

Traditional Uses and Biological Activities of *Verbascum* Species

İ. İrem TATLI*^o, Zeliha Ş. AKDEMİR**

Traditional Uses and Biological Activities of *Verbascum* Species **Summary**

The extracts, decoctions and infusions of *Verbascum*, commonly known as "mullein", have been used in traditional medicines for centuries in almost all parts of the world. In this paper, the usage (as expectorant, mucolytic, demulcent, diuretic, to treat respiratory disorders, hemorrhoids, rheumatic pain, inflammatory skin conditions, wounds, fungal infections and diarrhea) and biological (antiviral, antimicrobial, antimalarial, antioxidant, anti-inflammatory, antinociceptive, antitumor, anticancer, cytotoxic, immunomodulatory, antiulcerogenic, antihepatotoxic, antihyperlipidemic, antitussive and antigermination) activities of *Verbascum* species are reviewed.

Key Words: *Verbascum* species, Scrophulariaceae, biological activity, traditional medicine.

Received □ : □31.01.2007

Revised □ : □20.06.2007

Accepted □ : □16.07.2007

***Verbascum* Türlerinin Geleneksel Kullanılışı ve Biyolojik Aktiviteleri** **Özet**

Sığırkuyruğu olarak bilinen *Verbascum* türlerinin ekstre, dekoksion ve infüzyonları, çok eski zamanlardan beri hemen hemen tüm dünyada halk ilacı olarak kullanılmaktadır. Bu derlemede, *Verbascum* türlerinin kullanılışı (ekspektoran, mukolitik, demülsan, diüretik; solunum yolu rahatsızlıkları, hemoroit, romatizmal ağrılar, enflamasyonlu deri rahatsızlıkları, yaralar, mantar enfeksiyonları ve diyare tedavisinde) ve biyolojik aktiviteleri (antiviral, antimikrobiyal, antimalaryal, antioksidan, anti-enflamatuvar, antinosiseptif, antitümör, antikanser, sitotoksik, immunomodulator, antiülserojenik, antihepatotoksik, antihiperlipidemik, antitussif ve antigerminasyon aktiviteler) hakkında bilgi verilmiştir.

Anahtar Kelimeler: *Verbascum* Türleri, Scrophulariaceae, Biyolojik aktivite, Geleneksel tıp

INTRODUCTION

There are many Mediterranean and Irano-Turanian *Verbascum* species in Turkish flora¹. *Verbascum* L. is the largest genus of the family Scrophulariaceae, with about 2500 species worldwide. This genus is represented by 228 species, of which 185 are endemic in the flora of Turkey and the East Aegean Islands¹. *Verbascum* species contain biologically active compounds, such as flavonoids, phenylethanoid and neolignan glycosides, saponins, and iridoid and monoterpene glycosides².

The leaves and flowers of *Verbascum* are reported to have expectorant, mucolytic and demulcent proper-

ties, and are used to treat respiratory disorders such as bronchitis, dry coughs, tuberculosis and asthma in traditional Turkish medicine. The species are also used to treat hemorrhoids, rheumatic pain, superficial fungal infections, wounds and diarrhea, and have inhibitory activities against the murine lymphocytic leukemia and influenza viruses A2 and B. The oil made from the flowers is used to help soothe earache and can be applied externally for eczema and other types of inflammatory skin conditions. These species are reported to be mildly diuretic and to have a soothing and anti-inflammatory effect on the urinary tract, as well as acting as a mild sedative. They are

* Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Botany, S>hh>ye, Ankara, 06100, TURKEY

** Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, S>hh>ye, Ankara, 06100, TURKEY

^o Corresponding author e-mail: itatli@hacettepe.edu.tr

traditionally consumed as a tea to relieve abdominal pains³⁻⁵.

The roots and aerial parts of *Verbascum cheiranthifolium* Boiss. var. *cheiranthifolium* and the aerial parts of *V. chrysochaete* Stapff have been used for eczema, rheumatism, earache, hemorrhoids and menstrual pain. In addition to the above-mentioned common uses for the *Verbascum* genus, without referring to a particular species, the flowers of *V. lasianthum* as well as the flowers and leaves of *V. symes* Murb. et Rech fil. are reported to be used for hemorrhoids in southwest Anatolia^{6,7}.

The aerial parts of *V. pumilum* Boiss. and Heldr. have been used for anal fistula - they are boiled in a cauldron and the anus is exposed to the vapors as desiccant for wounds; dried and powdered leaves are spread on wounds; and for abdominal pain and bronchitis, leaves are first washed and then decocted to be used as a tea. The flowers of *V. orientale* (L.) All. have been used for pruritic conditions in urogenital organs, while dried or fresh flowers are boiled in milk and applied externally⁸.

In Europe, Asia and Northern America, several *Verbascum* species have been reported as antiseptic, astringent, demulcent, emollient, expectorant, sedative, narcotic, diuretic and antimalarial and as a treatment for tumors, inflammations, migraine, asthma and spasmodic coughs⁹.

