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Original article

TWO MAJOR FLAVONOIDS FROM THE FRUITS OF *VITEX AGNUS-CASTUS* L.

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Abstract

From the ethanolic extract of the fruits of Vitex agnus-castus L. (Verbenaceae), two main flavonoid constituents, casticin and artemetin, were isolated by means of chromatographic methods. The structure elucidation of the isolated compounds was established on the basis of spectroscopic methods (UV, IR, EIMS, 1D and 2D NMR). This is the first report for the presence of casticin and artemetin in V. agnus-castus growing in Turkey.

Key words: *Vitex agnus-castus, Verbenaceae, Flavonoid, Casticin, Artemetin*

Vitex agnus-castus L. Meyvalarından Major İki Flavonoit

Vitex agnus-castus L. (Verbenaceae) meyvalarının etanollü ekstresinden kastisin ve artemetin olmak üzere iki tane flavonoit kromatografik metotlarla izole edilmiştir. İzole edilen bileşiklerin yapı tayinleri spektral metotlara (UV, IR, EIMS, 1D ve 2D NMR) dayanarak aydınlatılmıştır. Türkiye'de yetişen V. agnus-castus'ta kastisin ve artemetin'in varlığı ilk defa bu çalışma ile bildirilmektedir.

Anahtar kelimeler: *Vitex agnus-castus, Verbenaceae, Flavonoit,Kastisin, Artemetin*

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INTRODUCTION

Vitex agnus-castus L. (Verbenaceae) is a small shrubby tree, widely distributed in the Mediterranean coastal area, Middle East and the South of Europe. This plant is also widely distributed along with Anatolian coastal region (1,2). It is also known by the common names as "chasteberry" and "chaste tree" and called as "hayıt, acı ayıt, ayıd, hayıd and beşparmak otu" in Turkey (3,4). The genus *Vitex* L. is represented by two species in the Flora of Turkey and East Aegean Islands, namely *Vitex agnus-castus* L. and *Vitex pseudo-negundo* (Haussk. ex Bornm.) Hand.-Mazz. (2).

The extracts of the fruits of *V. agnus-castus* are principally used as a botanical dietary supplement for the management of female gynecological disorders including corpus luteum insufficiency, premenstrual syndrome and several menstrual problems (4,5). In Turkish folk medicine, the infusion of the fruits has been used as diuretic, carminative and sedative (6). In addition, during field expeditions on Turkish folk medicine, seeds and sprouts of *V. agnus-castus* have also been recorded for stomachache as internally (7).

Previous phytochemical studies revealed the presence of flavonoids (8-13), iridoids (9,14,15), diterpenoids (16,17), essential and fatty oils (18-20), and ectysteroids (14) in the fruits, flowers and leaves of *V. agnus-castus*. In the previous studies on the leaves, fruits and seeds of *V. agnus-castus*, some flavonol and flavone derivatives (apigenin, artemetin, casticin, penduletin, eupatorin, vitexin, isovitexin, orientin, isoorientin and luteolin-7-*O*-glucoside) have been isolated (8-10,12,13). Hirobe et al. (11) reported that four new flavonoids, luteolin-6-*C*-(4"-methyl-6"-*O*-*trans*-caffeoylglucoside), luteolin-6-*C*-(6"-*O*-*trans*-caffeoylglucoside), luteolin-6-*C*-(2"-*O*-*trans*-caffeoylglucoside) and luteolin-7-*O*-(6"-*p*-benzoylglucoside) together with four known components, 4',5-dihydroxy-3,3',6,7-tetramethoxyflavone, luteolin, artemetin and isorhamnetin have isolated from the root bark of *V. agnus-castus*. In addition, Şarer and Gökbulut (21) reported that phenolic acids, caffeic and chlorogenic acid, content in the leaves and fruits of *V. agnus-castus* collected from Turkey have determined by HPLC.

In this study, the isolation and fully structural elucidation of the major flavonoids, casticin and artemetin, from the fruits of *V. agnus-castus* growing in Turkey were presented.

EXPERIMENTAL

General

The UV (MeOH) spectra were recorded on a Shimadzu UV-160 A spectrophotometer. The IR spectra were taken in KBr pellet on a BRUKER Vector 22 FT-IR Spectrophotometer. The ^{1}H-, ^{13}C-NMR, HMQC and HMBC spectra were recorded on a JEOL JNM-Alpha 500 FT-NMR Spectrometer (500 MHz for ^{1}H- and 125 MHz for ^{13}C-NMR) in CD_3OD for **1** and **2**, respectively. Tetramethylsilane (TMS) was used as an internal standard and chemical shifts were given as δ (ppm). The coupling constants (*J*) were reported as Hz. The Electron Impact Mass Spectrometry (EIMS) was measured on a HITACHI M-2500 using a 70 eV electron impact ion source. Column chromatography (CC) was performed using Silica gel (Kieselgel 60, 0.063-0.200 mm, Art. 7734, Merck) and Kieselgel 60 F_{254} (0.5 mm thickness, Art. 5554, Merck) was used for preparative thin layer chromatography (PTLC). Thin layer chromatography (TLC) was conducted on precoated plates (Kieselgel 60 F_{254} , Art. 5554, Merck). Compounds were detected by UV fluorescence and visualized by spraying with 1% vanillin-H_2SO_4 reagent, followed by heating at 105 °C for 1-2 min.

