Phytochemical Analysis of *Phillyrea latifolia* L., a New Source of Oleuropeoside

Cristina Recuero Carretero\(^a\), Ana M. Díaz Lanza\(^{a,*}\), Lidia Fernández Matellano\(^a\), Angel Rumbero Sánchez\(^b\), and Lucinda Villaescusa Castillo\(^a\)

\(^a\) Laboratorio de Farmacognosia, Departamento de Farmacología, Facultad de Farmacia, Universidad de Alcalá, 28871 Alcalá de Henares, Madrid, España.
\(^b\) Departamento de Química Orgánica, Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, España

* Author for correspondence and reprint request


**Phenolpropanoid Glycosides, *Phillyrea latifolia*, Secoiridoid Glycosides**

As a part of our studies on the biologically active substances from Spanish plants, we have undertaken an investigation of the chemical constituents of a typical Mediterranean species, *Phillyrea latifolia* L. (Oleaceae). Two secoiridoid glycosides, three phenylpropanoid glycosides, one lignane and two triterpenic acids were isolated from the leaves of this species and identified. The phytochemical analysis of the aerial parts of *P. latifolia* revealed that it is a rich source of oleuropeoside.

Oleaceae plants have been used for medicinal purposes and spices for many centuries. A number of biologically active substances have been isolated from plants of the Oleaceae. Secoiridoid glycosides are the major secondary metabolites in Oleaceae. Several plants of this family, e.g. *Olea europaea*, have been studied from a pharmacological point of view. Oleuropeoside is the major iridoid in olive leaves. Clinical data on the beneficial effects of olive leaves in the treatment of hypertensive disease has been available since the 1950s (Hansel et al., 1996). This compound increased coronary blood flow and showed antiarrhythmic and spasmyloytic effects (Ghisalberti, 1998). It showed a hypoglycemic effect and increased tolerance of orally administered glucose (Trovato et al., 1993). In fact, olive leaves are used in folk medicine as an antidiabetic drug (González et al., 1992). Oleuropeoside has also shown antimicrobial and has been shown to be a potent antioxidant endowed with antiinflammatory properties (Visioli et al., 1998a), it is a potent scavenger of superoxide radicals and inhibitors of neutrophil respiratory burst (Visioli et al., 1998b).

A previous study of the constituents of the leaves from *Phillyrea latifolia* revealed that it is a rich source of oleuropeoside. Because of the reported pharmacological activities of this compound, we have undertaken an investigation of this species. *Phillyrea latifolia* is found in Spain, in the Mediterranean Europe and in the north of Africa. *P. latifolia* leaves were well known in the Mediterranean historical medicine for their oropharyngeal antiinflammatory effects. At the present time the use of this species is as diuretic (Ballerio and Fresu, 1993), as antipyretic (Bellakhdar, 1997) and as antispasmodic against stomach aches (Merzouki et al., 1997). Aerial parts of *P. latifolia* showed antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* (Husain and Tobji, 1997).

No previous phytochemical study on *Phillyrea latifolia* leaves has been reported till now. This paper led to isolation of ursolic acid (1), oleanolic acid (2), phyllarin (3), ligustroside (4), oleuropeoside (5), salidroside (6), coniferin (7) and syringin (8) from the leaves of *Phillyrea latifolia*.

**Materials and Methods**

Plant material was collected in Jaén (Spain) (March, 1997) and identified by Pr. Carmen Bartolomé Esteban, Department of Vegetal Biology, University of Alcalá, Madrid, Spain. A voucher specimen (CR 97) is kept in the University of Alcalá.

Leaves of *Phillyrea latifolia* (800 g) were extracted with acetone at room temperature. The extract (46 g) was chromatographed on Sephadex LH-20 and eluted by MeOH to afford four frac-
Results and Discussion

In our study of the phytochemistry of this species we have isolated compounds 1-8 from the aces
tonic extract. Structures of isolated compounds are shown in Figure 1.