Verbascum species are used for different purposes in traditional medicine around the world; therefore, researchers have tested them for different types of biological activities. We present here an exhaustive review of the literature on the activity of *Verbascum* species throughout the world. Most tests have been performed on crude extracts without examining the nature of the active compounds. The results of these studies are listed below and include both positive and negative results.

Antiviral activity

The lyophilized infusion from the flowers of *V. thapsi-*

forme Schrad. (Scrophulariaceae) (FVI) showed antiviral activity in *in vitro* studies against fowl plague virus, several influenza A strains, influenza B strain, as well as against herpes simplex virus. Influenza virus titers decreased by 1-3 log units, and herpes simplex virus by 2.3 log. FVI has shown virucidal activity on herpes simplex virus at 300 µg/ml, but did not inactivate influenza viruses. Phytochemical investigations of FVI have shown the presence of flavonoids, iridoids, phenolic acids, saponins, amino acids and free sugars¹⁰.

On the other hand, the lyophilized infusion from the flowers of *V. thapsiforme* Schrad. (Scrophulariaceae) (FVI) reduced the infectious and hemagglutination yields of a range of influenza viruses in tissue cultures. Amantadine hydrochloride is an accepted and well-studied selective inhibitor of influenza virus reproduction. The combined application of the plant preparation FVI and three amantadine derivatives resulted in a marked enhancement of the inhibitory effect of FVI on the reproduction of influenza virus A/chicken/Germany/27, strain Weybridge (H7N7) in cell cultures of chicken embryo fibroblasts. The antiviral activity was determined by the difference in the infectious titers of control and treated viruses. The combined effect was defined on the basis of infectious viral yields. The most pronounced enhancement was shown for the combination of FVI and adamantanamine glucuronide¹¹.

The power of an antiviral agent is related to its capacity in being non-toxic for the host cell, since it is necessary to be sure that the inhibition observed is due only to a direct action in the viral replication. Thus, the toxic effect from the extracts was determined in monolayers of Vero cells. Positive toxicity was considered when the culture cells presented partial or complete loss of monolayer, rounding and shrinkage of cells, or granular appearance in the cytoplasm. On this basis, antiviral activity of the alcoholic extract of *V. thapsus* has been studied. The tests were carried out in Vero cells-pseudorabies virus strain RC/79 (herpes suis virus) system. Maximum non-cytotoxic concentration was 1.40 mg plant material *per* ml. The leaf extract of *V. thapsus* was able to inhibit the viral infectivity at 2

log¹².

In another study, 100 methanolic plant extracts were screened for their antiviral activity against seven viruses. Twelve extracts were found to have antiviral activity at the non-cytotoxic concentrations tested. The extracts prepared from *V. thapsus* exhibited antiviral activity against herpes virus type 1¹³.

Antimicrobial and antimalarial activities

The ever-increasing resistance of human pathogens to current antimicrobial agents is a serious medical problem, and has resulted in the need for novel antibiotic prototypes. Natural products derived from microorganisms have traditionally been the primary source for antibiotics, but recent research has proven that higher plants may serve as promising sources of novel antibiotic prototypes as well. Some species of the genus *Verbascum* have been used by mankind for millennia to treat internal and external infections. Hildegard of Bingen, the great abbess of the early 12th century, mentions the plant in the first book of her 'Physica' under 'De Wullenen', which probably referred to *Verbascum thapsus* L.^{14,15}.

The dichloromethane, ethanol:water (70:30 v/v), water and methanol extracts of *V. macrurum* leaves were tested for antimicrobial activity, and it was demonstrated that the ethanol:water extract was the most active¹⁶. In a similar study, the ethanolic extract of *V. qulebrum* was subjected to phytochemical screening and it was also evaluated against six microorganisms (*Staphylococcus aureus*, *Salmonella typhi*, *Saccharomyces pastorianus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*) in nutrient agar using disc agar method. This extract demonstrated the best spectrum of activity by inhibiting the growth of Gram (+) bacteria *B. subtilis* and the yeast *S. pastorianus*. The most sensitive was *S. pastorianus*, which was completely inhibited by *V. qulebrum* (10 mm)¹⁷. *V. sinaiticum* also exhibited intercellular broad spectrum antimicrobial activity against Gram (+) and Gram (-) bacteria, but no activity against the yeasts, *Candida albicans* and *C. tropicalis*¹⁸.

The extracts obtained from three *Verbascum* L. species (*V. olympicum* Boiss., *V. prusianum* Boiss., and *V. bombyciferum* Boiss.) were investigated for their antimicrobial activity. Growth inhibition, using agar disc diffusion assay, was determined against *E. coli* ATCC 11230, *Micrococcus luteus* La 2971, *S. aureus* ATCC 6538P, *S. typhi* ATCC 19430, *Klebsiella pneumoniae* UC57, *P. aeruginosa* ATCC 27893, *Corynebacterium xerosis* CCM 2824, *Bacillus cereus* ATCC 7064, *Bacillus megaterium* DSM 32, *Mycobacterium smegmatis* CCM 2067, *Proteus vulgaris* ATCC 8427, *C. albicans* ATCC 10231, *Rhodotorula rubra*, and *Saccharomyces cerevisiae* ATCC 9763. It was found that *Verbascum* L. species showed antimicrobial activity against the Gram (+) bacteria and the yeast, but no activity was seen against the Gram (-) bacteria. Antimicrobial activity was most consistently detected in the species *V. prusianum* Boiss., especially against *S. aureus* ATCC 6538P, *M. luteus* La 2971, *B. megaterium* DSM 32 and *C. albicans* ATCC 10231¹⁹.

