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Chemical constituents from the aerial parts of *Echinops integrifolius*

The paper investigated the chemical constituents from the aerial parts of *Echinops integrifolius* Kar. & Kir., four triterpenes, three flavonols, three phytosterol, along with three other compounds were isolated and identified by using of spectra. Among them, triterpenes of cycloartane type were found from the genus *Echinops* for the first time. In addition, anti-tumor activities of the extracts and some compounds were studied for the first time.

Key words: *Echinops integrifolius*; Flavonoids; Triterpenes;

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Echinops integrifolius өсімдігінің жер үсті бөлігінің химиялық құрамы

Бұл мақалада *Echinops integrifolius* Kar. & Kir., өсімдігінің жер үсті бөлігінің химиялық құрамы зерттеліп, Идентифицирлық спектр көмегімен төрт тритерпен, үш флавонол, үш фитостерин, сонымен қатар басқада үш қоспа бөлініп алынғаны баяндалады. Бұлардың ішінде Лакса туысынан циклоартандардың тритерпендері бірінші рет табылып, бұдан басқа ісікке қарсы белсенді экстракттар және кейбір қоспалар тұңғыш рет зерттеліп отыр.

Түйін сөздер: *Echinops integrifolius*; Флавоноидтар ; Тритерпендер;

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Химические составные от надземных частей *Echinops integrifolius*

В статье исследовали химические компоненты из надземных частей *Echinops integrifolius* Kar. & Kir., Четыре тритерпены , три флавонолы , три фитостерин , наряду с тремя другими соединениями были выделены и идентифицированы с помощью спектров . Среди них, тритерпены из циклоартанового типа были найдены из рода мордовника впервые . Кроме того, противоопухолевые активности экстрактов и некоторые соединения были изучены в первый раз .

Ключевые слова: *Echinops integrifolius*; Флавоноиды ; Тритерпены ;

Subject and Source

Echinops integrifolius Kar. & Kir. belonging to the genus *Echinops*, compositae family, is a kind of perennial herb growing in Mongolia, Altai of Russia and Xinjiang of China primarily. The aerial parts of the plant were collected from China, in August 2011 and authenticated by Prof. Bolat – **Previous work**

The genus *Echinops* (Asteraceae) belongs to the Cynareae Lam. tribe (¼ Cardueae Cass.) (Susanna et al., 2006) and consists of about 120

species that are distributed in tropical Africa and in temperate areas of Europe and Central Asia. This genus has been widely investigated, with numerous compounds isolated from various species. Including *Rhaponticum uniflorum* (L) DC., *Echinops grijisii* Hance, *E. latifolius* Tausch., *E. przewalskii* Iljin, *E. gmelinii* Turcz., *E. ritro* L., *E. talassicus* Golosk. and *E. integrifolius* Kar. & Kir. etc.

Echinops (Compositae) is widespread in north China and has been recorded in Chinese Pharmacopoeia (1995 Edition). *Echinops* is

one of the commonly used traditional Chinese medicine. It used an evil-heat clearing, detoxifying, swelling-subduing, pus-draining and lactation promoting agent (Morphological et al., 1994). The active constituents of the genus *Echinops* focuses on alkaloids, thiophenes, triterpenes and flavonoids with anti-tumor, anti-virus and so on (Wu, 2007). However, rarely phytochemical study has been undertaken on the species *Echinops integrifolius* (François Senejoux et al., 2013).

Present study

The powdered of *Echinops integrifolius* (10kg) were extracted with 70% ethanol (v/w) for 2 h. The ethanol extract was concentrated to syrup in vacuo after removal of solvents, the syrup was suspended in water and then partitioned sequentially with

petroleum ether, ethyl acetate and n-butanol for three times to yield three extracts.

The petroleum ether extract (256g) was subjected to a silica gel column chromatography using petroleum ether – ethyl acetate step gradient as eluent, petroleum ether – ethyl acetate (PE-EtOAc) (100:0) to yield β -amyrin acetate (**1**, 500mg); PE-EtOAc (100:1), Lupeol (**2**, 26mg); PE-EtOAc (100:2), β -sitosterol (**3**, 1g); PE-EtOAc (100:8), (23Z)-9,19-cycloart-23-ene-3 α ,25-diol (**4**, 18mg); The fraction of PE-EtOAc (7:1) was subjected to a silica gel column chromatography using petroleum ether – acetone step gradient as eluent, PE-acetone (5:1) to obtain Jaceidin(**5**, 13mg); The fraction of PE-EtOAc (6:1) was subjected to a silica gel column chromatography using petroleum ether – acetone

