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Biological and Phytochemical Studies on *Astragalus* Taxa from Anatolia

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Abstract: *Astragalus* L. (Fabaceae), one of the largest genera in Angiosperm, is represented with approximately 2500-3000 taxa. With the aim to study *Astragalus* taxa in Anatolia, Turkey, that is reported 425-450 taxa (endemism ratio 47-50%), we report anti-inflammatory effect of ethanolic extracts of roots from six *Astragalus* spp. and phytochemical investigation of *Astragalus strictispinus* Boiss. Phytochemical analysis of *Astragalus strictispinus* roots resulted in isolation and structural identification of 25 cycloartane type triterpenoids (1-25) including nine undescribed cycloartanes, eleven flavonoids and lignans (26-33), one pterocarbon and one pterocarbon glycoside (34-35), a steroidal glycoside (36).



Figure 1. Collected *Astragalus* spp. from Kumalar Mountain

Plant Material: Plant materials were collected from Kumalar Mountain, Afyonkarahisar, Turkey, altitude 1500-2000 m and identified by Fadime Aydoğan (Ph.D.) and Volkan Eroğlu (Ph.D.). Voucher specimens were deposited in the Ege University Herbarium of Faculty of Pharmacy (IZEF) and all samples were saved at NCNPR, University of Mississippi.

Binominal Name	NCNPR	Cytotoxicity	iNOS inh	NRF2 activation.
<i>A. strictispinus</i>	24183	NC	NA	NA
<i>A. angustifolius</i> subsp. <i>pungens</i>	24184	NC	NA	NA
<i>A. creticus</i>	24185	NC	NA	NA
<i>A. creticus</i>	24186	NC	NA	NA
<i>A. flavecens</i>	24187	NC	NA	NA
<i>A. angustifolius</i> subsp. <i>angustifolius</i>	24188	NC	NA	NA
<i>A. ponticus</i>	24189	NC	NA	NA
<i>A. strictispinus</i>	24190	NC	NA	NA
<i>A. strictispinus</i>	24191	NC	87 µg/mL	NA
<i>A. creticus</i>	24192	NC	NA	NA

Highest tested concentration for extract = 100 µg/mL, Parthenolide for iNOS, Curcumin for NRF2 were used for standard drug

Figure 2. Anti-inflammatory activity of ethanolic extracts from *Astragalus* spp.

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Introduction: *Astragalus* L. (Fabaceae) is represented with approximately 3000 taxa of annual or perennial stemmed herbs or small shrubs (up to 150–200 cm), leguminose flowers, a legume pod with kidney-shaped seeds in the world [1-3]. It's members are mainly distributed temperate region in Europe, Asia, and North America [2]. Guner et al described 452 taxa in *Astragalus* genus in Flora of Turkey and 224 of them are endemics in Turkey [2]. *Astragalus* root has been used for more than two millenniums in folk medicine to prevent and treat different illnesses as hepatoprotective, antioxidative, immunostimulant, antiviral, antidiabetic, cardioprotective, anti-inflammatory, wounds, leukemia, and anticancer. [3-8]. Many studies reported anticancer, anti-inflammatory, antiaging, immunomodulatory, antiviral, activity of *Astragalus* extract and main phytochemicals. The major phytochemicals of *Astragalus* taxa displaying beneficial properties include saponins, flavonoids, and polysaccharides [3-10]. Astragalosides are the main cycloartane-type saponins, with astragaloside IV as the quality control biomarker. Astragalosides IV and VII and cycloastragenol possess antioxidative, antiaging, anti-inflammatory, antiasthma, antidiabetes, and antiatherosclerosis activities, as well as cardioprotective and neuroprotective effects [9-15]. Herein, we report in-vitro anti-inflammatory activities of ethanolic extract of six *Astragalus* taxa and detailed investigation of phytoconstituents from roots of *Astragalus strictispinus* Boiss.

