SHORT REPORT

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Flavonoids and Terpenoids from *Phlomis cashmeriana* and their Chemotaxonomic significance

Javid Hussain[*1] Nausheen Bukhari[1], Hidayat Hussain[*2], Naseem Bano[1], Abdul Naeem[3] and Ivan R Green[4]

[1] *Department of Chemistry, Kohat University of Science and Technology N.W.F.P, Pakistan*

[2]*Department of Chemistry, Universität Paderborn, Warburger Straße 100, 33098 Paderborn, Germany*

[3] *National Centre of Excellence in Physical Chemistry, University Peshawar, Peshawar, Pakistan*

[4]*Department of Chemistry, University of the Western Cape, Bellville, 7530, South Africa*

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Abstract: The study of the chemical constituents of the whole plant of *Phlomis cashmeriana* (Lamiaceae) has resulted in the isolation and characterization of twelve compounds. These twelve compounds were identified as apigenin 7,4'-dimethyl ether (**1**), luteolin-7-methyl ether (**2**), bitalgenin (**3**), kaempferol 3-*O*-3'''-acetyl-α-L-arabinopyranosyl-(1'''-6''')-β-D-glucopyranoside (**4**), glutinol (**5**), oleanolic acid (**6**), *β*-amyrin (**7**), ursolic acid (**8**), 3-O-*p*-coumaroylshikimic acid (**9**), 3β-hydroxycycloart-24-one (**10**), and a mixture of *β*-sitosterol (**11**), and stigmasterol (**12**). The above compounds were individually identified by spectroscopic analyses and comparisons with reported data. The chemotaxonomic studies of isolated compounds have been discussed. This study constitutes the first phytochemical work on *P. cashmeriana*

Keywords: *Phlomis cashmeriana*; Lamiaceae; Natural Products; Chemotaxonomic studies.

1. Plant Source

The genus *Phlomis* L. (Lamiaceae) comprises more than 100 species distributed mainly in the Mediterranean region and eastwards to the western Himalayas [1]. *Phlomis cashmeriana* is among the

[*] Corresponding author: E- Mail:Hidayat110@gmail.com (H. Hussain), Phone +49-5251-602182 and E- Mail: javidhej@yahoo.com (J. Hussain)

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most distinguished Himalayan plants. It is found growing from Afghanistan to Kashmir, often in wastelands or on open slopes and blooms in the summer [2].

The whole plant of *P. cashmeriana* was collected at the Parachinar Kurram Agency, N.W.F.P Pakistan, in 2005, and identified by Dr. Jahandar Shah (plant taxonomist) at the Department of Botany, Islamia College, University of Peshawar. A voucher specimen has been deposited at the herbarium of the Botany Department, Kohat University of Science and Technology, Pakistan.

2. Previous Studies

There are no reports on phytochemical investigation of *P. cashmeriana*.

3. Present Study

The dried and powdered whole plant (8.5 kg) of *P. cashmeriana* were exhaustively extracted with MeOH at room temperature for two weeks and then filtered. The residue obtained (195 g) after the evaporation of MeOH was subjected to column chromatography on silica gel using *n*-hexane, *n*-hexane-EtOAc, EtOAc-MeOH and finally, pure MeOH as the mobile phase and yielded 10 fractions (F_{1-10}). Fraction no F_4 (5 g) yielded compounds apigenin 7,4'-dimethyl ether (**1**, 8 mg), luteolin-7-methyl ether (**2**), bitalgenin (**3**, 4.3 mg) by silica gel column chromatography [*n*-hexane-EtOAc (95:5 to 75:85)]. Similarly fractions F_6 (2 g) gave kaempferol 3-*O*-3'''-acetyl-α-L-arabinopyranosyl-(1'''-6''')-β-D-glucopyranoside (**4**, 40 mg) and 3-O-*p*-coumaroylshikimic acid (**9**, 9.5 mg) after column chromatography with *n*-hexane-EtOAc (85:15 to 70:30). Finally column chromatography of fraction F_5 (6 g) with with *n*-hexane-EtOAc (92:8 to 80:20) yielded glutinol (**5**, 26 mg), oleanolic acid (**6**, 10 mg), *β*-amyrin (**7**, 7 mg), ursolic acid (**8**, 8 mg), 3β-hydroxycycloart-24-one (**10**, 8 mg), and a mixture of *β*-sitosterol (**11**, 8 mg), and stigmasterol (**12**, 8 mg).