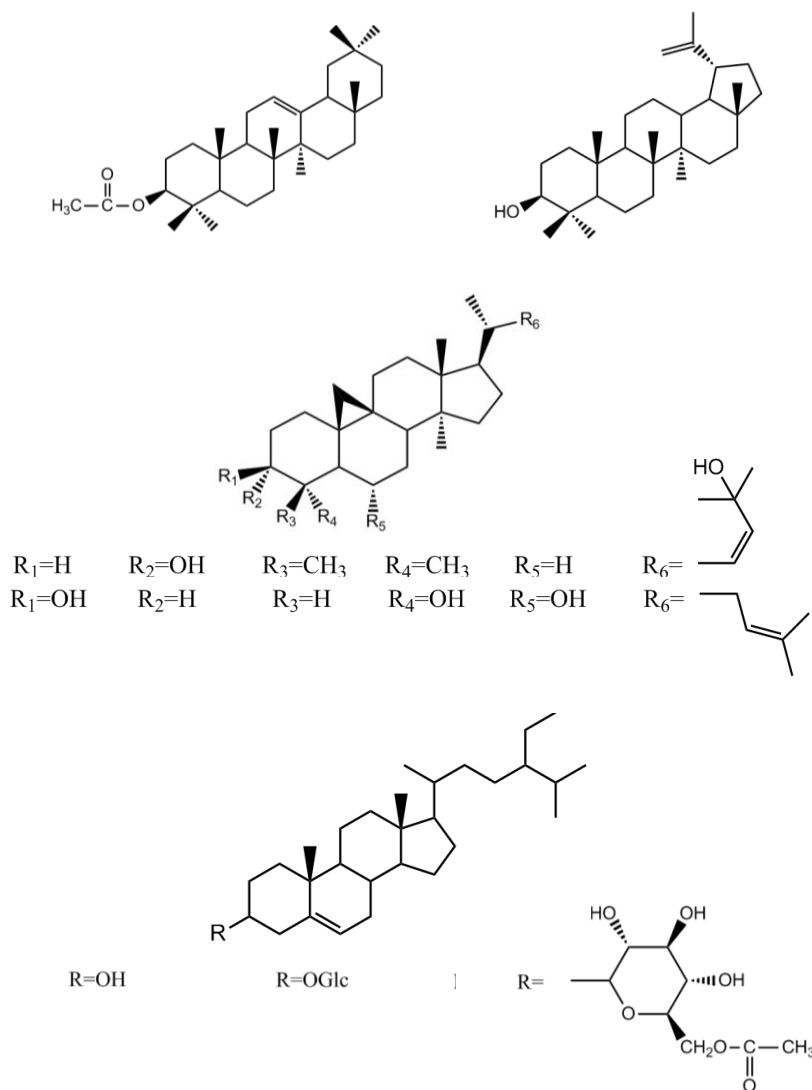


Figure 1 – Chemical structures of the constituents from *Echinops integrifolius*. (I)

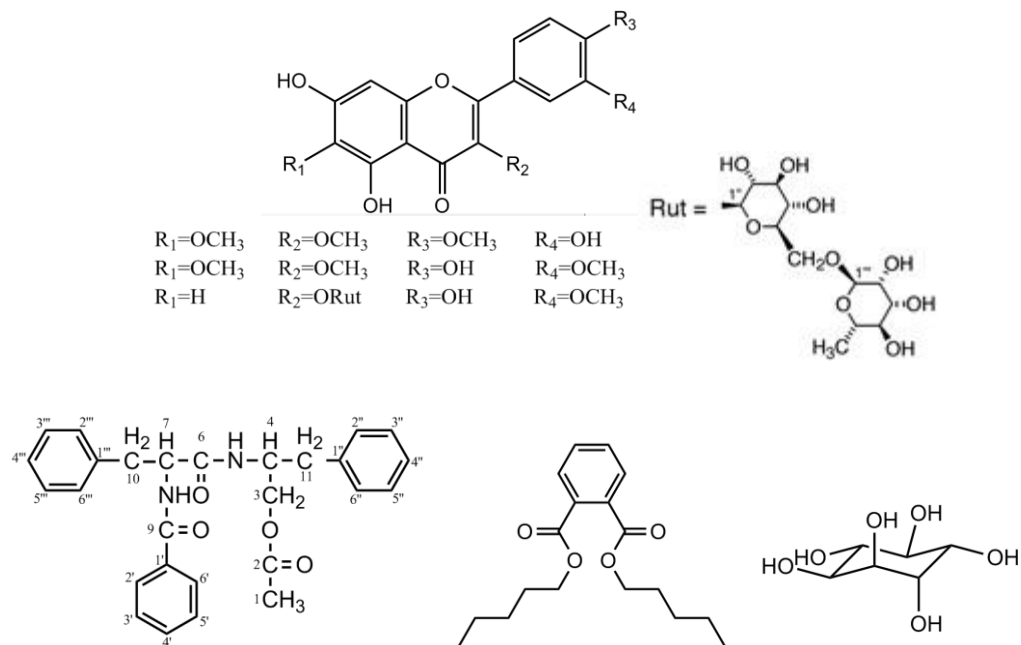


Figure 2 – Chemical structures of the constituents from *Echinops integrifolius*. (II)

