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Original Research

Terpenes from *Ilex Spinigera* (Loes.) Loes., an Endemic Plant of Iran

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Abstract

Ilex spinigera (Loes.) Loes widely distributed in north of Iran, has been traditionally used in malaria treatment. Up to now, this plant has not been subjected to detailed chemical constitution analysis. This paper reports the first phytochemical analysis of leaves of *I. spinigera*. collected in the Hyrcanian deciduous forests of N Iran. Chromatographic investigation of the methanol extract of the air dried leaves of *I. spinigera* afforded several triterpenes including lupeol, β-amyrin, lanosterol, taraxasterol, moretenol together with podosporin A and sandaracopimaradiene. Chemical structure of compounds were established using spectroscopic data (^{1}H-NMR, ^{13}C-NMR and MS), and by comparing with the literature. All of these compounds were isolated for the first time from this plant and have known pharmacological activities.

Keywords: *Ilex spinigera*, terpene, Aquifoliaceae, taraxasterol

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Introduction

Ilex spinigera (Loes.) Loes. (Aquifoliaceae) is an evergreen shrub in the Hyrcanian deciduous forest, N. Iran [1]. This endemic plant, similar to *Ilex aquifolium* in Europe [2], possesses relatively small, oblong, glossy, and spine margined leaves and ecologically confined in shady

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forests in various elevational belts of northern Iran [1-3]. *Ilex spinigera* has been used traditionally in northern Iran for malaria treatment. There is only one report on antibacterial activity of *I. spinigera* [4]. There are several phytochemical studies on other *Ilex* species. Various compounds such as anthocyanins, flavonoids, amino acids, alkaloids, fatty acids, alcohols, carotenoids, cyanogenic glucoside and phenols were isolated from *Ilex* species [5-9]. Among these compounds, triterpene saponins are commonly recognized major constituents of plant of *Ilex* genus [10, 11] and have been proved to be bioactive constituents closely related to the pharmacological activities of protecting the cardiovascular system [12, 13]. To our knowledge, there are not yet studies about chemical composition of *I. spinigera*. The present paper describes the isolation and identification of terpenes from the leave of *I. spinigera*.

Methods

General

NMR spectra were recorded using Bruker 500 MHz NMR spectrometers. MS spectra were performed on a Q-TOF MICRO spectrometer (Micromass, now Waters, Manchester, UK) equipped with an ESI source, that operated in the negative and/or positive ion mode. The flow rate of sample infusion was 10 μL/min. with 100 acquisitions per spectrum. Data were analysed by using the MassLynx software developed by Waters. All solvents used were of analytical grade.

Plant material

The studied samples were collected on March 2014 in the forests of Chamestan, Mazandaran province. The botanical identification of the species was performed by one of us (A. Naqinezhad). The voucher specimen of the studied plants is stored in our laboratory for further references and registered under the accession number 3019 MUH (Mazandaran University Herbarium).

Extraction and isolation

The air-dried and powdered leaves of *I. spinigera* (2 kg) were extracted with methanol (3*5 L, each 3 days) at room temperature and filtered. The filtrate was evaporated to give a residue, which was suspended in H_2O (2 L) and then extracted with petroleum ether (b.p. 60-90 ^oC, 3*1 L) and dichloromethane (DCM) (4*1 L), successively. The DCM residue was subjected to silica gel CC (100–200mesh, 1.5 kg), eluted with hexane-chloroform (1:0-0:1 gradient system)to obtain fractions A-D. After repeated silica gel CC (hexane-chloroform 1:1), Fraction A (16 g) afforded compounds 1 (213 mg), 2 (253 mg), 3 (187 mg), 4 (42 mg) and 5 (43 mg). Fraction C (21 g) was also applied to a silica gel eluted with (hexane-chloroform 1:1) to provide 6 (12 mg) and 7 (9 mg).

Results

The methanol extract of the leave of *I. spinigera* plant dry powder is partitioned successively with petroleum ether and dichloromethane (DCM). DCM fraction were subjected to repeated separation on silica gel column chromatography, affording lupeol (**1**) [14], β-amyrin (**2**) [15], lanosterol (**3**) [16], taraxasterol (**4**) [17] moretenol (**5**) [18] podosporin A (**6**) [19] and sandaracopimaradiene (**7**) [20] (Figure 1). Identification of compounds was done by comparison of their spectroscopic data with those reported in literatures. All of these compounds are known chemicals and isolated for the first time from this plant.