The \(^1\)H and \(^{13}\)C NMR data of all compounds were assigned using a variety of 2D-NMR experiments including \(^1\)H-\(^1\)H-COSY, HMOC, HMBC and NOESY experiments. Identification and assignment of the isolated compounds were performed by comparison of their spectroscopic data with those of authentic samples (oleanolic and u
rassic acids, Extrasynthese, France) and/or previously reported data (Damtoft et al., 1992; Inouye and Nishioka, 1972; Maillard et al., 1992; Chiba et al., 1980).

\(^1\)H NMR and \(^{13}\)C NMR spectra (in CD\(_3\)OD, TMS as int. standard; chemical shifts in \(\delta\) ppm) were obtained using Bruker WM spectrometer [300 MHz (\(^1\)H NMR) and 75 MHz (\(^{13}\)C NMR)].

The results obtained from analysis of isolated compounds were:

**Phillyrin (3):** \(^{13}\)C NMR: 51.2 (C-1), 83.3 (C-2), 72.0 (C-4), 55.8 (C-5), 89.0 (C-6), 70.7 (C-8), 132.8 (C-1'), 110.8 (C-2'), 147.8 (C-3'), 150.3 (C-4'), 112.8 (C-5'), 119.2 (C-6'), 137.5 (C-1''), 111.5 (C-2''), 149.5 (C-3''), 150.9 (C-4''), 118.0 (C-5''), 119.8 (C-6''), 56.7 (3'-OCH\(_3\)), 56.5 (4'-OCH\(_3\)), 56.4 (3'-
OCH\(_3\)), 102.8 (C-1''), 74.9 (C-2''), 78.2 (C-3''), 71.3 (C-4''), 77.8 (C-5''), 62.4 (C-6'').

**Ligustroside (4):** \(^{13}\)C NMR: 95.13 (C-1), 155.14 (C-3), 109.32 (C-4), 31.82 (C-5), 41.24 (C-6), 173.18 (C-7), 124.91 (C-8), 130.49 (C-9), 13.58 (C-10), 168.64 (C-11), 51.90 (C-12), 66.90 (C-1'), 35.19 (C-2'), 130.0 (C-3'), 130.99 (C-4'), 116.27 (C-7'), 130.99 (C-8'), 100.80 (C-1''), 74.77 (C-2''), 78.45 (C-3''), 71.48 (C-4''), 77.95 (C-5''), 62.78 (C-6'').

**Oleuropeoside (5):** \(^1\)H NMR: 5.90 (H-1), 7.5 (H-3), 3.95 (H-5), 2.43 and 2.69 (H-6g and H-6h), 6.06 (H-8), 1.65 (H-10), 3.70 (H-12), 4.19 and 4.09 (H-1' and H-1''), 2.75 (H-2'), 6.65 (H-4'), 6.67 (H-7'), 6.65 (H-8'), 4.79 (H-1''), 3.35 (H-2''), 3.40 (H-3''), 3.35 (H-4''), 3.35 (H-5''), 3.87 and 3.66 (H-6''a and H-6''b); \(^{13}\)C NMR: 95.3 (C-1), 155.1 (C-3), 109.4 (C-4), 31.8 (C-5), 41.3 (C-6), 173.2 (C-7), 124.9 (C-8), 130.8 (C-9), 13.5 (C-10), 168.7 (C-11), 51.9 (C-12), 66.8 (C-1''), 35.4 (C-2''), 130.8 (C-3''), 116.5 (C-4''), 146.2 (C-5''), 144.9 (C-6''), 117.1 (C-7''), 121.3 (C-8''), 101.0 (C-1''), 74.8 (C-2''), 78.0 (C-3''), 71.5 (C-4''), 78.4 (C-5''), 62.8 (C-6'').

**Salidroside (6):** \(^1\)H NMR: 4.00 and 3.68 (H-1' and H-1''), 2.81 (H-2'), 7.04 (H-4'), 6.67 (H-5), 6.67 (H-7), 7.04 (H-8), 4.26 (H-1''), 3.15 (H-2''), 3.32 (H-3''), 3.26 (H-4''), 3.23 (H-5''), 3.83 and 3.64 (H-6''a and H-6''b); \(^{13}\)C NMR: 72.1 (C-1), 36.4 (C-2), 130.7 (C-3), 130.9 (C-4), 116.1 (C-5), 156.6 (C-6), 116.1 (C-7), 130.9 (C-8), 104.4 (1''), 75.1 (C-2''), 78.1 (C-3''), 71.7 (C-4''), 78.0 (C-5''), 62.8 (C-6'').