step gradient as eluent, PE-acetone (8:1) to obtain Aurantiamide acetate (**6**, 9mg); The fraction of PE-EtOAc (5:1) was subjected to a silica gel column chromatography using petroleum ether – acetone step gradient as eluent, PE-acetone (100:2) to afford Dipentyl phthalate (**7**, 20mg); The fraction of PE-EtOAc (3:1) was subjected to a silica gel column chromatography using petroleum ether – acetone step gradient as eluent, PE-acetone (2:1) to afford 28,29-bis-Norcycloarten-3 β ,4 α ,6 α -triol (**8**, 10mg); The fraction of PE-EtOAc (2:1) was subjected to a silica gel column chromatography using petroleum ether – acetone step gradient as eluent, PE-acetone (5:1) to afford β -sitosterol-3-O- β -D-glucoside-6'-acetate (**9**, 17mg).

The ethyl acetate extract (60g) was subjected to a silica gel column chromatography using ethyl acetate – Ethanol step gradient as eluent, ethyl acetate – Ethanol (EtOAc-EtOH) (100:0) to yield Daucosterol (**10**, 500mg); The fraction of EtOAc-EtOH (100:0) was subjected to a silica gel column chromatography using Dichloromethane – Methanol step gradient as eluent, CH_2Cl_2 – CH_3OH (100:1) to obtain Centaureidin(**11**, 30mg); EtOAc-EtOH (100:10), L-chiro-Inosito (**12**, 30mg).

The butanol extract (190g) was subjected to a silica gel column chromatography using ethyl acetate – Ethanol step gradient as eluent. The

fraction of EtOAc-EtOH (100:5) was subjected to a silica gel column chromatography using Dichloromethane – Methanol step gradient as eluent, CH_2Cl_2 – CH_3OH (100:12) to obtain Isorhamnetin 3-rutinoside (**13**, 30mg).

The structures of all isolated compounds were elucidated by analysis of their spectral data (^1H NMR, ^{13}C NMR and mp.) and by comparison with the literature. They were identified as β -amyrin acetate (**1**) (Jin, Li et al., 2008), Lupeol (**2**) (Jamal et al., 2008), β -sitosterol (**3**) (XIE Qing-lan et al., 2006), (23Z)-9,19-cycloart-23-ene-3 \square ,25-diol (**4**) (Pi, Hui-Fang et al., 2009), Jaceidin(**5**) (Flamini et al., 2001), Aurantiamide acetate(**6**) (FENG Bao-min et al., 2011), Dipentyl phthalate (**7**) (W Suchanski et al., 1999) 28,29-bis-Norcycloarten-3 β ,4 α ,6 α -triol(**8**) (G. Brader et al., 1998), β -sitosterol-3-O- β -D-glucoside-6'-acetate (**9**) (LIU Gui-yan et al., 2005), Daucosterol(**10**) (Wang Jun-Ru et al., 1999), Centaureidin (**11**) (Flamini et al., 2001), L-chiro-Inositol (**12**) (Juan C. Argoti et al., 2013) and Isorhamnetin-3-rutinoside (**13**) (Yinrong Lu et al., 2000) (Fig. 1 and 2).

Chemotaxonomic significance

In the present study, three triterpenes (**1**, **4**, **8**), three phytosterol (**3**, **9**, **10**), one flavonols (**13**), along with three other compounds (**6**, **7**, **12**) were identified for the first time from *E. integrifolius*.

Simultaneously, in this study, we report for the first time, two triterpenes (**4**, **8**), one phytosterol (**9**), one flavonols (**13**), and three other compounds (**6**, **7**, **12**) were identified from the *Echinops* genus.

In addition, anti-tumor activities of the extracts and some compounds were studied for the first time. Part of above compounds was studied for the inhibiting the activity of lung cancer cells. Experimental results show that it have a very good effect to different type of lung cancer cells: the effect of inhibition of **4** is the most prominent than other compounds in 20 μ mol/L, the inhibition rate of H292 lung cancer cells reached 83.53%, and the inhibition rate of H1299 lung cancer cell lines was 65.89%, as well as the inhibition rate of H1944 lung

cancer cells was 61.17%. What's more, it should be noted that Cycloartane-type triterpene have not so far been reported to occur in other species of *E. integrifolius*, nor in other genera of the *Echinops* group, and could serve as the chemical marker to distinguish *E. integrifolius* from other genera of the *Echinops* group.

The mentioned information above might give some chemotaxonomic support for the investigation of the genus *Echinops*.

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