**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. orientale. 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 etopos—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.⁴,⁵ 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.⁶ 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.⁶,⁷⁸ Furthermore, it has been reported that *Linum* species such as *L. austriacum*, *L. narbonense*, *L. leontii*, *L. glaucum*, *L. album*, *L. flavum* and *L. mucronatum* subsp. *mucronatum* produce different lignans in their tissue and cell cultures.⁷,⁸,10-11 *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 glabrous leaves, erect pedicels in fruit and broadly ovoid capsule are the main morphological characteristics of the plant.¹¹ 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 F254 (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$_2$SO$_4$ in ethanol as spraying reagent. Lignans produced purple spots with H$_2$SO$_4$ 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, MeOH from 30% 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 alkanes of L. mucronatum

| NO. | 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   | Octadecanol               | -   | 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 1H, 13C-NMR spectroscopy. The 1H- 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). Obviously, the observed differences could be due to methoxy group which attached to C-6 position. The 13C-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. The aryltetralin lignans were isolated from various Linum species such as L. alba, L. flavum and L. capitatum. 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

![Structure of 6-MeO-α-Peltatin and dodecylcyclohexanone isolated from L. mucronatum DCM extract](image)

**Table 2.** 1H 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 | α-peltatin | β-peltatin |
|-------|------------------|------------|------------|
| 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      | 3.20 m     | 3.22 m     |
| 8     | 2.35, br. m      | 2.7 m      | 2.7m       |
| 9     | 4.41, br. dd; 3.71, br. dd | 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.             |            |            |
| OCH₂O | 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          | -          | -          |

* Data recorded in chloroform- d at 400 MHz; * overlapping signal; n.s.; not seen.

* Data extracted from this source.

**Table 3.** 13C-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'  |
| OCH₂O | 101.1   |         |     |
| 3',5'-OMe | 56.3 | 56.3 |     |
| 6-OMe | 61.1    |         |     |

* The signals may be interchangeable.
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 1H and 13C- 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 1H- 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 13C- 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 (1→6) linkage was confirmed, as well. On the other hand, presence of the anomeric proton and

**Table 4.** 1H and 13C- NMR data of the flavonoid glycosides IV and V in DMSO-d6. Coupling constant (J) in Hz in parentheses and Chemical shift (δ) in ppm.

|   | IV   | V    |
|---|------|------|
| 2 | -    | 164.16 |
| 3 | 6.91s| 101.15 |
| 4 | -    | 182.09 |
| 5 | -    | 159.31 |
| 6 | -    | 104.84 |
| 7 | -    | 162.44 |
| 8 | 6.88s| 93.70 |
| 9 | -    | 156.43 |
| 10| -    | 103.23 |
| 1’| -    | 120.88 |
| 2’| 7.97d (8.8)| 128.16 |
| 3’| 6.95d (8.8)| 116.01 |
| 4’| -    | 161.14 |
| 5’| 6.95d (8.8)| 116.01 |
| 6’| 7.97d (8.8)| 128.16 |
| OCH3| - | 3.68s | 56.02 |
| 1”| 4.65d (9.6)| 73.76 |
| 2”| -    | 69.52 |
| 3”| -    | 78.88 |
| 4”| -    | 70.90 |
| 5”| -    | 80.07 |
| 6”| -    | 70.82 |
| 1’’| -    | 103.16 |
| 2’’| 4.97d (7.2)| 101.15 |
| 3’’| -    | 73.73 |
| 4’’| -    | 73.85 |
| 5’’| -    | 77.19 |
| 6’’| -    | 70.93 |

On the other hand, presence of the anomeric proton and
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 (Mucronatose 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, Lamiastrum, Myrtaceae, Pteridaceae, Saxifragaceae and Betulaceae. 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 Syllinium including *L. flavum* and *L. album*. 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 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, it seems that this is the first report on the presence of compounds IV and V in the genus *Linum*.

**Ethical issues**

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

**Competing interests**

Authors declare no conflict of interests.

