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Antioxidant Activities of Chemical Constituents Isolated from *Echinops orientalis* Trauv.

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Abstract: The genus *Echinops* belonging to the Asteraceae family comprises 130 species. The dried leaves and seeds of *Echinops orientalis* Trauv. were extracted separately with methanol. Apigenin-7-*O*-(6"-trans-p-coumaroyl- -D-glucopyranoside **1**, and Apigenin-7-*O*- -D glucoside **2** were isolated from leaves and 1-methoxycarbonylindole **3** and beta-sitositerol **4** were isolated from seeds. The compounds were isolated by chromatographic techniques, based on column chromatography, preparative TLC and identified by spectroscopic methods including 1D-, 2D-NMR, UV, IR, HPLC-QTOF/MS. Isolated compounds and extracts were applied to antioxidant activity tests. While seeds and leaves extracts have high DPPH and moderate ABTS radical scavenging activities, the isolated flavones exhibited high cation radical scavenging activities.

Keywords: *Echinops orientalis* Trauv.; flavonoids; quinoline; antioxidant activities.

1. Introduction

Medicinal plants have been used for treatment of various illnesses in many nations for years [1]. After presented that synthetic chemicals are harmful, bioactive compounds isolated from plants, especially medicinal plants have been gained the great interest in use as pharmaceuticals, food additives, agrochemicals, fragrance ingredients and pesticides. The secondary metabolites playing a major role in adaptation of plants to their environment represent an important source of pharmaceuticals [2]. Antioxidants acting a vital role in food industry may be defined as compounds that inhibit or delay the oxidation of other molecules [3]. The genus *Echinops* is a member of Asteraceae family includes 130 species; 17 species, 2 subspecies and 3 varieties of which are grown in Turkey [4]. It survives in Africa, Mediterranean, and Asia. *Echinops* species have been used as traditional medicine for treatment of migraine, diuretic, heart diseases, urinary infection, as well as

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worm and hemorrhoid in Ethiopia [5]. Prior studies on the genus *Echinops* have displayed the presence of bioactive thiophenes [6], diterpenoids [7], and flavone and flavone glycosides [8].

Although plenty of researches have been carried out about the other *Echinops* species, the works on *Echinops orientalis* Trauv. are restricted. Herein, we isolated and elucidated four compounds from seeds and leaves (two from each) of *Echinops orientalis* Trauv. These compounds are known but they were isolated from that plant at first. The compounds were isolated by chromatographic techniques, such as column chromatography, preparative TLC and identified by spectroscopic methods (1D-, 2D-NMR, UV, IR, HPLC-QTOF/MS). The extract and isolated compounds were assayed antioxidant activities.

2. Materials and Methods

2.1. Plant Material

Echinops orientalis Trauv. was collected from Gaziosmanpasa University field during the period of investigation. The plant was identified by Assoc. Prof. Dr. Askin Akpulat, Deparment of Biology, Cumhuriyet University where the voucher specimen has been deposited (4580 AA).

2.2. Extraction and isolation

The leaves (750 g) and seeds (140 g) of plant were dried and powdered then extracted with methanol (24 h × 5 times) separately. Each filtered extract was evaporated in rotary evaporator and 5 g, 1.2 g of extracts were obtained for leaves and seeds respectively. Both extracts were subjected to column chromatography separately, using silica gel as the stationary phase and eluting with hexane and a gradients of ethyl acetate and methanol. After repeated column chromatograph and preparative TLC, Apigenin-7-*O*-(6"-*trans*-*p*-coumaroyl- -D-glucopyranoside **1** (15.0 mg), and Apigenin-7-*O*- -D glucoside **2** (12.0 mg) were isolated from leaves and 1-methylquinolin-4(*1H*)-one **3** (11 mg) and *β*-sitosterol **4**. (9 mg) were isolated from seeds. The stem of the plant was also extracted with methanol for activity assays.