**Coniferin (7):** \(^1\)H NMR: 4.21 (H-1), 6.32 (H-2), 6.54 (H-3), 6.74 (H-5), 6.74 (H-9), 4.86 (H-1''), 3.45 (H-2''), 3.40 (H-3'), 3.40 (H-4'), 3.20 (H-5'), 3.77 and 3.66 (H-6''a and H-6''b); \(^{13}\)C NMR: 63.5 (C-1), 130.1 (C-2), 131.3 (C-3), 135.0 (C-4), 105.6 (C-5), 154.4 (C-6), 153.5 (C-7), 154.4 (C-8), 105.6 (C-5), 57.1 (OCH\(_3\)), 105.4 (C-1''), 75.8 (C-2''), 77.9 (C-3''), 71.4 (C-4''), 78.4 (C-5''), 62.7 (C-6'').

These compounds have been isolated for the first time from *Phillyrea latifolia*.

The Phillyrea genus comprises three species: *P. media*, *P. angustifolia* and *P. latifolia*. Among these only *P. media* has furnished oleuropeoside (Popov et al., 1975).

Considering the bioactivity of oleuropeoside, and olive leaves, it is important to take into account the fact that *Phillyrea latifolia* also possesses a high amount of oleuropeoside. The presence of this compound, increases the possible pharmacological value of this species, because we now report that it is a potential source of oleuropeoside.
Fig. 1. Structures of isolated compounds.
Acknowledgements

This work was realized with the financial support of the following projects: PICASSO (Acciones Integradas Hispano-Francésas, Refs. H-211 and 98-B), FISS (Ministerio de Sanidad y Con-

sumo, Ref. 94/1671). CAM (Comunidad Autónoma de Madrid, Ref. C101/91), Universidad de Alcalá (E006/2001), a grant from Consejería de Educación y Cultura de la Comunidad Autónoma de Madrid (Convocatoria 1998).

Batello M. and Fresu I. (1993), Le pianti di uso officina-le nella Barbagia di Seui (Sardegna Centrale). Fito-
terapia 2, 141–150.
Chiba M., Hisada S., Nishibe S and Thieme M. (1980), $^{13}$C NMR analysis of symlocosin and (+)-epipinore-
Damtoft S., Franzyk H. and Jensen S. R. (1992), Excelsi-
oside a secoiridoid glucoside from Fraxinus excelsior. Phytochemistry 31, 4197–4201.
Ghisalberti E. L. (1998), Biological and pharmacological activity of naturally occurring iridoids and secoiri-
doids. Phytomedicine 5, 147–163.
Hansel K., Andersen A., Brogger Christensen S., Rosen-
dal Jensen S., Nyman U. and Wagner Smitt U. (1996), Isolation of angiotensin converting enzyme (ACE) in-
hibitor from Olea europaea and Olea lancea. Phyto-
tomedicine 2, 319–325.
Inouye H. and Nishioka T. (1972), Monoterpene glucos-
sides and related natural products. XIX. Structure of nuezhenide, a bitter tasting D-glucoside from Lig-
ustrum lucidum and Ligustrum japonicum. Tetrahe-
dron 28, 4231–4237.

Maillard M., Adewunmi C. O. and Hostettman K. (1992), A triterpene glycoside from the fruits of Tetra-
Merzouki A., Ed-Derfoufi F., El-Aatila A. and Molero-
Mesa J. (1997), Wild medicinal plants used by local Bouhmed population (Morocco). Fitoterapia 5, 444–
460.
Trovato A., Forestieri A. M., Iauk L., Barbera R., Montforte M. T. and Galati M. E. (1993), Hypoglyce-
Visioli F., Bellomo G. and Galli C. (1998), Free radical-
scavenging properties of olive oil polyphenols. Bio-