The whole plant extract of *P. cashmeriana* was fractionated by silica gel column chromatography to give several fractions, which were further chromatographed on silica gel to give four flavonoids (**1**-**4**), five triterpenes (**5-8,** and **10**), one shikimic acid derivative (**9**), and two steroids (**11** and **12**) for the first time from *P. cashmeriana* (Figure 1). These twelve compounds were identified as apigenin 7,4'-dimethyl ether (**1**) [3], luteolin-7-methyl ether (**2**) [4], bitalgenin (**3**) [5,6], kaempferol 3-*O*-3'''-acetyl-α-L-arabinopyranosyl-(1'''-6''')-β-D-glucopyranoside (**4**) [7], glutinol (**5**) [8], oleanolic acid (**6**) [9], *β*-amyrin (**7**) [10], ursolic acid (**8**) [11], 3-O-*p*-coumaroylshikimic acid (**9**) [12], 3β-hydroxycycloart-24-one (**10**) [13], and a mixture of *β*-sitosterol (**11**) [14], and stigmasterol (**12**) [14] by comparison of NMR and MS data with reported data.

Chemotaxonomic significance: Phlomis is a large genus in the Lamiaceae family, with over 100 species distributed throughout Euro-Asia and the North African countries [15]. Phytochemical investigations of the *Phlomis* species has been the subject of several studies, which resulted in the isolation of essential oils, flavonoids, iridoids, phenylethylalcohol glycosides and other components and with flavonoids being the major phyto-constituents, isolated from the *Phlomis* genus [15,16]. The present study reports on the isolation of four flavonoids (**1**-**4**), five triterpenes (**5-8,** and **10**), one shikimic acid derivative (**9**), and two steroids (**11** and **12**) for the first time from *P. cashmeriana* (Fig. 1). Since flavonoids are the major phyto-constituents isolated from the *Phlomis* genus it is no wonder that forty seven flavonoids have been isolated from *Phlomis sp* [15,16] to date. These include apigenin, luteolin, naringenin, eriodictyol, chryseriol, kaempferol, and their glycosides. This report describes for the first time the occurrence of flavonoids **1-4** in the genus *Phlomis* as well as for the title plant *P. cashmeriana*. Apigenin 7,4'-dimethyl ether (**1**) has been previously isolated from the genera *Teucrium*, *Dorystoechas*, *Salvia* [17], and *Ballota* [18] while luteolin-7-methyl ether (**2**) was reported to be isolated from the genera *Coridothymus*, *Thymbra* [19], *Isodon* [4], *Leonurus*, *Chaiturus*, and

Panzerina [19] of the family Lamiaceae. Similarly flavone **3** has been isolated from the genera *Scutellari*a [20], *Mentha* [21], and *Majorana* [22] of the family Lamiaceae.

On the other hand, compound **4** has earlier been reported to be found in *Thalictrum* [7], which belongs to the Ranunculaceae family and importantly, this compound is reported here for the first time to be isolated from the family Lamiaceae.