The free radical scavenging activities of leaves extract, seeds extract, stem extract, isolated compounds (**1, 2, 3, 4**) and standards were measured using 1,1-diphenyl-2-picryl-hydrazil (DPPH·) [9]. $ABTS^{\cdot+}$ cation radical scavenging activity was determined according to the literature [10]. The reducing power of samples was determined according to method of Oyaizu [11]. Total soluble phenolic compounds in extract of leaves and seeds were determined with Folin-Ciocalteu reagent [12] using gallic acid as a standard for calibration curve.

3. Results and Discussion

3.1. Structure elucidation

Compound **1** was obtained as white solid. It exhibited a molecular ion peak at *m/z* 577.1376 as $[M-H]^-$ corresponding to the molecular formula $C_{30}H_{26}O_{12}$ (calcd 577.1344) in HPLC-QTOF. The ^{13}C (APT) NMR spectrum confirmed the presence of 30 carbons consisting of one methylene, eighteen methines and eleven quaternary carbons, verifying the flavonoid structure that the sugar moiety linked on it. The UV spectroscopy showed the presence of a flavanonol at 292, 336 nm. IR absorptions suggested the presence of a hydroxy group (3350 cm^{-1}), an α, β-unsaturated carbonyl group (1638 cm^{-1}). In ^1H NMR spectrum, signals at δ 7.94 (2H, d, J = 8.8 Hz, H2', H6') and δ 6.92 (2H, d, J = 8.8 Hz, H3', H5') are characteristic for the B ring as well as δ 6.66 (2H, J = 8.6 Hz, H5'", H9'") and δ 7.36 (2H, J = 8.6 Hz, H6'", H8'") for the ring A. The coupling constant between the protons at δ 7.49 and δ 6.33 as 15.8 Hz indicated that H2'" and H3'" oriented as *trans* fashion.^1H NMR spectrum at 12.99 and 10.39 ppm, in accordance with the HMBC correlation signals indicated the presence of free 5- and 4'-hydroxyl groups. The HMBC correlation signals between the anomeric proton of glucose and C-7 at δ_C 163.2 ppm indicated the glucoside at position 7. The HMBC correlation signals between the 6"a at

3.46 ppm and cumaroyl carbonyl carbon at 166.89 revealed that *p*-coumaroyl is on position 6" and also the COSY correlation of 6"a and 6"b protons at 3.46 and 4.19 ppm, respectively indicated the *p*-coumaroyl is on position 6". A HETCOR experiment revealed direct correlations between protons and carbons. Thus compound **1** was elucidated as apigenin 7-O-(6"-*trans*-*p*-coumaroyl-*β*-D-glucopyranoside) [13].

