Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(1):491-494
(http://derpharmachemica.com/archive.html)

Chemical constituents of *Vitex agnus-castus* (Verbenaceae)

[1]Hanane Aissaoui, [1,2]Methaq Algabr,[1]Samia Mezhoud, [1,]*Ratiba Mekkiou, [1]Ouahiba Boumaza, [1]Ramdane Seghiri, [1]SamirBenayache and [1]FadilaBenayache

[1]Unité de Recherche : Valorisation des Ressources Naturelles, Molécules Bioactives et Analyse Physico-chimiques et Biologiques (VARENBIOMOL), Université des frères Mentouri Constantine, Algérie.
[2]Laboratory of Chemistry, Faculty of Applied Sciences, University of Hajjah, P.O.Box 80004, Yemen.

ABSTRACT

*Chemical investigation of the ethyl acetate extract of the aerial parts (leaves and flowers) of Vitex agnus-castus led to the isolation of casticin (**1**), luteolin 7-methyl ether (**2**) and luteolin 7-O-β-glucopyranoside (**3**). The structures of these compounds were identified by comparison of their MS, UV, ^{1}H and ^{13}C NMR data with those reported in the literature as well as acid hydrolysis for compound (3).*

Key words: Verbenaceae, *Vitex agnus-castus*, flavonoids.

INTRODUCTION

The species *Vitex agnus-castus* L (VAC) once phylogenetically classified in the Verbenaceae family, but now situated within the Lamiaceae Family [1], also known as chaste berry, is widely distributed in both temperate and tropical zones like Central Asia, the Mediterranean region, and Southern Europe [2]. Aromatic leaves are used as a spice and fruits as a substitute for pepper [3, 4]. Organs of this species have a long history (over 2000 years) of use as herbal medicine [5]. In the Algerian Sahara, *Vitex agnus*-castus is known as "Kef Meriem" and used in traditional medicine to relieve rheumatic pains. Traditionally, VAC has been used since ancient Greek times as a treatment for menstrual problems. In addition, it has been used to treat pain, swelling, inflammation, headaches, rheumatism, and sexual dysfunction [6]; menstrual disorders (amenorrhoea, dysmenorrhoea), premenstrual syndrome (PMS), corpus luteum insufficiency, infertility, acne, menopause and disrupted lactation [7-9]. The most thoroughly studied mechanism is through dopamine receptors in the anterior pituitary. Several studies have indicated that VAC acts on dopamine D2 receptors to decrease prolactin levels [10-14].

A literature survey showed that the chemical constituents of *vitex agnus-castus* include flavonoids and their biological activities have been widely studied in earlier years and many flavonoids and flavonoids glycosides have been reported from this plant [9-10]. In continuation of our study on Algerian medicinal species and in the aim to find other secondary metabolites not described until now, we report here the isolation and the structure elucidation of flavonoids from the EtOAc fraction of the soluble part of the aqueous MeOH extract of the leaves of *Vitex agnus-castus* from Algeria. The structures of the isolated compounds were identified on the basis of spectroscopic studies, acid hydrolysis and comparison with literature data.

MATERIALS AND METHODS

General Procedures

The UV Spectra were recorded on an Evolution 300 spectrophotometer. The ^{1}H and ^{13}C NMR Spectra were recorded at 250 and 62.9 MHz with a Bruker DPX-250, 250 MHz spectrometers. Mass spectra Q-TOF micro (waters) spectrometer. Chemical shifts (δ) are given in ppm using TMS as internal standard and coupling constants (*J*) are given in Hz. Column chromatography (CC) was carried out on silica gel 230-400 mesh (Merck). TLC was performed on pre-coated silica gel plates 60F254 (Merck) and the chromatograms were visualized under UV light (at 254 and 366 nm) before and after exposure to ammonia vapour, as well as spraying with anisaldehyde-sulphuric acid spray reagent.

Plant material

The aerial parts of *Vitex agnus-castus* have been collected from Bechar region in the south–west of Algeria in April 2006 and the plant has been authenticated by Mr Benabdelhakem (Director of the protection of nature agency, Bechar, Algeria). A voucher specimen of the plant material (VACV/06) has been deposited in the Herbarium of the VARENBIOMOL research unit, University of Frères Mentouri Constantine.