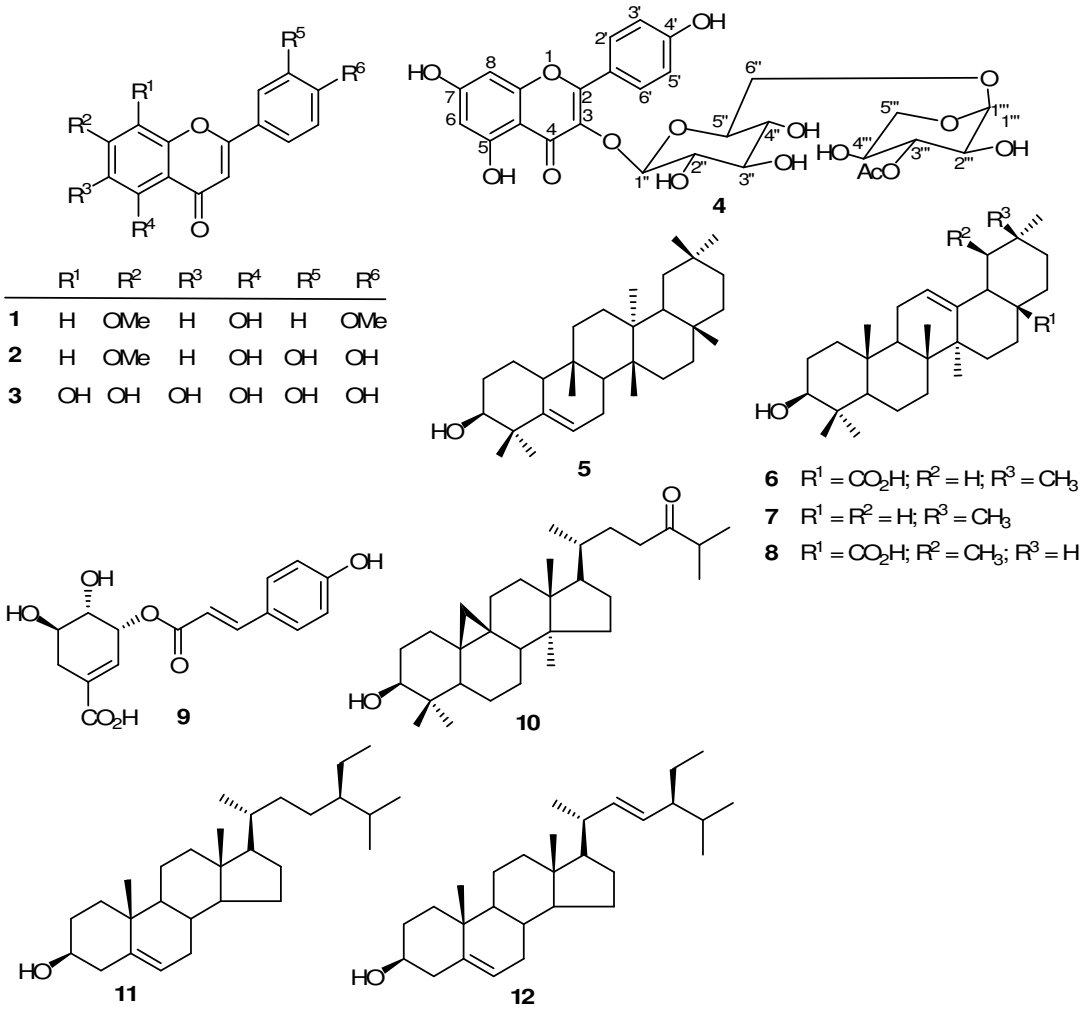

Figure 1. Compounds **1–12** isolated from *P. cashmeriana*.

Flavonoids have proved at a lower level to be chemotaxonomically useful, within families, genera and species. Table 1 illustrates a comparison for a wide range of flavonoids occurring in some species of *Phlomis*. These include apigenin, luteolin, naringenin, eriodictyol, chryseriol, kaempferol and their glycosides [15,16]. The majority of flavonoids identified in the *Phlomis* genus are flavones, flavonols and frequently as their 7- or 3-glycosides. Luteolin-7-*O*-β-glucopyranoside, chrysoeriol-7-p-coumaroylglucoside and chrysoeriol-7-glucoside constitute the most commonly glycosylated flavonoids found in the *Phlomis* genus [15,16]. *P. fruticosa* is however somewhat different since it has a greater variety of flavonoids (less common compounds) compared to other species of *Phlomis*.

Table 1. Occurrence of flavonoids in some species of *Phlomis*

Compound	**Source (Reference)**
Acacetin-7-O- β -glucopyranoside	*Phlomis aurea* [27]
Apigenin	*Phlomis lychnitis* [28]
	Phlomis samia [29]
Apigenin-7-glucoside	*Phlomis aurea* [30]
	Phlomis floccose [30]
	Phlomis lychnitis [28]
Apigenin-7-rutinoside	*Phlomis aurea* [30]
	Phlomis floccose [30]
Apigenin-7-p-coumaroylglucoside	*Phlomis aurea*[30]
	Phlomis floccose [30]
	Phlomis lychnitis [28]
Astragalin	*Phlomis spinidens*28]
Chryseriol	*Phlomis lychnitis* 28]
	Phlomis samia [29]
	Phlomis fruticosa [31]
Chryseriol-7-glucuronide	*Phlomis fruticosa* [31]
Chrysoeriol-7-glucoside	*Phlomis aurea* [30]
	Phlomis floccosa [30]
	Phlomis lychnitis[28]
	Phlomis fruticosa [31]
	Phlomis caucasica [32]
Chrysoeriol-7-rutinoside	*Phlomis aurea* [30]
	Phlomis floccose [30]
	Phlomis caucasica [32]
Chryseriol-7-rhamnosylglucoside	*Phlomis fruticosa* [31]
Chrysoeriol-7-p-coumaroylglucoside	*Phlomis aurea* [30]
	Phlomis floccose [30]
	Phlomis lychnitis [28]
Chrysoeriol-7-O- β -glucopyranoside	*Phlomis aurea* [27]
	Phlomis integrifolia [33]
	Phlomis lunariifolia [34]
	Phlomis brunneogaleata[34]
Eriodictyol	*Phlomis fruticosa* [31]
Ermanin	*Phlomis samia*[29]
Hesperetin	*Phlomis fruticosa* [31]
Hispidulin-7-glucoside	*Phlomis aurea* [30]
Isoquercitrin	*Phlomis spinidens* [35]
Isorhamnetin-3-p-coumaroylglucoside	*Phlomis purpurea* [36]
Kaempferol-3-glucosides	*Phlomis spectabilis*[37]
	Phlomis caucasica [32]
Kaempferol-3-p-coumaroylglucoside	*Phlomis purpurea* [36]
Kaempferol-3-(6″-(*E*)-p-coumaroyl)glucosides	*Phlomis spectabilis*[37]
Kaempferol (7,4′-dimethyl ether)-3-glucoside	*Phlomis spectabilis*[37]
Kaempferol (7,4′-dimethyl ether)-3-(6″-(E)-p-coumaroyl)glucosides	*Phlomis spectabilis* [37]
Kaempferol-3-O- β-D-glucopyranosyl-(1-6)- β -D-glucopyranoside	*Phlomis aurea* [38]

