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RESEARCH ARTICLES

Search for Antifungal Compounds from Some *Verbascum* Species Growing in Turkey

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Search for Antifungal Compounds from Some* Verbascum *Species Growing in Turkey

Summary : *Turkish medicinal plants provide a rich source of biologically-active natural products. With the use of simple bioassays, it is possible to evaluate the bioactivity of the natural compounds. Antifungal screening of 16 compounds in a matrix format from two* Verbascum *species growing in Turkey was conducted directly on thin-layer chromatographic (TLC) plates sprayed with a spore suspension. Compounds possessing strong antifungal activity produced a clear zone of inhibition bounded by a sharp margin regardless of the size of the inhibitory zone. Ilwensisaponin A and C from* Verbascum pterocalycinum *var. mutense Hub.-Mor. were found to be active. Bioautographic assay indicated that the saponins appeared to be the most effective against* Colletotrichum acutatum, C. fragariae *and* C. gloeosporioides

Keywords: Medicinal plants, *Verbascum lasianthum, Verbascum pterocalycinum* var. mutense, *Scrophulariaceae*, Bioactive Compounds, Saponins, Ilwensisaponin A, Ilwensisaponin C, Antifungal Activity

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Türkiye'de Yetişen Bazı* Verbascum *Türlerinden Elde Edilen Antifungal Bileşiklerin Araştırılması

Özet: *: Türkiye'de yetişen tıbbi bitkiler, biyolojik olarak aktif doğal ürünlerce zengin bir kaynak oluşturmaktadırlar. Basit biyolojik tarama metodları kullanarak, doğal bileşiklerin biyolojik aktivitelerini belirlemek mümkündür. Türkiye'e yetişen iki* Verbascum *türünden elde edilen 16 bileşiğin antifungal aktivite taraması, spor süspansiyonu püskürtülmüş ince tabaka kromatografisi (İTK) plaklarına direkt olarak uygulanmaları ile yapılmıştır. Kuvvetli antifungal aktiviteye sahip bileşikler, inhibisyon zonlarının boyutları gözetilmeksizin keskin sınırları olan temiz bir zon vermişlerdir.* Verbascum pterocalycinum *var. mutense'den elde edilen ilwensisaponin A ve C aktif bulunmuştur. Biyootografik metot ile, saponinlerin* Colletotrichum acutatum, C. fragariae *ve* C. gloeosporioides*'e karşı en fazla etkiyi gösterdiği görülmüştür*

Anahtar kelimeler: Tıbbi Bitkiler, *Verbascum lasianthum, Verbascum pterocalycinum* var. *mutense, Scrophulariaceae,* Biyoaktif Bileşikler, Saponinler, İlvensisaponin A, İlvensisaponin C, Antifungal Aktivite

INTRODUCTION

In many developing countries, plants constitute the main medicines used in health care practice[1]. Most of the plants are regularly used as traditional medicine for the treatment of various diseases in Turkey[2,3]. Consequently, it is important to evaluate the utilized plants and remedies in order to gain more knowledge about the efficacy of this approach. The choice of plant material for such studies can be guided by consulting traditional healers, herbalists or the documented information[1].

Verbascum, commonly known as "mullein", is a widespread genus of the family Scrophulariaceae, and is represented by 228 species (185 of which are endemic) in the flora of Turkey[4]. Some *Verbascum* species have been used as piscicide, antiseptic, astringent, demulcent, emollient, expectorant, sedative, narcotic, diuretic and antimalarial and as a treatment for tumors, inflammations, migraine, asthma and spasmodic coughs in Turkey, Europe, Asia and Northern America[3,5].

Some phytochemical studies on *Verbascum* species

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of Turkish origin have revealed the presence of iridoids, phenylethanoids[6], saponins and monoterpenoids[7]. Iridoids display an interesting spectrum of biological activity such as antiinflammatory[8] and antimicrobial[9]. Likewise, phenylethanoid glycosides also show a wide range of biological activity. They are known to possess antibacterial and antifungal activities[10]. Saponins are also well-documented as exhibiting a variety of biological activities, including antibacterial and antifungal activity[11].

Owing to the continuing development of the microbial-resistance in medicine and agriculture, the discovery of new antimicrobial substances is important[12]. Plant-derived constituents offer potential leads for the development of antifungal drugs effective against human pathogenic fungi[13]. In addition, the desire for safer agrochemicals with less environmental and mammalian toxicity is a major concern. Particularly desirable is the discovery of novel antimicrobial agents representing new and natural chemical classes that operate by different models of action from existing antifungal agents[12,14,15].

There is a growing interest in antifungal compounds as the occurrence of systematic mycoses associated with immunodeficiency diseases (such as AIDS) and the use of immunosuppressors is continually incrasing[1].

Therefore, it would be interesting to study the effect of the compounds from *Verbascum* species on medically important fungi for the development of new antifungal agents for the treatment of serious fungal infections, especially in immunosuppressed and immunocompromised patients.

As a part of our continuing search for bioactive agents from natural sources, we here have report the results of the antifungal activity by thin-layer chromatography (TLC)-bioautographic assay of the compounds from *Verbascum lasianthum* and *V. pterocalycinum* var. *mutense* in different classes.

MATERIALS and METHODS

Plant Materials

The research materials were collected from the following locations.