Extraction and isolation

Aerial parts (leaves and flowers) of *Vitex agnus-castus* were air-dried and macerated separately with EtOH/H_2O (7/3; v/v) for 48 hours three times. The crude extract was concentrated at room temp. and diluted with distillate water. After precipitation of chlorophyll with $Pb(OAc)_4$ and filtration, the remaining aqueous solution was extracted successively with $CHCl_3$, EtOAc and *n*-BuOH. The organic layers were dried with Na_2SO_4 giving after removal of solvents under red. pressure, $CHCl_3$, EtOAc and *n*-BuOH extracts respectively.

A part of the EtOAc extract (8g) was chromatographed with 230-400 mesh silica gel by CC using chloroform with increasing percentages of methanol to yield 24 fractions (1–24) obtained by combining the eluates on the basis of TLC analysis. Fraction 5 which contained a major component was resubmitted to preparative TLC ($CHCl_3$/MeOH; 9:1) to afford compound **1**. The fractions 7 and 8 were gathered and subjected to preparative TLC ($CHCl_3$/MeOH; 9.8:1.2) to afford 4 subfractions (7-1; 7-4). The subfraction 7-2 contained a pure compound **2**. Fraction 16 contained a precipitate was washed with MeOH three times to afford a pure yellow product, compound **3**.

Acid hydrolysis

Solution of compound **3** in 2 ml (HCl 4N) was heated for 2 h and left to cool. The mixture was extracted with EtOAc and the EtOAc fraction was used for detection of theaglycone. The aqueous fraction was concentrated and using for identification of sugars. The sugars were identified by TLC using solvent system (acetone–water; 90:10) by comparison with authentic samples.

RESULTS AND DISCUSSION

From the EtOAc extract obtained from *Vitex agnus-castus* leaves, three flavonoids (**1-3**) (figure 1), were isolated by chromatographic methods than identified on the basis of their UV and NMR spectral data and comparison with literature data for similar structures.

Compound 1: yellow powder soluble in chloroform. The molecular formula of this compound, was confirmed by the study of the mass spectrum in ionization electron impact (EI^+) at *m/z* 374 which correspond to the molecular ion $M^{+\cdot}$ Which led to the formula $C_{19}H_{18}O_8$ indicating a compound containing 11 unstaurations, UV λmax (nm); MeOH: 258, 350; + NaOH: 271, 383; +$AlCl_3$: 270, 377; + $AlCl_3$/HCl: 268, 371, 281; +NaOAc: 258, 349, 271; + NaOAc/H_3BO_3: 258, 349, 268.^{1}H NMR ($CHCl_3$-d_1, 400MHz, :δppm, *J*:Hz): 7.71 (1H, *bs*, H-2'), 7.75 (1H, *bd*, *J*=8.5, H-6'), 6.99 (1H, *d*, *J*=8.5, H-5'), 6.53 (1H, *s*, H-8), 3.89 (3H, *s*, OCH_3), 3.94(3H, *s*, OCH_3), 3.98 (3H, *s*, OCH_3), 4.01 (3H, *s*, OCH_3). This compound was identified as casticin [15].

Compound 2: yellow powder soluble in acetone, UV λ_{max} (nm): MeOH: 269, 349; + NaOH: 269, 402; +$AlCl_3$: 273, 418; + $AlCl_3$/HCl: 275, 388, 356; +NaOAc: 270, 363; + NaOAc/H_3BO_3: 263, 374. ^{1}H NMR (acetone-d_6, 250 MHz, δ:ppm, *J*:Hz): 7.52 (1H, *d*, *J* = 2.1, H-2'),7.48 (1H, *dd*, *J* = 8.6, 2.1, H-6'), 7.02 (1H, *d*, *J* = 8.6, H-5'), 6.60 (1H, *s*, H-3), 6.55 (1H, d, *J*=2.1, H-8), 6.27(1H, *d*, *J* = 2.1, H-6), 3.33(3H, s, OCH_3). This compound was characterized as 5, 3', 4' -trihydroxy 7-methoxyflavone, named luteolin7-methyl ether [16].

