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*Research Article*

# *New Phenolics* from *Linum mucronatum* subsp. *orientale*

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**Abstract**

***Introduction:*** Lignans and flavonoids are widely distributed phenolics in the plant kingdom. Aryltetralin type lignans (podophyllotoxin derivatives) as the major secondary metabolites of *Linum* species play an important role in the production of chemotherapy drugs. In the present study, lignans and flavonoid glycosides from aerial parts of *Linum mucronatum* subsp. *orientale* were isolated and identified.

***Methods:*** The phytochemical investigation has been carried out on Hexane, DCM and MeOH extracts of the plant. Separation of chemical constituents was done using different chromatography (CC, prep-TLC, GC/MS and HPLC) methods. The major compounds of dichloromethane (DCM) and methanol extracts were isolated and their structures were elucidated using co-chromatography in the presence of known lignans, HPLC and NMR techniques.

***Results:*** Our results showed that podophyllotoxin and *6-MeO-α- peltatin*, as new compound, are the major lignans of the DCM extract of *L. mucronatum* subsp. o*rientale*. Two new flavonoid glycosides were also elucidated in the methanolic extract.

***Conclusion:*** The DCM and methanol extracts of *L. mucronatum* were found to contain aryltetralin-type lignans and flavonoids. The occurrence of *6-MeO-α- peltatin* and flavonoids in *L. mucronatum* has been reported for the first time.

## Introduction

Lignans are considered as a class of secondary plant metabolites, which are produced by oxidative dimerization of two phenylpropanoid units.[1] In recent years, lignans have attracted a growing attention because of their numerous pharmacological activities, mainly as strong antineoplastic and antiviral agents.[2,3]

During the last few decades, lignans especially podophyllotoxin derivatives have been the objective of various studies engaged in production of new anticancer drugs. Moreover, semisynthetic derivatives of podophyllotoxin—etoposide, teniposide and etophos—are currently used in chemotherapy of various types of cancer. *Podophyllum* species are the main source of podophyllotoxin, for which there is a high demand in international market. However, it should be considered that these species are endangered and their frequency in nature has declined considerably due to unscheduled and unorganized collecting.[4-6] Therefore, we should seek other alternative sources for podophyllotoxin production.

*Linum* (Linaceae) comprises of 230 species which are widely distributed throughout the world. The genus is divided into the sections *Syllinum*, *Cathartolinum*, *Dasylinum*, *Linum* and *Linastrum* based on their morphological characteristics.[7] The sections of *Syllinum* and *Cathartolinum* produce a wide spectrum of aryltetralin lactone type lignans among which 6-methoxy podophyllotoxin, its glycosides and ester derivatives are most abundant.[8,9] Furthermore, it has been reported that *Linum* species such as *L. austriacum, L. narbonense*, *L. leonii*, *L. glaucum, L. album, L. flavum* and *L. mucronatum* subsp. m*ucronatum* produce different lignans in their tissue and cell cultures.[7,8,10-13] *L. mucronatum* subsp. *orientale* belongs to the section *Syllinum* and grows in Northwest of Iran as an endemic species. Erect or divergent flowering stems, yellow flowers, linear very acute and glabrus leaves, erect pedicels in fruit and broadly ovoid capsule are the main morphological characteristics of the plant.[14]

In the present study, we describe the isolation and the structural determination of a new lignin, *6-MeO-α-peltatin* and some other known compounds from the aerial parts of *L. mucronatum* subsp. *orientale,* as well.

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## Materials and methods

### *Materials*

All solvents were of analytical grade and purchased from Sharlau or Caledon Company. Other reagents and materials were provided by Merck (Merck Co., Germany).

### *General experimental procedures*

NMR spectra were recorded in Methanol-$d_4$ or Chloroform-d solvents on a Buker Avance 400 MHz spectrometer (400 MHz for $^1$H and 100 MHz for $^{13}$C). Gas chromatography-mass spectrometry analyses (GC/MS) were performed on a Shimadzu GC-MS-QP 5050A gas chromatograph fitted with a DB-1 (polydimethylsiloxane, 60 m × 0.25 mm i.d.) capillary column. Carrier gas, helium with a column flow rate of 0.9 mL/min, total flow: 8.4 mL/min; injector temperature, 280 ˚C interface temperature, 310 ˚C; injection volume: 0.1 µL of extract in n-hexane (2%); split ratio, 1:5; oven temperature program: 50 ˚C (keep time: 2 min) to 305 ˚C (keep time: 10 min) with rate of 10 ˚C/min, solvent cut time: 5 min were used in the experiment. A Shimadzu LC-10A prep-HPLC coupled with a SPD-M20A detector (190-500 nm) and a prep-C18 column (CLC Shim-pack C18 column, 22 × 250 mm, 15µm) was used for separations. Column chromatography was conducted with silica gel 60 F254 (mesh; 0.063-0.200 mm) (Merck No: 1.10757.1000). Analytical and preparative thin layer chromatography (TLC) was performed on pre coated silica gel 60 $F_{254}$ (0.25 mm and 2 mm, respectively) plates.

