomnia, cough, cold and flu. Some biological and pharmacologic properties such as antioxidant, antimicrobial, gastroprotective, spasmylytic, anxiolytic, wound healing, analgesic and anti-inflammatory effects have been documented for S. lavandulifolia. Moreover, the results of previous clinical trials were indicative to its beneficial effects in abnormal uterine bleeding management (caused by polycystic ovary syndrome) and in oxidative stresses. The use of this plant, however, has been cautioned during pregnancy period because of its abortive potential and teratogenic effects.

There are some reports in the literature on phytochemical constituents of various extracts of S. lavandulifolia in literature. Four phenylethanoid glycosides including lavandulifoliosides A and B, verbascoside, and leucosceptoside A, together with one iridoid glycoside named 5-allosyloxy-aucubin have been isolated from the aerial parts of S. lavandulifolia by Delazar et al. In another study, acetyl- and butyryl-Cholinesterase and tyrosinase inhibitory activity of different extracts of S. lavandulifolia were assessed and monomelittoside, melittoside, stachysolone and arbutin were isolated and reported as its major compounds. Recently, Delnavazi et al. reported the isolation of eight methoxylated flavonoids from chloroform extract of this species, of which chrysoplenitinum, kumatakenin and viscose were found to have higher preferential toxicity against MDA-MB-23 cells in comparison with tamoxifen. The present study was an attempt to more investigate phytochemical constituents of this medicinal species, specially its flavonoid glycosides content using various chromatographic and spectroscopic methods.

Materials and Methods
Plant material
Aerial parts of S. lavandulifolia were collected from Ghotour region (Khoy, West-Azerbaijan Province, Iran) in its flowering stage. The plant voucher specimen was deposited at the herbarium of Institute of Medicinal Plants, ACECR (Academic Center for Education, Culture and Research), Karaj, Iran (Voucher no. 2014 MPIH).

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Extraction and fractionation
The shade-dried and ground plant aerial parts (0.5 kg) were macerated with methanol-water (7:3) (3×8 L each) at the room temperature. The concentrated hydroalcoholic extract was dispersed in water and fractionated successively with chloroform and n-butanol using liquid-liquid fractionation method. Fractions were then dried using a rotary evaporator under low pressure at 45 °C.

Isolation and purification of compounds
A portion of n-butanol fraction (3 g) was divided to nine subfractions (A-I) by column chromatography on Sephadex LH-20 (5×30 cm) using methanol as eluent. Compounds 1 (8 mg) and 2 (3 mg) were isolated from subfractions B (0.3 g) and C (0.1 g), respectively, by Sephadex LH-20 column chromatography (2×80 cm) using MeOH-H₂O (8:2). Subfraction E (0.3 g) was eluted on RP18 column (2×20 cm) by MeOH-H₂O (2:8) to get compound 3 (32 mg). Compound 4 (6 mg) was obtained from subfraction G (0.5 g) by chromatography on Sephadex LH-20 column (2×70 cm) using MeOH-H₂O (8:2) as solvent system. Column chromatography of subfraction F (0.5 g) on Sephadex LH-20 column (2×70 cm) using MeOH-H₂O (8:2) resulted in isolation of compound 5 (13 mg). Subfraction F2 (35 mg) was also moved on a RP-18 column (1×20 cm) and eluted with MeOH-H₂O (2:8to 4:6) to get compound 6 (12 mg). In all chromatography steps, fractions were monitored by thin layer chromatography under 254 and 366 UV wavelengths and those with same spots were combined. NMR (Varian INOVA 500 MHz spectrometer (500 MHz for ¹H-NMR and 125 MHz for ¹³C-NMR) and UV (Shimadzu UV-1800) spectral analyses were applied for structure elucidation of isolated compounds.

Results
Phytochemical investigation of the hydroalcoholic extract obtained from S. lavandulifolia aerial parts led to the isolation and structure elucidation of four flavone glycosides; Chrysoeriol-7-O-β-D-glucopyranoside (Thermopsoside) (1), Apigenin-7-O-(6″-O-Acetyl)-β-D-glucopyranoside (2), Luteolin-7-O-β-D-glucopyranoside (Cynaroside) (3), Apigenin-7-O-β-D-glucopyranoside (Apietin) (4), along with Apigenin (5) and Chlorogenic acid (6) (Figure 1). The structures of compounds were established by ¹H-NMR, ¹³C-NMR and UV spectral analyses comparing with those published in the literature.¹⁹-²¹

