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Neo-clerodanes from Teucrium divaricatum and their potential antiinflammatory and antimicrobial activities

Sophie Fadime Aydoğan
Dicle University; University of Mississippi, faydogan@olemiss.edu

Zulfiqar Ali
University of Mississippi

Shabana I. Khan
University of Mississippi

Canan Karaalp
Ege University

Ikhlas A. Khan
University of Mississippi

Neo-clerodanes from Teucrium divaricatum subsp. divaricatum and their biological activity evaluation

Fadime Aydoğan[1,2], Zulfiqar Ali[2], Shabana I. Khan[2], CananKaraalp[3], Ikhlas A. Khan[2], Erdal Bedir[4]

[1]Department of Pharmaceutical Botany, Faculty of Pharmacy, Dicle University, 21280, Sur, Turkey. [2]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA. [3]Department of Pharmaceutical Botany, Faculty of Pharmacy, Ege University, 35040, Izmir, Turkey, [4] Department of Biotechnology and Bioengineering, Izmir Institute of Technology, 35430, Izmir, Turkey

Abstract: *Teucrium* L., one of the 245 genera in the Lamiaceae family, is represented with approximately 370 taxa in the world. Herein, the isolation and structural identification of 17 secondary metabolites including two undescribed *neo*-clerodane glycosides (**1-2**), 13 *neo*-clerodane diterpenoids (**3-15**), an iridoid glycoside (**17**), and a phenylpropanoid glycoside (**16**) from the whole plant of *T. divaricatum* subsp. *divaricatum* is reported. *Neo*-clerodane diterpenoids were evaluated for their potential anti-inflammatory and antimicrobial activities.

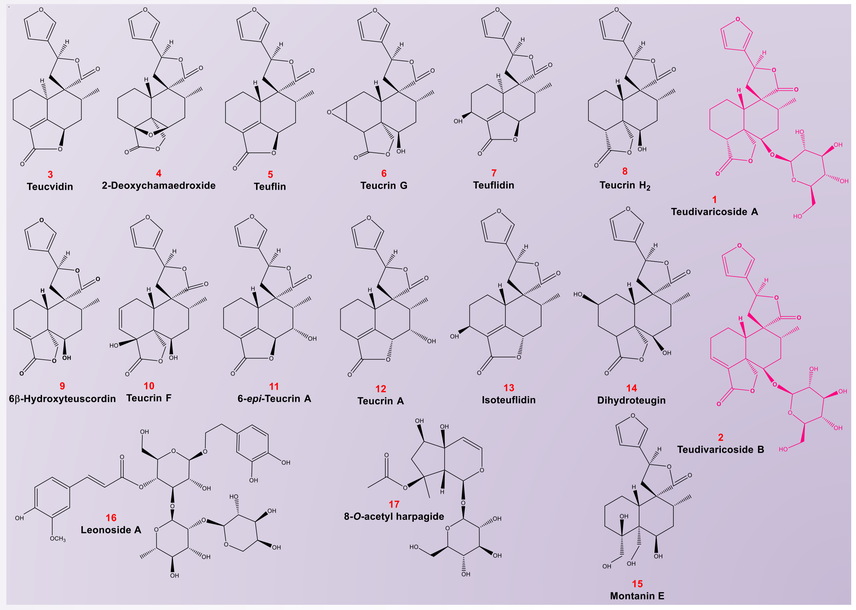

Figure 1. Structures of compounds 1-17.

Introduction: *Teucrium* L. (Lamiaceae) is represented with approximately 370 taxa of perennial, bushy, shrubs or herbs in the world [1,2]. It's members are mainly distributed in the Mediterranean region with a considerable number of species occurring in Spain, Algeria, Morocco, Italy, Greece, and Turkey [2]. Çeçen et all described the last taxa as *T. turcicum* and by last revision of the genus, 50 *Teucrium* taxa (38 species) are found in the Flora of Turkey and 19 of them are endemics in Turkey [2]. *T. divaricatum* and *T. chamaedrys* are familiar and common species known as 'germander' worldwide and have traditionally been used as tonic, carminative, spasmolytic, diuretic, antiseptic, antirheumatic, antipyretic, and anthelmintic [3-8]. We report the isolation and structural identification of 15 *neo*-clerodane diterpenoids including two undescribed *neo*-clerodane glycosides, an iridoid glycoside and a phenylpropanoid glycoside from the whole plant of *T. divaricatum* subsp. *divaricatum*. *Neo*-clerodane diterpenoids were evaluated for their potential anti-inflammatory and antimicrobial activities.