### *Plant material*

Aerial parts of *L. mucronatum* BERTOL. subsp. *orientale* were collected in June 2012 during the flowering period (Badlou-Miyaneh, Kaghazkonan Protected Area, Iran). A voucher specimen (Tbz-FPh-735) has been deposited in the Herbarium of the Tabriz University of Medicinal Sciences, Tabriz, Iran.

### *Extraction and isolation*

The air-dried and grounded samples of *L. mucronatum* (100 g) were Soxhlet-extracted with n-hexane-DCM and methanol (1:1 L), respectively. The extracts were concentrated using a rotary evaporator under vacuum and a maximum temperature of 40 ˚C.

The DCM extract (0.9 g) was loaded on a column of silica gel (5×90 cm) and elution was done by gradient mixtures of chloroform in n-hexane (0→100). The eluted fractions (136 fraction; 10 ml each) were monitored by TLC on silica gel under the UV light (254 nm) before and after applying 70% $H_2SO_4$ in ethanol as spraying reagent.[15] Lignans produced purple spots with $H_2SO_4$ reagent following heating in 120 ˚C for 5 min. The similar fractions were combined together and finally 11 sub-fractions were yielded. Lignan-containing sub-fractions were further purified using preparative TLC by double running in methanol-chloroform (10:90 v/v) and toluene-acetone (65:35 v/v), respectively. The isolated materials were extracted from silica gel and purified by crystallization/re-crystallization procedure. This procedure afforded two off-white lignans: *I* (1.8 mg) and *II* (2.3 mg). The structure of compounds *I* and *II* were elucidated using NMR spectroscopy and co-chromatography with known lignans on silica gel plate. Isolation and identification of compound *III* from mother liquor of crystallization was done using GC/MS technique. On the other hand, the volatile compounds of less polar sub fractions were analyzed by GC/MS technique.

A portion of the MeOH extract (2 g) was subjected to Sep-Pak fractionation using a step gradient of MeOH-water mixtures (10:90, 20:80, 40:60, 60:40, 80:20 and 100:0). Analysis of the 20% methanolic Sep-Pak fraction (234 mg) by preparative HPLC (Shim-Pak ODS column, 22×250 mm, 15 µ; mobile phase: 0-50 min, MeOH from 10% to 30% in water; 50-62 min, 30% MeOH in water; 62-64 min, MeOH from 30% to 10%; flow rate 20 mL/min; detection at 220, 280 and 350 nm) afforded compound *IV* (18 mg). A similar analysis of the 40% methanolic Sep-Pak fraction (312 mg) (mobile phase: 0-50 min, MeOH from 30% to 50% in water; 50-62 min, 50% MeOH in water; 62-64 min, from 50% to 30%; flow rate 20 mL/min) yielded compound *V* (32 mg).

## Results

### *Structural characterization*

The DCM extract of *L. mucronatum* aerial parts was fractioned as described previously. The lignan-containing fraction was separated by a combination of CC and preparative TLC and a novel aryltetralin lignan (compound *I*) together with podophyllotoxin (compound *II*) were yielded. Some known alkanes were also isolated from the mother liquors of the crystallization along with the compound *III* (Table 1).

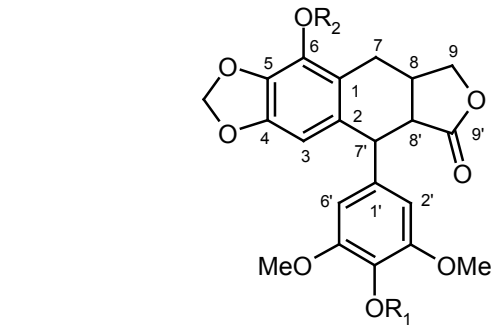
**Table 1.** Composition of the alkans of *L. mucronatum*