Compound 3: yellow powder soluble in DMSO, The molecular formula of this compound was determined as $[M+Na]^+$ corresponding to $C_{21}H_{20}O_{11}Na$ on the basis of HRESI-MS+ spectrum at *m/z* 471.0902 (100%) which led to the formula $C_{21}H_{20}O_{11}$ (448 Da) indicating a compound containing 12 unsaturations. UV λ_{max} (nm): MeOH: 270,

349; + NaOH: 270, 405; +$AlCl_3$: 272, 420; + $AlCl_3$/HCl: 272, 387,356; +NaOAc: 270, 407;+NaOAc/H_3BO_3: 270, 375. ^{1}H NMR (DMSO-d_6, 250MHz, δ:ppm, *J*:*Hz*): 7.48 (1H, *dd*, *J* =8.3, 2.1, H-6'), 7.44 (1H, *d*, *J*=2.1, H-2'), 6.91 (1H, *d*, *J* = 8.3, H-5'), 6.81 (1H, *d*, *J* = 2.1, H-8), 6.77 (1H, *s*, H-3), 6.46 (1H, *d*, *J* = 2.1, H-6), 5.10 (1H, *d*, *J* =7.1, H-1" Glucose), (3.20- 3.70 sugar protons). ^{13}C NMR (DMSO-d_6, 62.9 MHz, δ : ppm): 163.4 (C-2), 103.6 (C-3), 182.3 (C-4), 161.5 (C-5), 99.9 (C-6), 164.9 (C-7), 95.1 (C-8), 157.4 (C-9), 105.7 (C-10), 121.7 (C-1'), 113.9 (C-2'), 146.2 (C-3'), 150.4 (C-4'), 116.4 (C-5'), 119.6 (C-6'), 100.3(C-1"), 73.5 (C-2"), 76.8 (C-3"), 69.9 (C-4"), 77.6 (C-5"), 61.02 (C-6"). Acid hydrolysis of compound 3 produced luteolin and glucose while the configuration of anomeric sugar was deduced by its J_{H-H} coupling constant. This compound was characterized as luteolin-7-*O*-*β*-glucopyranoside [17].

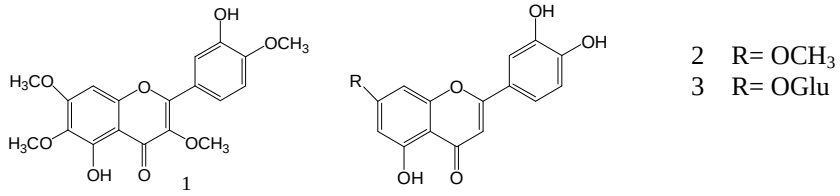

Figure 1: Structures of Compounds 1- 3

Literature search revealed that the isolated compounds (**1-3**) have diverse biological activities such as Casticin, which is one of the bioactive flavonoids obtained from polyphenol plants; it is a main active compound in roots, aerial parts, leaves and fruits of variety of plants. It has been reported to be responsible for a wide spectrum of biological and pharmacological activities including immunomodulatory [18, 19] anti-hyperprolactinemia [20, 21], anti-tumor [22-24], neuroprotetective [19], anti-inflammatory [25, 26] and analgesic activities [27].

Luteolin and its derivatives are common in edible plants and plants used in traditional medicine to treat a wide variety of diseases. Luteolin and its derivatives have been reported to possess strong anti-oxidative and anti-inflammatory activities [28-30].

CONCLUSION

This study relates to the phytochemical investigation of the EtOAc extract of the aqueous-alcoholic extract obtained from the leaves of *Vitex agnus- castus*. This contribution led to the isolation of three known flavonoids named as Casticin, luteolin7-methyl ether and luteolin 7-*O*-glucopyranoside.