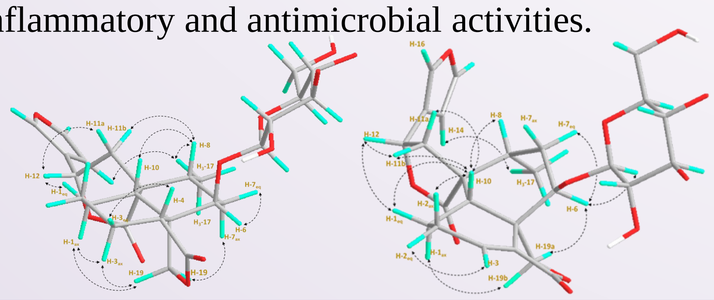

Figure 4: Key NOESY correlations of 1 and 2.

Table 1: NMR data of compounds 1 and 2.

		1[a]		2[a]
	δ_C	δ_H (δ ppm, J= Hz)	δ_C	δ_H (δ ppm, J= Hz)
1	23.04	1.23 (*m*)† 1.79 (*d*, *J*=11.1)	21.18	1.90 (*t*, *J*=12.4) 1.25 (*m*)†
2	25.10	1.95 (*m*)† 1.70 (*m*)†	27.38	2.29 (*m*) 2.18 (*p*, *J*=12.4)†
3	24.90	1.18 (*m*) 1.51 (*m*)	136.89	6.75 (*t*, *J*=4.7)
4	44.73	3.36 (*dd*, *J*=12.3, 6.4)	149.98	-
5	47.68	-	50.36	-
6	75.21	3.97 (*t*, *J*=2.8)	74.69	4.36 (*bs*)
7	31.59	2.22 (*dd*, *J*=12.5,3.9) 1.97 (*ddd*, 18.4, 9.1, 5.2)	32.03	2.30 (*m*)† 2.05 (*m*)†
8	33.18	2.33 (*m*)	34.02	2.24 (*m*)†
9	51.53	-	50.73	-
10	42.93	2.20 (*m*)	43.61	2.86 (*dd*, 13.2, 1.9)
11	41.69	2.15 (*d*, *J*=8.3)	41.13	2.30-2.17 (2H, *m*)†
12	72.05	5.43 (*t*, 8.7)	72.19	5.53 (*t*, *J*=8.6)
13	126.23	-	125.80	-
14	109.13	6.55 (*d*, *J*=1.9)	108.97	6.58 (*d*, *J*=1.9)
15	145.06	7.69 (*t*, *J*=1.8)	144.93	7.70 (*t*, *J*=1.8)
16	140.83	7.78 (*s*)	140.93	7.82 (*s*)
17	16.97	0.95 (*d*, *J*=7.5)	16.46	0.98 (*d*, *J*=5.4)
18	180.6	-	170.66	-
19	71.21	4.73 (*d*, *J*=10.9) 4.61 (*d*, *J*=10.9)	72.65	4.79 (*dd*, *J*=8.7, 5.4)† 4.18 (*d*, *J*=8.0) †
20	178.0	-	178.16	-
1'	103.05	4.78 (*d*, *J*=7.7)	102.79	4.79 (*dd*, *J*=8.7, 5.4)†
2'	75.33	4.05	74.88	3.99 (*dd*, *J*=9.0, 7.7)
3'	78.61	4.23 (*t*, *J*=8.9)	78.26	4.19 (*t*, *J*=9.0) †
4'	71.95	4.14 (*t*, *J*=9.2)	71.48	4.07 (*t*, *J*=9.2)
5'	78.45	3.89 (*td*, *J*=6.0, 2.9)	78.00	3.84 (*m*)
6'	63.46	4.53 (*dd*, *J*=12.0, 2.0) 4.32 (*dd*, *J*=12.2, 6.1)	62.80	4.48 (*dd*, *J*=12.0, 2.7) 4.32 (*dd*, *J*=12.0, 2.7)