**References**

1. Petersen M, Alfermann AW. The production of cytotoxic lignans by plant cell cultures. Appl Microbiol Biotechnol 2001;55: 135-42.
2. Schmidt TJ, Hemmati S, Klaes M, Konuklugil B, Mogaheghzadeh A, Ionkova I, et al. Lignans in flowering aerial parts of *Linum* species--chemodiversity in the light of systematics and phylogeny. Phytochemistry 2010;71: 1714-28. doi: 10.1016/j.phytochem.2010.06.015
3. Zanwar AA, Hegde MV, Bodhankar SL. Cardioprotective activity of flax lignan concentrate extracted from seeds of *Linum usitatissimum* in isoprenalinduced myocardial necrosis in rats. Interdiscip Toxicol 2011;4: 90-7. doi: 10.2478/v10102-011-0016-8
4. Botta B, Delle Monache G, Misiti D, Vitali A, Zappia G. Arylketinal Lignans: Chemistry, Pharmacology and Biotransformations. Current Medicinal Chemistry 2001;8: 1363.
5. Chaurasia O, Baleb B, Tayade A, Kumar R, Phani Kumar G, Singh S. Podophyllum L.: an endangered and anticancerous medicinal plant- an overview. Indian Journal of Traditional Knowledge 2012;11: 234-41.
6. Gordaliza M, Castro MA, Miguel Del Corral JM, San Feliciano A. Antitumor Properties of Podophyllotoxin and Related Compounds. Curr Pharm Des 2000;6: 1811-6.
7. Mogaheghzadeh A, Dehshahri S, Hemmati S. Accumulation of lignans by in vitro cultures of three *Linum* species. Z Naturforsch C 2009;64: 73-6.
8. Hemmati S, Von Heimendahl CB, Klaes M, Alfermann AW, Schmidt TJ, Fuss E. Pinoresinol-lariciresinol reductases with opposite enantiospecificity determine the enantiomeric composition of lignans in the different organs of *Linum usitatissimum*. Planta Med 2010;76: 928-34. doi: 10.1055/s-0030-1250036
9. Vasilev N, Ebel R, Edrada R, Fuss E, Alfermann AW, Ionkova I, et al. Metabolic profiling of lignan variability in *Linum* species of section Syllinium native to Bulgaria. Planta Med 2008;74: 273-80. doi: 10.1055/s-2008-1034298
10. Konuklugil B, Ionkova I, Vasilev N, Schmidt TJ, Windhovel J, Fuss E, et al. Lignans from Linum species of sections Syllinum and Linum. Nat Prod Res 2007;21: 1-6. doi: 10.1080/1478640600798385
11. Mohagheghzadeh A, Schmidt TJ, Alfermann AW. Arylnaphthalene lignans from in vitro cultures of Linum austriacum. J Nat Prod 2002;65: 69-71.
12. Vasilev N, Ionkova I. Lignan accumulation in cell cultures of Linum strictum ssp. strictum L. Acta Pharm 2004;54: 347-51.
13. Mohagheghzadeh A, Gholami A, Soltani M, Hemmati S, Alfermann AW. Linum mucronatum: organ to organ lignan variations. Z Naturforsch C 2005;60: 508-10.
14. Rechinger K. Flora Iranica. Verlagsanstalt, Graz-Austria: Akademische Druck; 1974.
15. Wagner HH, Sabine B. Plant Drug Analysis: A Thin Layer Chromatography Atlas. Berlin, Heidelberg: Springer, Verlag; 1996.
16. Broomhead AJ, Dewick PM. Aryltetralin lignans from Linum flavum and Linum capticum. Phytochemistry 1990;29: 3839-44.
17. Agrawal PK. Carbon-13 NMR of Flavonoids (Studies in Inorganic Chemistry). B.V. Amsteram: Elsevier Science Publishers; 1989.
18. Bosabalidis A, Gabrieli C, Niopas I. Flavone aglycones in glandular hairs of linum x intercedens. Phytochemistry 1998;49: 1549-53.
19. O’rourke C, Byres M, Delazer A, Kumarasamy Y, Nahar L, Stewart F, et al. Hirsutanonol, oregonin and genkwanin from the seeds of Alnus glutinosa (Betulaceae). Biochem Syst Ecol 2005;33: 749-52.
20. Anwar F, Przybylski R. Effect of solvents extraction on total phenolics and antioxidant activity of extracts from flaxseed (Linum usitatissimum L.). Acta Sci Pol Technol Aliment 2012;11: 293-301.
21. Ibbahim RK, Shaw M. Phenolic constituents of the oil flax (Linum usitatissimum). Phytochemistry 1970;9: 1855-8.
22. Dave OB, Mazza G, Kenaschuk EO. Flavonoid content of flaxseed. Influence of cultivar and environment. Euphytica 1996;90: 163-7.
23. Ilić S, Konstantinović S, Todorović Z. Flavonoids from flower of Linum capitatum Kit. Facta Universitatis 2004;3: 67-71.
24. Ilić S, Konstantinović S, Todorović Z. Antimicrobial activity of bioactive component from flower of Linum capitatum Kit. Facta Universitatis 2004;3: 73-7.