| N0. | Compound | Rt | MW | Formula | Composition (%) |
|---|---|---|---|---|---|
| 1 | Dodecane | 13.9 | 170 | C12H26 | 0.42 |
| 2 | 9-Dodecen-1-ol acetate | 26.0 | 226 | C14H26O2 | 94.2 |
| 3 | Z-9- Dodecenylacetat | 27.3 | 226 | C14H26O2 | 0.42 |
| 4 | Tetradecane | 27.5 | 198 | C14H30 | 0.42 |
| 5 | n-Octyl-ether | 27.9 | 242 | C16H34O | 1.07 |
| 6 | Benzyl alcohol | 28.0 | 108 | C7H8O | 0.42 |
| 7 | Pentadecane | 28.6 | 212 | C15H32 | 0.42 |
| 8 | 10, 12-Octadecadiyonic acid | 34.1 | 276 | C18H28O2 | 1.49 |
| 9 | Octadecanal | - | 268 | C18H36O | 0.21 |

Compound *I* (*6-MeO-α- peltatin)*, an off-white powder together with a minor impurity of 3-dodecylcyclohexanone *III*, was separated and purified by repeated crystallization (Fig. 1). Its structure was determined by $^{1}H$, $^{13}C$-NMR spectroscopy. The $^{1}H$- NMR of compound *I* was very similar to spectroscopic data of β-peltatin with minor differences, especially in chemical shifts of H-3 and H-7 signals (Table 2).[16] Obviously, the observed differences could be due to methoxy group which attached to C-6 position. The $^{13}C$-NMR data of compound *I* is shown in Table 3.

Podophyllotoxin was identified by direct comparison with authentic sample on TLC, according to Wagner *et al*, 1996.[15] The aryltetralin lignans were isolated from various *Linum* species such as *L. alba*, *L. flavum* and *L. capitatum*.[16] However, phytochemical studies are continuing on this section. This is the first report on occurrence of *6- MeO-α-peltatin*, in the aerial parts of *Linum* species.

After fractionation of methanol extract on a SPE (C18) column, preliminary analyses of the yielded fractions with HPLC and on-line UV spectra (diode array detection: range of 220-500 nm.) revealed the presence of several flavones derivatives. The prep-HPLC of fractions




***6-MeO-α- peltatin (I)*** $R_1$=H , $R_2$=Me

***α- peltatin*** $R_1$ , $R_2$ = H

***β- peltatin*** $R_1$= Me , $R_2$= H

*III*

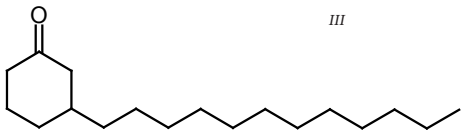


**Fig. 1.** Structure of *6-MeO-α- Peltatin* and dodecylcyclohexanone isolated from *L. mucronatum* DCM extract

**Table 2.** $^{1}H$ NMR data of 6-methoxy alpha-peltatin, alpha-peltatin and beta-peltatin in CDCl3, chemical shifts and coupling constans *J* are given in ppm and Hz respectively.

| H | *6-MeO-α- peltatin* [a] | *α- peltatin* [c] | *β- peltatin* [c] |
|---|---|---|---|
| 3 | 6.51, s | 6.25 | 6.24 |
| 2' | 6.37 | 6.37 | 6.36 |
| 6' | 6.37 | 6.37 | 6.36 |
| 7 | 2.35, br. m[b] | 3.20 m | 3.22 m |
| 8 | 2.35, br. m[b] | 2.7 m | 2.7m |
| 9 | 4.41, br. *dd*[b]; 3.71, br. *dd*[b] | 4.48, ca. *dd*; 3.95. *dd* | 4.48, ca. *dd*; 3.96. *dd* |
| 7' | 4.60, br. *d* | 4.60. d (3.7) | 4.61. *d* (3.7) |
| 8' | n.s. | | |
| $OCH_2O$ | 5.98, *d* ( 1.5); 6.09, *d* (1.5) | 5.94 , *d* (1.4); 5.96 , *d* (1.4) | 5.94 , *d* (1.4); 5.95 , *d* (1.4) |
| 3',5'-OMe | 3.76, s | 3.79, s | 3.76, s |
| 4'- OMe | - | - | 3.81, s |
| 6-OMe | 3.81 | - | - |

[a] spectra recorded in chloroform- d at 400 MHz; [b] overlapping signal; n.s.; not seen.