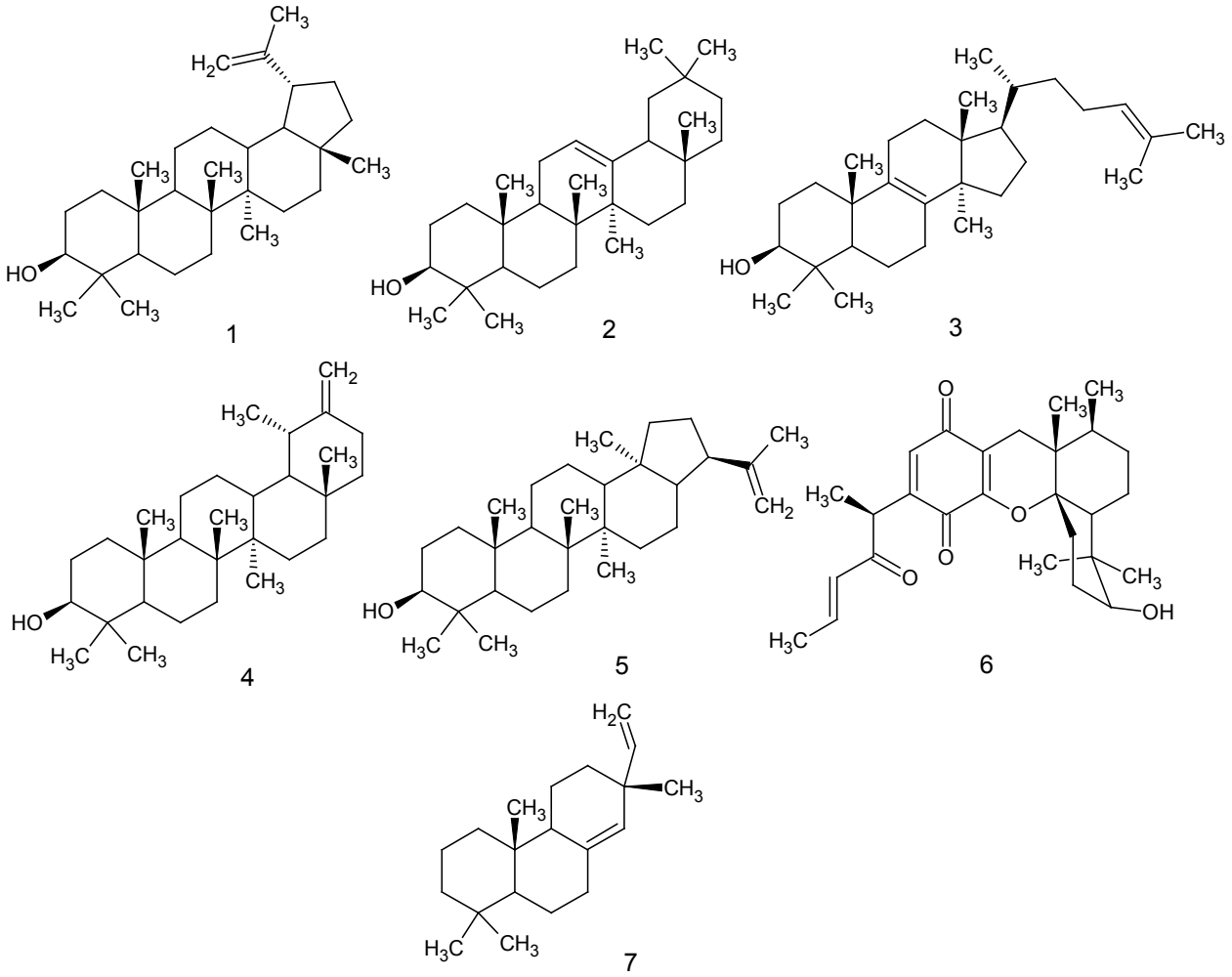

Figure 1: Chemical components of *I. spinigera*

Discussion

In this research, two groups of terpenes were isolated from *I. spinigera* including triterpenes (lupeol, β-amyrin, lanosterol, taraxasterol), diterpenes (sandaracopimaradiene) and one tetracyclic sesquiterpene quinone (Podosporin A). Isolated sterols at this study including Lupeol, β-amyrin and lanosterol have vast occurrence in diverse plant families [21] and display widely pharmacological activities [14, 22, 23]. Taraxasterol is found in alcoholic beverages. Taraxasterol is a constituent of dandelion roots (*Taraxacum officinale*), Roman chamomile flowers (*Anthemis nobilis*). This compound has been shown to exhibit anti-microbial function. The distribution of taraxasterol in plants is not extensive, but the biological activity of this compound is very interesting [24]. Podosporin A displays potent activity against the earlysuccessional coprophilous fungi [18] and also displays potent brine shrimp toxicity [25]. Sandaracopimaradiene have significant antimicrobial and antispasmodic properties [19]. These findings suggest that this plant can be a potentially valuable source of antimicrobial and cytotoxic agents. In relation with using of this plant as an antimalarial agent, various studies showed that terpens exhibit antiplasmodial activities [26, 27]. The results of the current study indicate that afro-mentioned terpens might display anti-

malarial activity.

On the other hand, triterpene saponins are commonly recognized major constituents of *Ilex* species [10, 11]. Several isolated compounds from the genus *Ilex* have been reported to possess interesting bioactivities. Triterpenes from *I. kudincha* and *I. cornuta* [28] exhibit inhibitory activity on acyl CoA cholesteryl acyl transferase (ACAT). Adenosine, isolated from *I. cornuta*, enhances coronary blood flow [29]. Triterpene saponins from *I. oblonga* inhibit tobacco mosaic virus (TMV) [30]. The more researches on this endemic plant are on-going in our laboratory.

Conflict of Interests

Authors have no conflict of interests.

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None.

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