Spectral data for isolated flavone glycosides
Compound 1; Chrysoeriol-7-O-β-D-glucopyranoside (Thermopsoside), ¹H-NMR (DMSO-d₆, 500 MHz): δ ppm 7.60 (1H, br d, J= 8.1 Hz, H-6′), 7.59 (1H, br s, H-2′), 7.01 (1H, s, H-3), 6.95 (1H, d, J= 8.1 Hz, H-5′), 6.88 (1H, br s, H-8), 6.46 (1H, br s, H-6), 5.07 (1H, d, J= 7.2 Hz, H-1″), 3.90 (3H, s, OCH₃), 3.1-3.8 (6H, overlapped peaks, H-2″ to H-6″); ¹³C-NMR (DMSO-d₆, 125 MHz): δ ppm 181.84 (C-4), 164.04 (C-2), 162.96 (C-7), 161.03 (C-5), 156.92 (C-9), 151.8 (C-4′), 146.84 (C-3′), 122.86 (C-1′), 118.73 (C-6′), 113.11 (C-5′), 112.13 (C-2′), 105.35 (C-10), 103.75 (C-3′), 99.92 (C-1″), 99.54 (C-6″), 94.72 (C-8), 77.12 (C-5′″), 76.35 (C-3′″), 73.06 (C-2′″), 69.54 (C-4′″), 60.58 (C-6′″), 55.73 (OCH₃). UV spectrum (MeOH, λₘₐₓ nm): 268, 342.²¹,²²

Figure 1. Flavone glycosides isolated from the hydroalcoholic extract of S. lavandulifolia aerial parts.
Flavone Glycosides from Stachys lavandulifolia

Compound 2: Apigenin-7-O-(6′-O-Acetyl)-β-D-glucopyranoside, 1H-NMR (DMSO-d₆, 500 MHz): δ ppm 13.03 (1H, s, OH-5), 8.00 (2H, d, J= 8.5 Hz, H-2′,6′), 6.96 (2H, d, J= 8.5 Hz, H-3′,5′), 6.84 (1H, s, H-3), 6.75 (1H, br s, H-8), 6.62 (1H, br s, H-6), 5.01 (1H, d, J= 7.5 Hz, H-1′), 4.32 (1H, br d, J= 11.7 Hz, H-6′a), 4.11 (1H, dd, J= 11.7, 7.5 Hz, H-6′b), 3.73 (1H, m, H-5′), 3.2-3.6 (3H, overlapped peaks, H-2′ to H-4′), 2.06 (3H, s, COCH₃); 13C-NMR (DMSO-d₆, 125 MHz): δ ppm 181.95 (C-4), 172.57 (COCH₃), 167.58 (C-2′), 164.43 (C-7), 162.75 (C-4′,5′), 156.82 (C-9), 129.58 (C-2′,6′), 122.66 (C-1′), 116.04 (C-3′,5′), 106.57 (C-10), 105.32 (C-3), 102.63 (C-1′′), 98.51 (C-6), 94.99 (C-8), 77.10 (C-3′′), 75.78 (C-5′′), 74.71 (C-2′′), 71.59 (C-4′′), 64.00 (C-6′′), 20.52 (C-3COCH₃). UV spectrum (MeOH, λmax nm): 259, 335, 324.

Compound 3: Luteolin-7-O-β-D-glucopyranoside (Cynaroside), 1H-NMR (DMSO-d₆, 500 MHz): δ ppm 13.00 (1H, s, OH-5), 7.45 (1H, d, J= 8.3, 1.6 Hz, H-6′), 7.43 (1H, d, J= 1.6 Hz, H-2′), 6.90 (1H, d, J= 8.3 Hz, H-5′), 6.80 (1H, d, J= 1.2 Hz, H-8), 6.76 (1H, s, H-3), 6.45 (1H, d, J= 1.2 Hz, H-6′), 5.09 (1H, d, J= 7.5 Hz, H-1′), 3.0-3.8 (6H, overlapped peaks, H2′ to H6′); 13C-NMR (DMSO-d₆, 125 MHz): δ ppm 181.76 (C-4), 174.52 (C-2), 162.89 (C-7), 161.09 (C-5), 156.88 (C-9), 150.36 (C-4′), 145.87 (C-3′), 120.98 (C-1′), 119.09 (C-6′), 116.00 (C-5′), 113.31 (C-2′), 105.30 (C-10), 103.00 (C-3′), 99.97 (C-1′), 99.55 (C-6), 94.76 (C-8), 77.12 (C-5′), 76.37 (C-3′), 73.08 (C-2′), 69.58 (C-4′), 60.63 (C-6′). UV spectrum (MeOH, λmax nm): 256, 267 (sh), 346.