Plant material

The fruits of *Vitex agnus-castus* L. (Verbenaceae) were collected from the vicinity of Bağarası, Söke-Aydın, in July 1999. Plant material was identified by one of us (Assoc. Prof. Dr. Nurgün Küçükboyacı). A voucher specimen (GUE 2111) was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey.

Extraction, Isolation and Purification

Air-dried and powdered fruits of *Vitex agnus-castus* (400 g) were extracted 3 times with ethanol at 40 °C (3 x 2.5 L). After filtration, the combined extracts were evaporated under reduced pressure. The residue (42 g) was extracted with *n*-hexane to remove the lipid material and then with chloroform. The chloroform soluble portion (12 g) was evaporated to dryness and subjected to column chromatography on silica gel (70-230 mesh). Elution was carried out with increasing polarities of $CHCl_3$:CH_3OH mixtures. The fractions were combined according to their UV spectra and TLC analysis. The main fraction eluted by $CHCl_3$:CH_3OH (98:2) mixture was applied to preparative TLC to give **1** (65 mg) and **2** (38 mg) as major compounds.

Casticin (1): Pale yellow powder; $C_{19}H_{18}O_8$; UV (MeOH) λ_{max}: 257, 270, 350 nm; IR ($CHCl_3$) ν cm^{-1}: 3200-3100 (OH), 1660 (C=O), 1600 (C=C) ; ^{1}H-NMR (CD_3OD, 500 MHz) δ , ^{13}C-NMR (CD_3OD, 125 MHz) δ , HMQC, HMBC and NOE were given in Table 1; EIMS: 374 (100) $[M]^+$, 373 (41) $[M-1]^+$, 359 (57) $[M-15]^+$, 331 (16) $[M-43]^+$.

Artemetin (2): Light-yellow powder; $C_{20}H_{20}O_8$; UV (MeOH) λ_{max}: 256, 271, 349 nm; IR ($CHCl_3$) ν cm^{-1} : 3250-3100 (OH), 1665 (C=O), 1605 (C=C) ; ^{1}H-NMR (CD_3OD, 500 MHz) δ , ^{13}C-NMR (CD_3OD, 125 MHz) δ , HMQC, HMBC and NOE were given in Table 2; EIMS: 388 (100) $[M]^+$, 387 (90) $[M-1]^+$, 373 (88) $[M-15]^+$, 345 (28) $[M-43]^+$.

RESULTS AND DISCUSSION

The ethanol extract of the air-dried and powdered fruits of *V. agnus-castus* was extracted with *n*-hexane and chloroform. The chloroform fraction was subjected to column chromatography and preparative TLC to afford the flavonoids **1** and **2**.

The structure of compound **1** (Figure 1) was elucidated to be casticin by comparing of those reported spectral data in the literature (9,10,22). In addition, casticin was confirmed by extensive 2D NMR methods, which spectral data have not been fully reported previously to our knowledge. The detailed spectral data of **1** was shown in Table 1. Casticin (5,3'-dihydroxy-3,6,7,4'-tetramethoxyflavone) was found to be one of the most abundant constituents in the chloroform extract of *V. agnus-castus*. It was previously isolated from *Vitex agnus-castus* (9,10,12) and other *Vitex* species such as *V. negundo* (10), *V. trifoliate* (10), *V. rotundifolia* (10) and *V. trifolia* (23) as well as other plants such as *Parthenium* sp., *Brickellia* sp. and *Chrysosplenium americanum* (24).