[c] Data extractedfrom this source.[16]

**Table 3.** $^{13}C$-NMR data of 6-methoxy alpha-peltatin, in CDCl3.

| *C* | *ppm* | *ppm* | *C* |
|---|---|---|---|
| 1 | 117.4 | 134.9 | 1’ |
| 2 | 142.1 | 106.8 | 2’ |
| 3 | 103.8 | 149.3 | 3’ |
| 4 | 150.1 | 130.5 | 4’ |
| 5 | 138.9 | 149.3 | 5’ |
| 6 | 143.3 | 106.8 | 6’ |
| 7 | 25.7 | 45.7* | 7’ |
| 8 | 36.8 | 45.9* | 8’ |
| 9 | 71.2 | 173.2 | 9’ |
| $OCH_2O$ | 101.1 | | |
| 3’’- OMe | 56.3 | | |
| 5’-OMe | 56.3 | | |
| 6-OMe | 61.1 | | |

*The signals may be interchangeable.

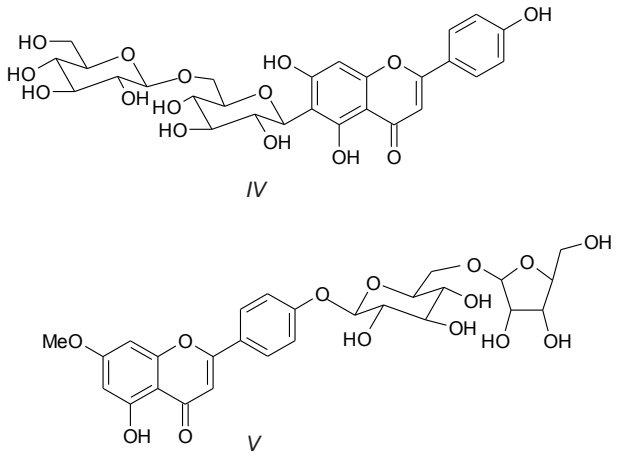



**Fig. 2.** Structure of two flavonoid glycosides from *L. mucronatum*

afforded 2 major compounds *IV* and *V*, which were fully characterized by spectroscopic techniques (Fig. 2). The UV spectrum of IV( λmax: 270 and 336 nm) was resemble to that of apigenin and was identified as 6-C-[β-D-glucuronopyranosyl (1→6)-O-β-D-glucuronopyranoside] apigenin (*Mucronatoside1*) by comparison of its $^{1}$H and $^{13}$C- NMR spectra (Table 4) with previously reported literature data.[17]

The UV spectra of *V* and *IV* were very similar to each other, but their NMR data was different. The $^{1}$H- NMR spectrum of *V* had two doublets at δ6.50, 1.8 Hz (H6) and δ6.88, 1.8Hz (H8) typical of a 5,7 disubstituted flavonoid moiety. The $^{13}$C- NMR spectrum (Table 4) was in agreement with the structure of the 7-methyl apigenin, and the connection of glycosyl moiety to position C4' and the interglycosidic (l→6) linkage was confirmed, as well. On the other hand, presence of the anomeric proton and

**Table 4.** $^{1}$H and $^{13}$C- NMR data of the flavonoid glycosides *IV* and *V* in DMSO-$d_6$. Coupling constant (*J* ) in Hz in parentheses and Chemical shift (δ) in ppm.

| | ***IV*** | | ***V*** | |
|---|---|---|---|---|
| Position | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 2 | - | 164.16 | - | 163.96 |
| 3 | 6.91s | 101.15 | 6.92s | 104.84 |
| 4 | - | 182.09 | - | 182.09 |
| 5 | - | 159.31 | - | 156.43 |
| 6 | - | 104.84 | 6.50d (1.8) | 100.82 |
| 7 | - | 162.44 | - | 164.15 |
| 8 | 6.88s | 93.70 | 6.88d (1.8) | 93.69 |
| 9 | | 156.43 | | 159.31 |
| 10 | | 103.23 | | 107.46 |
| 1′ | | 120.88 | | 120.91 |
| 2′ | 7.97d (8.8) | 128.16 | 7.98 d (8.7) | 128.68 |
| 3′ | 6.95d (8.8) | 116.01 | 6.95 d (8.7) | 115.99 |
| 4′ | | 161.14 | | 162.441 |
| 5′ | 6.95d (8.8) | 116.01 | 6.95 d (8.7) | 115.99 |
| 6′ | 7.97d (8.8) | 128.16 | 7.98 d (8.7) | 128.61 |
| OCH3 | | | 3.68s | 56.02 |
| 1″ | 4.65d (9.6) | 73.76 | 4.98 d (7.2) | 101.15 |
| 2″ | | 69.52 | | 73.58 |
| 3″ | | 78.88 | | 77.19 |
| 4″ | | 70.90 | | 70.93 |
| 5″ | | 80.07 | | 76.83 |
| 6″ | | 70.82 | | 69.53 |
| 1‴ | 4.97 d(7.2) | 103.16 | 5.21 d (4.3) | 107.46 |
| 2‴ | | 75.72 | | 73.76 |
| 3‴ | | 77.10 | | 72.62 |
| 4‴ | | 72.62 | | 80.90 |
| 5‴ | | 77.15 | | 60.62 |
| 6‴ | | 60.23 | | |