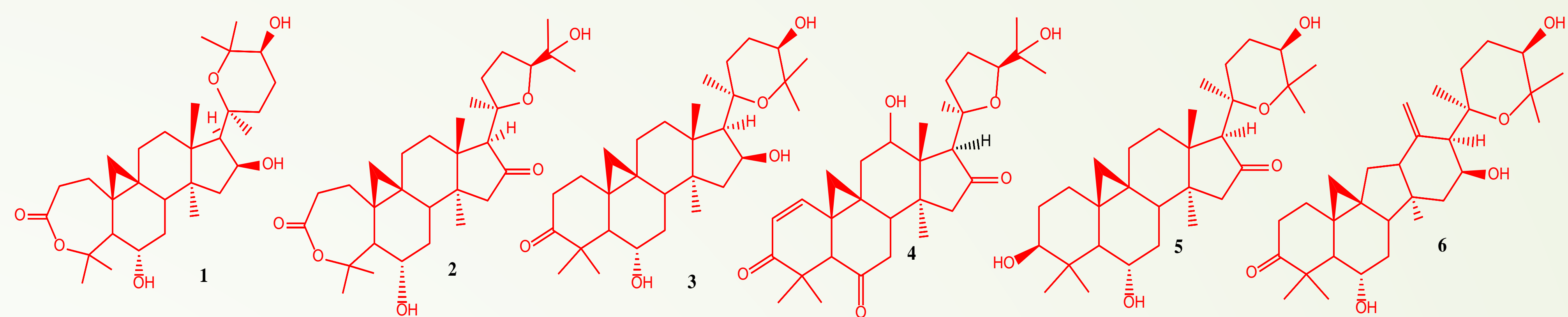


Figure 3. Structure of isolated undescribed triterpene derivatives from *A. strictispinus*