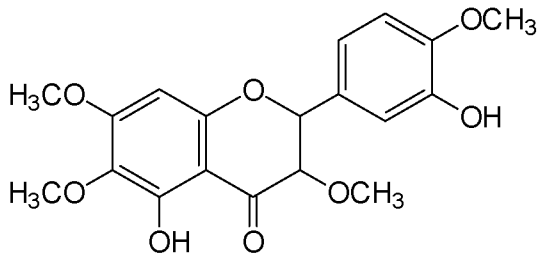

Figure 1. The molecular formula of Casticin **(1)**

Table 1. NMR Spectral data of Casticin **(1)**

Position	^{1}H (J,Hz)	^{13}C (HMQC)	HMBC	NOE
2	-	154.1	-	-
3	-	137.6	-	-
4	-	177.9	-	-
5	-	151.4	-	-
6	-	131.2	-	-
7	-	155.1	-	-
8	6.43 s	90.8	C-7, C-9, C-6, C-10	7-OCH_3
9	-	158.2	-	-
10	-	105.3	-	-
1'	-	122	-	-
2'	7.60 d (2)	111.4	C-2, C-6'	3'-OH, 3-OCH_3
3'	-	146.0	-	-
4'	-	149.9	-	-
5'	6.87 d (8.6)	111.7	C-3', C-1'	4'-OCH_3, 6' H
6'	7.64 dd (8.6, 2)	120.1	C-2	-
3- OCH_3	3.83 s	59.7	-	-
6- OCH_3	3.87 s	60.1	-	-
7- OCH_3	3.93 s	55.4	-	-
4'-OCH_3	3.90 s	55.1	-	-

The structure of compound **2** (Figure 2) was determined to be artemetin by comparing of spectral data with the literature data (10,21,25). To our knowledge, the structure of artemetin, the spectral data of which have not been fully reported previously, has been determined by using extensive two-dimensional NMR methods. The detailed spectral data of artemetin was given in Table 2. Artemetin (5-hydroxy-3,6,7,3',4'-pentamethoxyflavone) was previously obtained from *V. agnus-castus* (9) and other *Vitex* species, *V. trifolia* (25), *V. negundo* (10) and *V. trifoliate* (10). Besides, it was also isolated from other plants such as *Artemisia* sp., *Kuhnia eupatorioides*, *Achillea* and *Brickellia* species (24). The above-mentioned compounds showed complete agreement of their physical and spectroscopic data with the literature values. In addition, the structure of the compounds was confirmed by HMQC and HMBC methods.

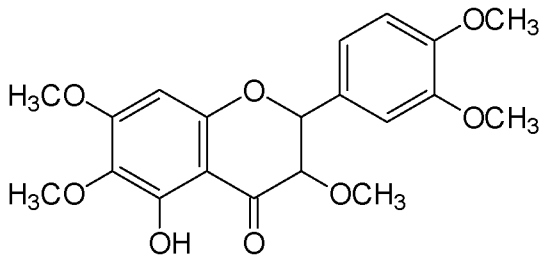

Figure 2. The molecular formula of Artemetin (**2**)

A great number of pharmacological effects have been ascribed to flavonoids such as anti-inflammatory, analgesic, antihepatotoxic, antiallergic, antiosteoporotic, antitumour, antimicrobial, antiviral, enzyme inhibiting, antioxidant and central vascular system effects so far (26-28). Two flavonoids derivatives found as the major ingredients in the present study, casticin and artemetin were previously shown to possess such activities. Hadju et al. (12) reported that casticin showed marked inhibitory activity against lipid peroxidation in rat brain homogenate, but proved to be inactive in the DPPH assay. Hu et al. (29) described that casticin has potent analgesic and anti-hyperprolactinemia properties and may have a role in treating premenstrual syndrome. Casticin has also markedly inhibited the growth of KB cells (30). Artemetin has found antiproliferative activity in human myeloid leukemia HL-60 cells and antioxidant activity against peroxyl radicals (31,32).

V. agnus-castus is described to have medicinal importance in human health. Currently, the extracts of the fruits of *V. agnus-castus* are primarily used to treat premenstrual syndrome (5). In previous studies on the secondary metabolites of *V. agnus-castus*, in particular, *C*-glycosyl flavones have been found to be common in genus *Vitex* (33). This is the first report for the isolation of casticin and artemetin as the major flavonoids from the fruits of Turkish *Vitex agnus-castus*.

Table 2. NMR Spectral data of Artemetin (**2**)

Position	^{1}H (J,Hz)	^{13}C (HMQC)	HMBC	NOE
2	-	151.4	-	-
3	-	138	-	-
4	-	180	-	-
5	-	151.9	-	-
6	-	131.5	-	-
7	-	155	-	-
8	6.43 s	89.8	C-7, C-9, C-6, C-10	7-OCH_3
9	-	158	-	-
10	-	105.7	-	-
1'	-	122.1	-	-
2'	7.67 d (2)	110.7	C-6', C-2	3'-OCH_3, 3-OCH_3
3'	-	148.0	-	-
4'	-	150.8	-	-
5'	6.98 d (8.6)	110.3	C-1', C-3'	6' H, 4'-OCH_3
6'	7.71 dd (8.6, 2)	121.5	C-2	-
3- OCH_3	3.85 s	59.7	-	-
6- OCH_3	3.87 s	60.1	-	-
7- OCH_3	3.93 s	55.4	-	-
3'-OCH_3	3.85 s	55.5	-	-
4'-OCH_3	3.91 s	55.7	-	-

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