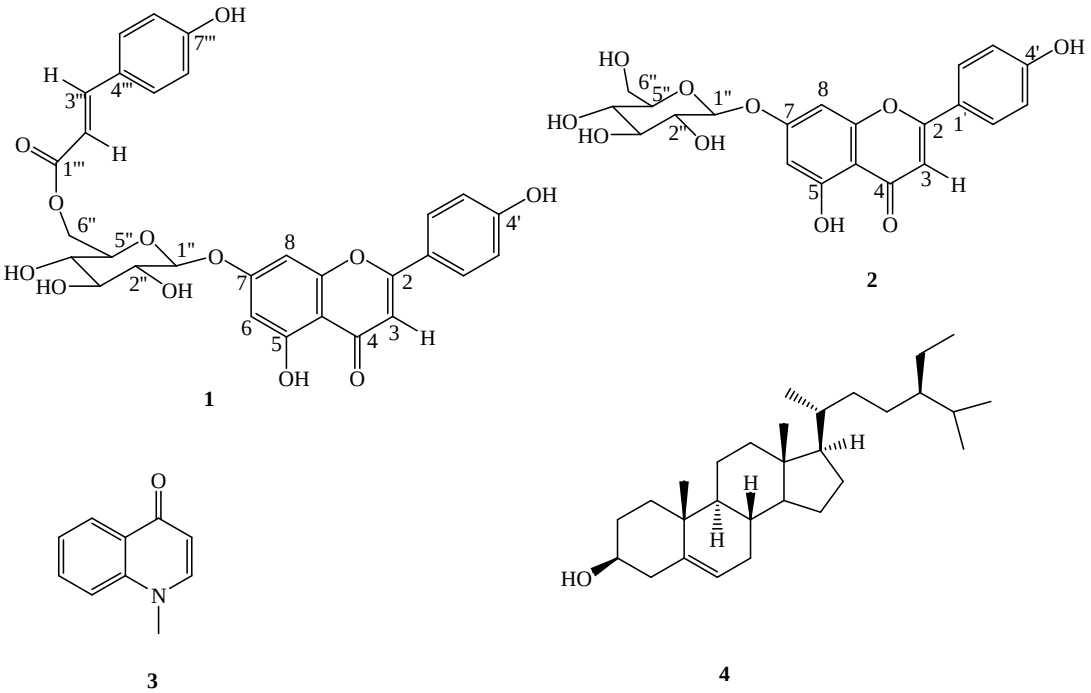

Figure 1. Structures of compounds isolated from *Echinopsorientalis* Trauv.

Compound **2** was obtained as yellow solid. Its molecular formula was established as $C_{21}H_{20}O_{10}$ by HPLC-QTOF (*m/z* 431.1160 $[M-H]^-$), calcd. (431.0976). IR (KBr) spectrum showed the presence of hydroxy group (3345 cm^{-1}), carbonyl group (1635) and aromatic ring (1605, 1512, 1485 cm^{-1}). The ^{13}C (APT) NMR spectrum confirmed the presence of 21 carbons. The comparison of the spectroscopic data with the literature also confirmed the proposed structure [14]. A well-known compound, existing in many plants, isolated from the seeds was *β*-sitosterol **4** [15, 16] (Figure).

3.2. Antioxidant activity

Antioxidant capacity is a significant indication for medicinal bioactivity and functional components in food industry. In this study, the antioxidant activities of the crude extract (seeds, leaves and stem) and isolated compounds (**1**, **2** and **3**) from the corresponding extract were assayed and were compared to BHA, BHT, trolox for positive control.

3.3. DPPH free radical scavenging activity

The DPPH assay was based on the measurement of altering the purple color to yellow of DPPH radical at 517 nm after reaction with antioxidant compound. The effect of antioxidants on DPPH radical was thought to be due to their hydrogen donating ability. DPPH·is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [17]. Even though the isolated compounds (1, 2, 3, 4) didn't exhibit the significant activities, the extracts of seeds, leaves were potentially active and presented consequential and concentration-dependent DPPH radical-scavenging ability. This may be due to the synergic effect of molecules in seeds and leaves.

3.4. ABTS radical cation decolorization assay

The antioxidant ability of extracts and isolated compounds to scavenge the blue-green colored ABTS radical cation was measured relative to the radical scavenging ability of BHA, BHT and Trolox. The result clearly indicates that flavone **2** has an interesting ABTS radical cation scavenging activity besides flavone **1**. Flavonoids are very effective antioxidants and they protect the cardiovascular and oxidative disease. They have the ability to modulate the activity of various enzymes and interactions with specific receptors [18]. The presence of hydrogen of hydroxyl groups in flavones **1** and **2**, able to reduce free radicals and delocalization of unpaired electron leads to the formation of a stable phenoxyl radical. The seeds and leaves extracts also exhibited the moderately activities of IC_{50}. Total phenolic compounds decreased in an order of seeds > leaves > steam, therefore exhibition of high ABTS radical cation scavenging activity of seeds extract compared to the leaves and steam extracts could be attributed to the phenolic compounds which the seeds extract has the most.

3.5. Ferric ions (Fe^{3+}) reducing antioxidant power assay

The reducing capacity of a sample may serve as a significant indicator of its potential antioxidant activity. Reducing Power activity of an antioxidant compound has been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. In reducing power assay, the extracts and isolated compounds didn't exhibit significant activity.

3.6. Determination of total phenolic compounds

Phenols and related compounds have antioxidant potentials due to the hydroxyl groups which the acidic protons could be donated easily [19]. 76.59, 45.18 and 9.19 g gallic acid equivalent of phenols were detected in the seeds, leaves and stem extracts respectively. Among the extracts, seeds extracts contain the most phenolic compounds; therefore it exhibited the most antioxidant activity.

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Supporting Information

Supporting Information accompanies this paper is available.

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