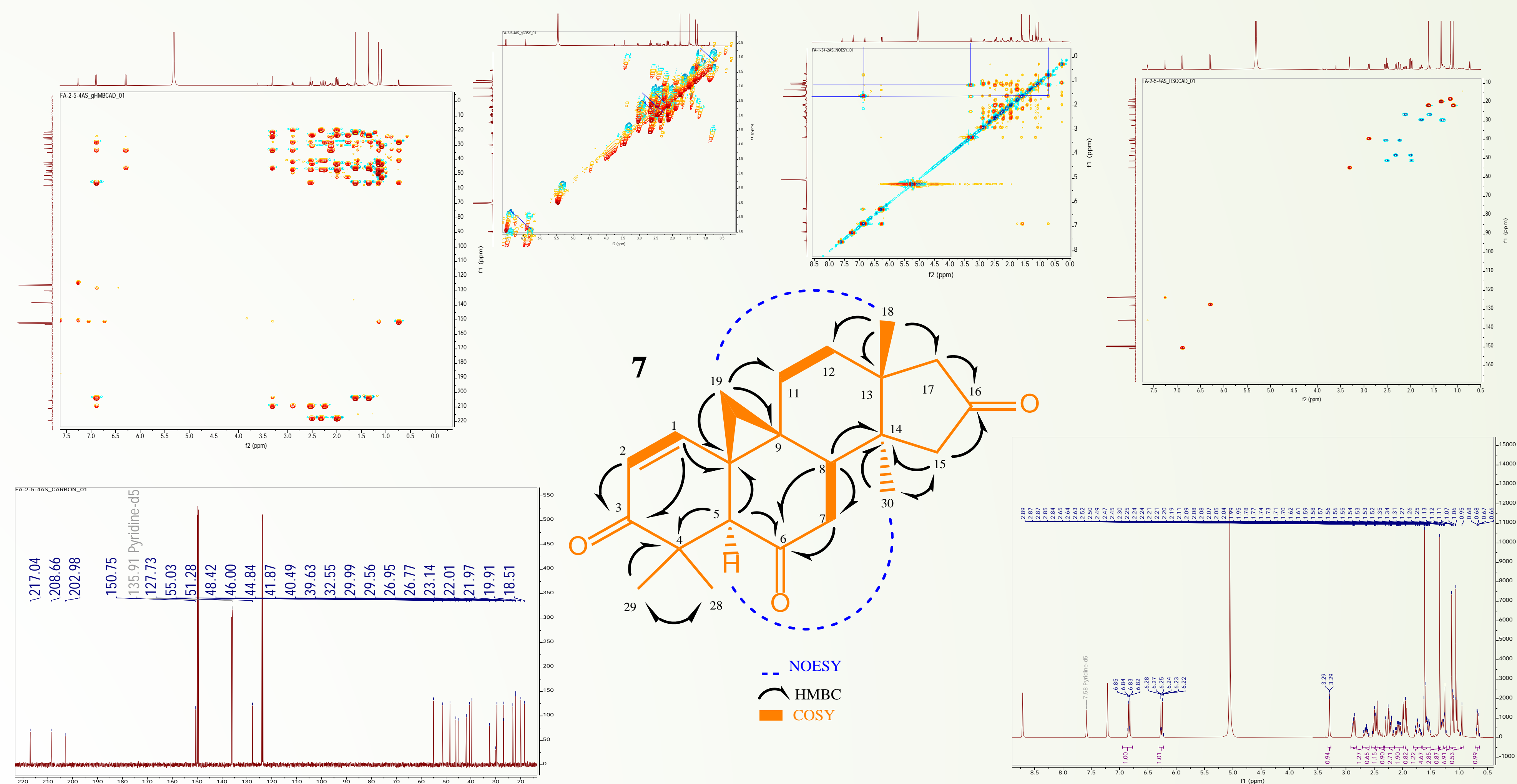


Figure 4. 1D and 2D NMR spectra of Compound 7

Results and Discussion: Air-dried-grounded roots of 10 *Astragalus* samples (10 g each) were extracted with ethanol by Dionex™ ASE™ 350 accelerated solvent extractor. Filtered extracts were dried under the vacuum and freeze dried. Extracts were screened for antiinflammatory activities in terms of iNOS inhibition and NRF-2 activation as well as for cytotoxicity. Only *A. strictispinus* extract inhibited iNOS with IC₅₀ values of 87 µg/mL. The roots of *Astragalus strictispinus* (1.1 kg) was extracted with MeOH (5L x 24 h x 3) at room temperature. The dried MeOH extract (137 g) was subjected to column chromatography on silica gel, Sephadex LH-20, and C18 semi-preperative HPLC to purify 36 compounds. Structure elucidation of the isolated compounds was achieved by analyses of their NMR and mass spectral data. Chemical shifts were assigned using HSQC, COSY, and HMBC, NOESY spectra (Figure 3). Seven new triterpenes (1-7) (Figures 3 and 4), astrastriospins A-G and two new triterpene glycosides (8 and 9): astrastriospinoids I and II together 16 known saponins, isolated from the root extract, were identified as cycloastragenol (10), cycloasagenin (11), 20(R),25-epoxy-3β,6α,16β,24α-tetrahydroxycycloartane (12), cyclopycnanthogenin (13), 17-epi-cycloasagenin (14), cycloadsurgenin (15), cycloasagenin C (16), cycloastragenol-6-O-β-D-glucopyranoside (17), cycloastragenol-16-O-β-D-glucopyranoside (18), cycloasagenin-6-O-β-D-glucopyranoside(19), astragaloside IV (20), astragaloside II (21), astragaloside VII (22), astrasieversianin X (23), cyclosieversioside I (24), astragaloside VI (25), with other phytochemical class known compounds as vestitol (26), sissotrin (27), ononin (28), acacetin (29), artocarpetin (30), isoliquiritigen (31), lariciresinol (32), 3',4',7-trihydroxyflavone (33), and maackianin (34), maackianin-3-O β-D-glucopyranoside (35) and sitosterol-3-O-β-D-glucopyranoside (36).