Verbascum lasianthum Boiss. ex Bentham: B1: İzmir: Urla, Üçahırlar Mevkii, August 1999.

Verbascum pterocalycinum var. *mutense* Hub.-Mor.: C4: İçel: Between Mut and Karaman, *Pinus brutia* and *Pinus nigra* fields, 930-1100 m, July 2000.

Voucher specimens were deposited in the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 99130 and HUEF 00184, respectively).

Microorganisms

Three fungal strains, *Colletotrichum acutatum* Simmonds, *C. fragariae* Brooks, and *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz., which are important plant pathogenic fungi, were used in the assay. The three *Colletotrichum* species were isolated from strawberry (*Fragaria x ananassa* Duchesne). Commercial fungicides vinclozolin, chlorothalonil and thiabendazole (Chem. Service, West Chester, PA) were used as validation controls in a microbioassay[15].

Inoculum Preparation

Conidial suspensions were prepared according to published procedures[8]. Conidial concentrations were determined spectro photometrically[16,17] from a standard curve, and suspensions were then adjusted with sterile distilled water to a concentration of 1.0 x 106 conidia ml^{-1}.

Antifungal Assay

Inhibition of fungal growth on chromatographic plates was evaluated by modifications of TLC bioa-

utographic assays[18,19]. Each sample was dissolved in MeOH and commercial fungicide standards in 95% EtOH. Each test compound was applied on glass-backed silica gel plates from stock solutions to achieve a final amount of 2 µg using a disposable glass micropipette for each sample. To detect biological activity directly on the TLC plate, silica gel plates with a fluorescent indicator were sprayed with conidial suspensions of *Colletotrichum acutatum, C. fragariae* and *C. gloeosporioides.* Aliquots of 25-50 ml of inoculum spray solution were prepared for each test fungus with liquid potato dextrose broth (PDB) containing 12 g /500 ml (PDB), 0.1% bacto agar, and 0.1% Tween-80. Using a 50 ml chromatographic sprayer, each plate was sprayed lightly (to a damp appearance) three times with conidial suspension. Clear inhibition zones were observed against a dark-grey background after four days incubation at room temperature in a humid atmosphere.

RESULTS AND DISCUSSION

Previously isolated iridoid glucosides, 6-*O*-(α-L-rhamnopyranosyl)-catalpol **(1)**, verbascoside A **(2)**, pulverulentoside I **(3)**, buddlejoside A_5 **(4)**, aucubin **(5)**, unduloside III **(6)**, 8-*O*-acethylharpagide **(7)**, harpagoside **(8)** and vanilloylajugol **(9)**, along with two phenylethanoid glycosides, verbascoside **(10)** and poliumoside **(11)**, from the roots of *Verbascum lasianthum*[6,20], and two saponins, ilwensisaponin A **(12)** and C **(13)**, together with two iridoid glycosides, ajugol **(14)** and picroside IV **(15)** and a monoterpene glucoside, 1-(β-D-glucopyranosyl)-8-hydroxy-3,7-dimethyl-oct-2(E),6(E)-dienoate **(16)**, from the flowers of *Verbascum pterocalycinum* var. *mutense*[7] were used in this study.

Compounds **1-16** were further evaluated for in vitro antifungal activity on TLC-bioautographic assay[18,19]. Active compounds appeared as clear spots against a colored background. In the test for fungicidal activity, the saponins, ilwensisaponin A and C, were found to exhibit some *in vitro* activity against *Colletotrichum acutatum, C. fragariae* and *C. gloeosporioides.*

The results of this survey thus showed that *Verbascum* species contain potentially bioactive saponins and that it is worth studying this antifungal assay for saponins.

CONCLUSION

To date, many saponins have been isolated and characterized from *Verbascum* species[7,21]. In Turkey and other countries, *Verbascum* species have long been utilized for medicinal purposes, and saponins are considered to have the active principles possessing such physiological activities[11]. Triterpene saponins are described as having interesting biological activities such as anti-inflammatory, molluscicidal and fungustatic[22]. Some structural features for antimicrobial activity of triterpene saponins have been documented in the literature: The strongest activities are exhibited by the monodesmosidic saponins. The availability of ester groups and an increase in the number of sugar units lead to an increase in activity[23]. Maximum activity is shown by monodesmosides with four or five monosaccharides. Glycosides with monosaccharide moiety in the position C-3 of the aglycone were strongly antimycotic. One theory is that the saponins themselves are inactive and comprise only water-soluble transport forms. In the presence of cell membrane glycosidases, there is formation of the aglycone which is the active membranolytic component. It must be emphasized that other mechanisms must also be involved, since there are exceptions to the parallel between cholesterol binding and fungicidal properties[11].

Therefore it is proven that the antifungal activity of ilwensisaponin A and C is closely related to their structure having a monodesmosidic with tetrasaccharidic moiety at C-3.

To our knowledge this is first report of the antifungal activity of ilwensisaponin A and C.

In order to investigate the plant species for other activities related to their traditional use, further bioassays will need to be performed.

Natural product research will contribute to the long-range improvement of US agriculture by enhancing our knowledge about disease control measures and host-pathogen interactions and may provide an opportunity for the discovery of new pharmaceutical agents to treat human diseases[16].

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