Compound 4: Apigenin-7-O-β-D-glucopyranoside (Apietin), 1H-NMR (DMSO-d₆, 500 MHz): δ ppm 12.99 (1H, s, OH-5), 7.89 (2H, d, J= 8.5 Hz, H-2′,6′), 6.90 (2H, d, J= 8.5 Hz, H-3′,5′), 6.82 (1H, s, H-3), 6.79 (1H, br s, H-8), 6.41 (1H, br s, H-6), 5.06 (1H, d, J= 7.4 Hz, H-1′), 3.1-3.7 (6H, overlapped peaks, H2′ to H6′); 13C-NMR (DMSO-d₆, 125 MHz): δ ppm 182.3 (C-4), 164.2 (C-7), 163.1 (C-2), 161.7 (C-9), 153.1 (C-5), 150.1 (C-4′), 128.4 (C-2′,6′), 121.4 (C-1′), 116.2 (C-3′,5′), 105.6 (C-10), 103.1 (C-3), 101.1 (C-1′), 99.3 (C-6), 95.2 (C-8), 76.8 (C-5′), 75.8 (C-3′), 73.2 (C-2′), 69.6 (C-4′), 60.7 (C-6′). UV spectrum (MeOH, λmax nm): 268, 332.

Discussion
Thermopsoside (1), Apigenin-7-O-(6′-O-Acetyl)-β-D-glucopyranoside (2), Cynaroside (3), Apigenin (4), Apigenin (5) and Chlorogenic acid (6) are the compounds isolated from S. lavandulifolia in the present study. Although phytochemical constituents of S. lavandulifolia aerial parts have been investigated in some previous studies, this is the first report on isolation of four flavone glycosides (1-4) and chlorogenic acid (6) from this medicinal species. A literature review on isolated compounds showed that some of them have been documented to possess pharmacological activity and health promoting effects. Apigenin-7-O-glucoside (apigenin) and luteolin-7-O-glucoside (cynaroside) have been shown to have protective activity against carbon tetrachloride-induced hepatic injury, possibly through their antioxidant properties as ROS scavengers. Moreover, significant protective effects of luteolin-7-O-glucoside and its aglycon have been demonstrated against oxidative damage induced by t-butyl hydroperoxide in HepG2 cultured cells. In 2013, Chung and Young-Sun showed luteolin and luteolin-7-O-glucoside inhibit lipopolysaccharide-induced inflammatory responses through modulation of NF-κB/AP-1/P3K-Akt signaling cascades in RAW 264.7 cells. Luteolin was found more potent than its glucoside for anti-inflammatory activity in former study. In another study, productions of nitric oxide and prostaglandin E2 (PGE2) were significantly suppressed by luteolin-7-O-glucoside (20 μM) in bacterial lipopolysaccharide activated-mouse macrophage RAW264.7 cells without cytotoxic effects. Luteolin-7-O-glucoside has been reported to have a protective effect against cisplatin-induced kidney injury using HK-2 cell (human proximal tubule cell line) and an animal model. It has also been reported that pretreatment of luteolin-7-O-glucoside (25 mg/kg, p.o.) for 4 days had significant gastroprotective effect against ethanol induced gastric injury in rats.

Anxiolytic potential of apigenin-7-O-glucoside isolated from Stachys tibetica Vatke was evaluated by Kumar and Bhar using Elevated plus-maze test in rat. The results showed oral administration of apigenin-7-O-glucoside (2.5 and 5 mg/kg) had a significant anxiolytic activity, comparable to apigenin (5 mg/kg) and diazepam (1 mg/kg). Modulation of GABA_{A} receptors has been considered as a mechanism for anxiolytic activity of apigenin and its derivatives. Interestingly, Apigenin has been identified as an anxiolytic principle of S. lavandulifolia in a bioactivity guided isolation approach by Rabbani et al. Chlorogenic acid (5-O-Caffeoylquinic acid) is a most caffeoylquinic acid derivative in natural resources. This polyphenol compound has lately received special attention by researchers due to its beneficial health effects in cardiovascular disease (CVD), diabetes, obesity, cancer prevention, and hepatic steatosis, which are related to antioxidant and anti-inflammatory effects, as well as its potential in regulation of glucose and lipid metabolism.

Conclusion
The result of this study introduce Stachys lavandulifolia as valuable source of natural phenolic compounds. The presence of mentioned bioactive compounds (1-6) in S. lavandulifolia may be correlated with some biological activities such as gastroprotective, antioxidant, anti-inflammatory and anxiolytic effects reported in literature for this species and also provide scientific rationales for some traditional uses of S. lavandulifolia as liver tonic, sedative and in treatment of gastrointestinal conditions.

Conflict of Interests
The authors claim that there is no conflict of interest.
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