REFERENCES

[1] P Chantaranothai, *Trop. Nat. Hist.*, **2011**, 11 (2), 91–118.
[2] TG Tutin, VH Heywood, NA Burges, DH Valentine, SM Walters, DA Webb, Flora Europaea, vol. 3, Cambridge University Press, Cambridge, **1972**, pp. 122.
[3] P Hanelt, Mansfeld's Encyclopedia of Agricultural and Horticultural Crops (Except Ornamentals), Springer, Berlin, **2001**.
[4] D Stojkovi´c, M Sokovi´c, J Glamo˘clija, A D˘zami´c, A´Ciri´c, M Risti´c, D Grubi˘si´c, *Food Chem.*, **2011**, 128 ,1017–1022.
[5] SN Chen, BJ Friesen, D Webster, D Nikolic, RB van Breemen, ZJ Wang, HHS Fong, NR Farnsworth, GF Pauli, *Fitoterapia*, **2010**, 82 528–533.
[6] R Upton, (Ed.), Chaste Tree Fruit, *Vitex agnus-castus*: Standards of Analysis, Quality Control, and Therapeutics. *American Herbal Pharmacopoeia*, Santa Cruz, CA, **2001**.
[7] C Daniele, C Thompson Coon, M Pittler, E Ernst. *Drug Safety*, **2005**, 28, 319–332.
[8] V N Prilepskaya, AV Ledina, AV Tagiyeva, FS Revazova, *Maturitas* (Suppl. 1), **2006**, S55–S63.
[9] SA Brahim, Int. J. Pharm. Pharm. Sci, **2013**, 766–767.
[10] H Jarry, S Leonhardt, C Gorkow, W Wuttke, *Experimental and Clinical Endocrinology*, **1994**, 448–454.
[11] E Hoberg, Swiss Federal Institute of Technology Zurich, Zurich, **1999**, p. 129.
[12] D Berger, W Schaffner, E Schrader, B Meier, A Brattstrom, *Archives of Gynecology and Obstetrics*, **2000**, 264, 150–153.
[13] B Meier, D Berger, E Hoberg, O Sticher, W Schaffner, *Phytomedicine*, **2000**, 7, 373–381.
[14] W Wuttke, H Jarry, V Christoffel, B Spengler, D Seidlova-Wuttke, *Phytomedicine*, **2003**, 10, 348–357.
[15] Anonymous, Chaste Tree. In: Dombek C, Ed. Lawerence Review of Natural Products. St. Louis: *Facts and Comparisons*, **1998**

[16] E Wollenweber, et al. *Z. Natur-forsch*, **1992**, 47c, 782.
[17] J Raynaud, et al. Prescription et conseil en phytothérapie, Ed. Tec et Doc, **2005**, 97-9.
[18] MA Mesaik, S Murad, KM Khan, RB Tareen, A Ahmed, Atta-ur-Rahman, MI Choudhary, *Phytother Res*., **2009**, 23, 1516-20.
[19] Y Ling, J Zhu, M Fan, W Bin, Q Luping, H Chenggang, *Biomed Chromatogr*., **2012**, 26, 1502-8.
[20] Y Hu, HL Xin, QY Zhang, HC Zheng , K Rahman, LP Qin, *Phytomedicine,* **2007**, 14, 668-74.
[21] Q Ye, QY Zhang, CJ Zheng, Y Wang, LP Qin, *Acta Pharmacol Sin.,* **2010**, 31, 1564-8.
[22] K Haidara, L Zamir, QW Shi, G Batist, *Cancer Lett*, **2006**, 242, 180-90.
[23] F Zeng, L Tian, F Liu, X. Sheng, *Acta Biochim Biophys Sin (Shanghai)*, **2012,** 44, 442-9.
[24] JK Shen, HP Du, M Yang, YG Wang, J Jin, *Ann Hematol.*, **2009**, 88, 743-52.
[25] MI Choudhary, S Jalil, SA Nawaz, KM Khan , RB Tareen, Atta-ur-Rahman, *Phytother Res.*, **2009**, 23, 1336-9.
[26] T Velpandian, P Gupta, AK Ravi, HP Sharma, NR Biswas, *BMC Complement Altern Med.,* **2013,** 13, 1.
[27] SM Lee, YJ Lee, YC Kim, et al. *Inflammation*, 2012, **35**, 584-93.
[28] G Seelinger, I Merfort, CM Schempp, *Planta Med*, **2008,** 74,1667-77.
[29] CY Chen, WH Peng, KD Tsai, SL Hsu, *Life Sci.,* **2007**, 81, 1602-14.
[30] JT Hwang, OJ Park, YK Lee, MJ Sung, HJ Hur, MS Kim, JH Ha, DY Kwon, *Int J Mol Med.*, **2011**, 28:25–31.