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B R I E F C O M M U N I C A T I O N S

HPLC METHOD FOR THE ANALYSIS OF SALICIN AND CHLOROGENIC ACID FROM *Viburnum opulus* AND *V. lantana*

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The genus *Viburnum* (Caprifoliaceae) comprises more than 230 species distributed from South America to Southeast Asia, the majority of them being endemic [1].

The plant is represented by four species in the flora of Turkey: *Viburnum opulus* L., *V. lantana* L., *V. orientale* Pallas, and *V. tinus* L. [2, 3].

In inner Anatolia, a traditional drink named gilaburu has been prepared from the fruits of *V. opulus*. The fruit has a dark-red color and is edible. The barks of *V. lantana* have been used in folk medicine as rubefiant and analgesic [4]. The bark and root of *Viburnum prunifolium* (black how) are used for complaints of dysmenorrhea in folk medicine [5]. The preventive effect of *V. dilatatum* on oxidative damages was found in rats subjected to stress [6] and in streptozotocin-induced diabetic rats [7]. In addition, the effect of *V. dilatatum* on antioxidant enzymes in plasma, liver, and stomach was examined, and the results suggested that ingestion of the fruits of this plant might contribute to reducing the consumption of antioxidant enzymes, such as superoxide dismutase, catalase, glutathion peroxidase, and glutathion [8]. Some iridoid aldehydes isolated from *V. luzonicum* exhibited moderate inhibitory activity against He La S3 cancer cells [9].

The genus *Viburnum* is known to contain triterpenoids [10] diterpenoids [11], sesquiterpenes [12], iridoids [9], and polyphenols [13].

The aim of this study was to determine the appropriate HPLC method. Good separation and determination of salicin and chlorogenic acid in leaves, branches, and fruits of *Viburnum opulus* L. and *Viburnum lantana* L. were performed by using a mobile phase consisting of bidistilled water, tetrahydrofuran, and *ortho*-phosphoric acid (97.7: 1.8: 0.5) (v/v/v) and an Exsil ODS column (150 × 4.6 mm, 5 μm), at a flow rate of 0.5 mL/min in an isocratic elution.

The chromatograms of standards and samples were plotted by an HPLC system consisting of a Hewlett Packard Series 1100 model (Agilent Technologies, Inc., California, USA). The UV-VIS detector was set at 270 nm, and peak areas were integrated automatically by computer using Agilent software. The chromatograms were plotted and processed using the above-mentioned software.

The injection volume was 20 μL, and triplicate injections were used for each sample. At a flow rate of 0.5 mL/min the retention times for salicin and chlorogenic acid were observed to be 3.22 and 4.68 min, respectively.

V. opulus was collected from Kayseri, Turkey and *V. lantana* was collected from Ankara, Turkey. Voucher specimens were deposited at the Herbarium of Ankara University, Faculty of Pharmacy (AEF), with herbarium numbers AEF 23696, AEF 23543, respectively.

Standard salicin (Aldrich; S-30-5) (11.85 mg) and chlorogenic acid (Sigma; C-3878) (11.87 mg) were accurately weighed into a 10 mL volumetric flask, dissolved in the mobile phase, and filled up to volume with mobile phase.

Three grams of dried and powdered samples (leaves, branches, and fruits) of *V. opulus* and *V. lantana* were macerated with methanol at room temperature for 8 h. The resulting extracts were combined and evaporated to dryness. The residues were dissolved in 25 mL mobile phase. The solution was passed through a 0.45 μm filter, and 20 μL of the extract was directly injected into the HPLC. The assay results were obtained from the mean value of three separate injections.

The equation of the regression line was determined as $Y = 6.4248 X + 147.25$ for salicin and as $Y = 23.606 X + 189.59$ for chlorogenic acid.

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TABLE 1. Contents of Salicin and Chlorogenic Acid in *Viburnum* Species

Species	Salicin % (n = 3, mean) Mean ± SD	Chlorogenic acid % (n = 3, mean) Mean ± SD
V. opulus leaves	0.903±0.0239 (2.641)*	0.682±0.0177 (2.594)*
V. opulus branches	1.250±0.0563 (4.507)*	0.356±0.0074 (2.068)*
V. opulus fruits	1.266±0.0585 (4.621)*	1.240±0.0355 (2.862)*
V. lantana leaves	0.390±0.0125 (3.202)*	0.759±0.0064 (0.839)*
V. lantana branches	0.782±0.0096 (1.229)*	0.222±0.0097 (4.378)*
V. lantana fruits	0.459±0.0230 (5.021)*	0.010±0.0004 (5.222)*

*RSD % values are given in parentheses; RSD % = (Standard Deviation/Mean) × 100;
SD = Standard Deviation.

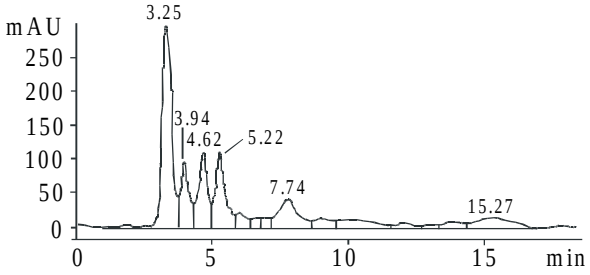

Fig. 1. Chromatogram of the *Viburnum lantana* branches: salicin (3.94), chlorogenic acid (4.62).

Excellent linearity was obtained for compounds between peak areas and concentrations of 17.775–474 μg/mL with r^2 = 0.9997, and 17.808–474.8 μg/mL with r^2 = 0.9991 for salicin and chlorogenic acid, respectively.

The LOD (limits of detection) was calculated to be 5.925 and 5.936 μg/mL and the LOQ (limits of quantification) was calculated to be 17.775 and 17.808 μg/mL for salicin and chlorogenic acid, respectively.

Quantitative determination of salicin and chlorogenic acid in the leaves, branches, and fruits in *V. opulus and V. lantana* were carried out by RP-HPLC using the external standard method (Fig. 1). Results of salicin and chlorogenic acid contents in different organs of *V. opulus* and *V. lantana* are given in Table 1.

According to our results, *V. opulus* branches (1.250%) and fruits (1.266%) can be considered as a good salicin source compared to the other samples. *V. opulus* fruits (1.240%) also can be evaluated as a good chlorogenic acid source. However, the salicin content of the leaves (0.390%) and fruits (0.459%) of *V. lantana* are low; and the chlorogenic acid content of the fruits (0.010%) of *V. lantana* are very low. Hence it is not productive to use *V. lantana* to obtain salicin and chlorogenic acid.

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