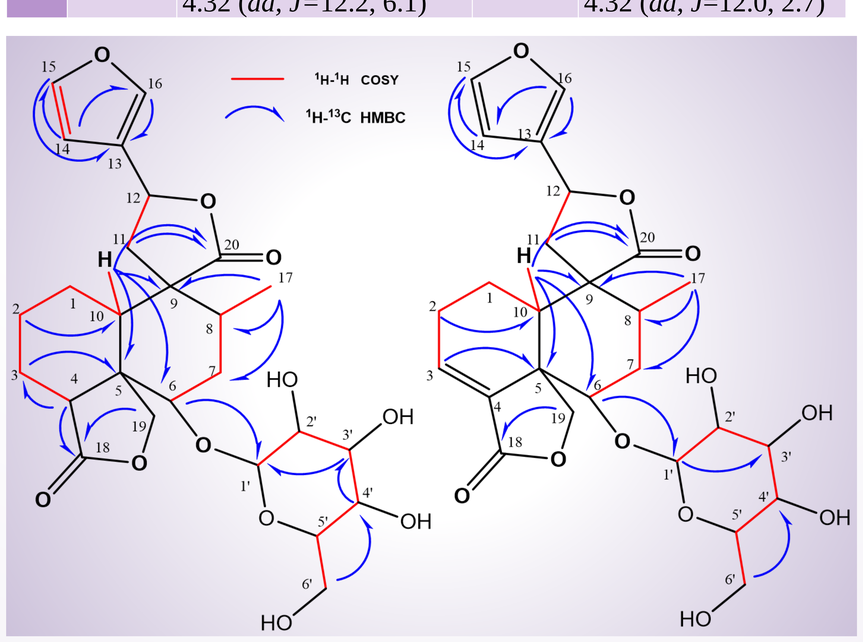

Figure 2: Key COSY and HMBC correlations of compounds 1 and 2.

Plant Material: Plant material was collected from Göynük Canyon, Antalya, Turkey, altitude 574 m and identified by Fadime Aydogan (Ph.D.) and Volkan Eroğlu (Ph.D.). Voucher specimen was deposited in the Ege University Herbarium of Faculty of Pharmacy (IZEF) (# IZEF 6613).

Results and Discussion: The $CHCl_3$ and H_2O fractions of *T. divaricatum* subsp. *divaricatum* extract was subjected to column chromatogeaphy on silica gel, Sephadex LH-20, and C-18 semi-preperative HPLC to purify 17 compounds (Figure 1). Structure elucidation of the isolated compounds was achieved by analyses of their NMR and mass spectral data analysis. Chemical shifts were assigned using HSQC, COSY, and HMBC spectra (Figure 2). Relative stereochemistry at stereogenic centers was determined using CD (Figure 3) and NOESY correlations (Figure 4). Two new *neo*-clerone gylcosides and 13 known *neo*- and *nor*-clerodane diterpenoids isolated from the $CHCl_3$ fraction were identified as teudivaricosides A (**1**) B (**2**), teucvidin (**3**), 2-deoxychamaedroxide (**4**), teuflin (**5**), teucrin G (**6**), teuflidin (**7**), teucrin H2 (**8**), 6*β*-hydroxyteuscordin (**9**), teucrin F (**10**), 6-epi teucrin A (**11**), teucrin A (**12**), isoteuflidin (**13**), dihydroteugin (**14**), montanin E (**15**). Two known glycosides isolated from the aqueous extract were identified as leonoside A (**16**) and 8-*O*-acetyl-harpagide (**17**). All *neo*-clerodane derivatives were screened for *in-vitro* antimicrobial activity and antiinflammatory activities in terms of iNOS and NF-κB inhibition as well as for cytotoxicity. None of them showed significant antimicrobial activitiy (upto 20 μg/mL-lower doses) and antiinflammatory activity (upto 50 μM- lower doses).

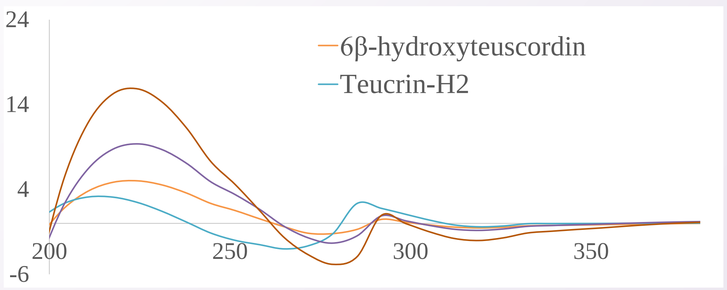

Figure 3: ECD spectra of compounds 1, 2, 8, and 9.

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