Table 1 (*Continued*)

Compound	Source (Reference)
Lucenin-2	*Phlomis aurea* [30]
	Phlomis floccose [30]
Luteolin	*Phlomis lychnitis*[28]
	Phlomis crinita [39]
Luteolin-7-glucoside	*Phlomis aurea* [30]
	Phlomis floccosa [36]
	Phlomis lychnitis [28]
	Phlomis purpurea [36]
	Phlomis fruticosa [31]
Luteolin-7-rutinoside	*Phlomis aurea* [31]
	Phlomis floccose [30]
Luteolin-7-diglucoside	*Phlomis aurea* [30]
	Phlomis floccose[30]
Luteolin-7-p-coumaroylglucoside	*Phlomis aurea* [30]
	Phlomis floccose [30]
	Phlomis lychnitis[28]
	Phlomis fruticosa [31]
Luteolin-7-glucuronide	*Phlomis fruticosa* [31]
Luteolin-7-rhamnosylglucoside	*Phlomis fruticosa* [31]
Luteolin-7-O-[4-O-acetyl-α-rhamnopyranosyl-(1→ 2)]-β-glucuronopyranoside	*Phlomis lunariifolia* [34]
Luteolin-7-O-β-glucopyranoside	*Phlomis aurea* [27]
	Phlomis lunariifolia [34]
	Phlomis brunneogaleata [40]
	Phlomis crinita [39]
	Phlomis tuberosa [41]
	Phlomis younghusbandii [42]
Naringenin	*Phlomis angustissima* [43]
	Phlomis fruticosa [31]
	Phlomis caucasica[32]
Naringenin-7-glucoside	*Phlomis aurea* [30]
Naringenin-7-p-coumaroylglucoside	*Phlomis aurea* [30]
Phlomisflavosides A	*Phlomis spinidens* [28]
Phlomisflavosides B	*Phlomis spinidens* [28]
Quercetin-3-O- β -D-glucopyranoside	*Phlomis aurea* [38]
Rutin	*Phlomis caucasica* [32]
Tricin-7-glucoside	*Phlomis fruticosa* [31]
Vicenin-2	*Phlomis aurea* [30]
	Phlomis floccose [30]
	Phlomis fruticosa [31]

Triterpenes glutinol (**5**) and *β*-amyrin (**7**) were characterized for the first time from the genus *Phlomis* but had previously been isolated from *Sideritis* of Lamiaceae family [23,24]. This study, in our view, can be used to establish an intertribal relationship between the two genera *Phlomis* and *Sideritis* of the Lamiaceae family. Oleanolic (**6**) and ursolic acid (**8**) have been reported from 88 taxa of the Lamiaceae family including the genus *Phlomis* but the plants belonging to the subfamily Nepetoideae produced significantly higher amounts of both these triterpenes than those in the subfamily Lamioideae (Janicsak et al., 2006). *β*-Sitosterol (**11**) has been reported to be isolated from *P. cashmeriana* [25] but this is the first report of the presence of 3-*O*-*p*-coumaroylshikimic acid (**9**) and 3*β*-hydroxycycloart-24-one (**10**) in the genus *Phlomi*s as well as in the Lamiaceae family. Interestingly, compound **9** has been isolated from the Thelypteridaceae family [12] and compound **10**

from Compositae [13] and Pinaceae [26]. The isolation and identification of twelve compounds from *P. cashmeriana* represents a most significant phytochemical analysis of the components of the plant and may be used as a foundation for further chemotaxonomic studies on the genus *Phlomis* and Lamiaceae family.

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