carbon signals at δ 5.21 (d 4.3 Hz) and 103.87, respectively, confirmed the arabinose structure for the second sugar. Thus the structure of *V* is as 7-O-methyl[-4'-O-β-D-arabinofuranosyl (1→6)-O-β-D-glucuronopyranoside] apigenin (*Mucronatoside 2*). According to the published data, the 5, 4'-dihydroxy-7-methoxyflavone (genkwanin) derivatives were previously found in some genera of the families *Aspleniaceae, Asteraceae, Cistaceae, Eupomataceae, Lamiaceae, Myrtaceae, Pteridaceae, Saxifragaceae* and *Betulaceae*.[18,19] However, there have been so far no reports on the occurrence of compound *V* in any *Linum* species.

## Discussion

In the present study *6-MeO-α- peltatin*, as a new compound, along with a known lignan podophyllotoxin were isolated from *Linum mucronatum* subsp. *orientale* (Fig. 1). In fact, the isolation of *6-MeO-α- peltatin* has never been reported till now. The isolation of the podophyllotoxin and a number of other aryltetralin lignans from this species is in fair agreement with previous results reporting 5-methoxypodophyllotoxin, podophyllotoxin, α-peltatin, and β-peltatin in *Linum* species, especially those produced by section Syllinum including *L. flavum* and *L. album*.[16]

Many lignans and neolignans have served as lead compounds for the development of new anticancer and antivirus drugs. Perhaps the best known example is podophyllotoxin, an antimitotic compound that binds to tubulin. Traditionally, podophyllotoxin serves as a starting material for semi-synthesis of etoposide, teniposide and other podophyllotoxin derivatives/ analogs, under development as potential anticancer drugs. Its antitumoural activity is due to inhibition of topoisomerase II.[6] At present, the commercial source for podophyllotoxin is *Podophyllum* species which are growing in North America, India, Pakistan and some parts of Afghanistan and Tibet.[1] Investigations have revealed that the genus *Linum* could be a new and reliable source for podophyllotoxin.[2,6-9,14] Aryltetralins are widely distributed in the genus *Linum* and could be used as chemotaxonomic markers. Moreover, the podophyllotoxin and its two known analog lignans, α-peltatin and β-peltatin, were reported previously from different species of *Linum*. Podophyllotoxin analogs were reported to induce cancer cell death with signs of apoptosis. For example, GL-331, a C7-modified 4'-demethyl epipodophyllotoxin, could increase cellular protein tyrosine phosphatase (PTP) activity significantly and in addition, GL-331- induced inter nucleosomal cleavage was efficiently prevented by two PTP inhibitors. Other pathways, such as p53-dependent pathway and Bax- dependent pathway might also be involved in the apoptosis induced by podophyllotoxin analogs. Furthermore, phytochemical investigation of the aerial parts of *L. mucronatum* afforded two new flavon glycosides and some known hydrocarbons together with lignans. Although a large number of flavonoids including anthocyanosides and apigenin-C-glycosides from flax species have been reported,[20-24] it seems that this is the first report on the presence of compounds *IV* and *V* in the genus *Linum*.

## Conclusion

The present study deduces that *L. mucronatum* is a good source of lignans, especially podophyllotoxin and a novel aryltetralin lignan, *6-MeO-α- Peltatin.* An efficient column and preparative TLC chromatography method with a two-phase solvent system was developed for the isolation of these compounds. The structure of *6-MeO-α- Peltatin* was determined by NMR analysis. Furthermore, two apigenin glycosides were reported as new compounds from *L. mucronatum*. Obviously, more studies should be carried out to elucidate the relationship between the structural features of the identified compounds and their bioactivity.

## Ethical issues

Authors declare ethical issues are not applicable in the present study.

## Competing interests

Authors declare no conflict of interests.

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