The methanol extracts obtained from endemic *V. gypsicola* Vural & Aydogdu, *V. pseudoholotrichum* Hub.-Mor., *V. cymigerum* Hub.-Mor., *V. cholorostegium* Bornm. & Murb., *V. linguifolium* Hub.-Mor., *V. pellitum* Hub.-Mor., *V. protractum* Fenel ex Tchihat., *V. bellum* Hub.-Mor., *V. dalamanicum* Hub.-Mor. *V. chionophyllum* Hub.-Mor., *V. cilicium* Boiss., *V. trapifolium* (Stapf) Hub.-Mor., *V. meinckeanum* Murb. and *V. lyratifolium* Köchel were investigated for their antimicrobial activities. Antimicrobial activity was revealed against *E. coli* ATCC 11230, *S. aureus* ATCC 6538P, *K. pneumoniae* UC57, *P. aeruginosa* ATCC 27853, *P. vulgaris* ATCC 8427, *B. cereus* ATCC 7064, *M. smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, *M. luteus* CCM 169, *C. albicans* ATCC 10231, *R. rubra* DSM 70403, and *Kluyveromyces fragilis* ATCC 8608 by disc diffusion method. The *Verbascum* L. extracts had a strong antimicrobial activity against the Gram (+) bacteria and the yeast cultures used in this study²⁰⁻²³.

The methanolic extracts of the leaves, flowers, roots and seeds of *V. blattaria*, *V. bombyciferum*, *V. chaixii*, *V. dumulosum*, *V. nigrum*, *V. olympicum*, *V. phlomoides*, *V. phoeniceum* and *V. roripifolium* were studied for their antimicrobial activities. The extracts had a strong

antimicrobial activity against *E. coli* ATCC 11230, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 6538P and *C. albicans* ATCC 102¹⁴.

Barbour et al.²⁴ tested *in vitro* antimicrobial efficacy of the water and methanol extracts derived from different parts of indigenous wild plant species that have been commonly used in Lebanese folk medicine. The antimicrobial efficacy was determined using the single disc diffusion method, with 10 and 20 µl load extract volume *per disc*. Nine test microorganisms were used, namely: *E. coli*, *Proteus sp.*, *P. aeruginosa*, *Shigella dysenteriae*, *Salmonella enteritidis*, *S. typhi*, *S. aureus*, *Streptomyces faecalis*, and *C. albicans*. The percentage of test organisms that were susceptible to one of the most efficacious methanol plant extracts (10 and 20 µl/disc) was as follows: *V. leptostychem* flowers (11.1% and 99.9%, respectively) and leaves (22.2% and 66.6%, respectively). The water extract of *V. leptostychem* did not result in inhibition of the test organisms.

The hydroalcoholic extract of *V. sinaiticum*, traditionally used in the treatment of various skin disorders, was screened for its antimicrobial activity against *S. aureus* ATCC 6538, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *Aspergillus niger* ATCC 10535, *Trichophyton mentagrophytes* ATCC 18748, and *C. albicans* (clinical isolate), which are known to cause different types of skin infections. The tests were carried out using agar well diffusion method at three concentration levels (100, 50 and 25 mg/ml) of the crude extract. This work revealed that this species has a strong antibacterial activity against *S. aureus* and *P. aeruginosa*²⁵.

Turker and Camper⁴ studied the biological activity of common mullein (*V. thapsus* L.) extracts and commercial Mullein products using selected bench top bioassays, including antibacterial. The extracts were prepared in water, ethanol and methanol. Antibacterial activity (especially the water extract) was observed with *K. pneumoniae*, *S. aureus*, *Staphylococcus epidermis*, and *E. coli*.

Antimicrobial activities of the ethyl acetate and methanol extracts of five *Verbascum* species (the aerial parts

of *V. chionophyllum* Hub.-Mor., *V. cilicicum* Boiss., *V. pterocalycinum* var. *mutense* Hub.-Mor., *V. pycnostachyum* Boiss. & Helder. and *V. splendidum* Boiss.) as well as 22 secondary metabolites isolated from the methanolic extracts of *V. cilicicum* Boiss., *V. lasianthum* Boiss. ex Benth and *V. pterocalycinum* var. *mutense* Hub.-Mor. (Scrophulariaceae) were studied. The materials were tested *in vitro* against *C. albicans* (ATCC 90028), *Cryptococcus neoformans* (ATCC 90113), *S. aureus* (ATCC 29213), methicillin-resistant *S. aureus* (ATCC 43300), *P. aeruginosa* (ATCC 27853), *Aspergillus fumigatus* (ATCC 90906) and *Mycobacterium intracellulare* (ATCC 23068) using a 96-well microplate assay. Amphotericin B, ciprofloxacin and rifampin were used as positive controls. Ilwensisaponin A and C showed antimicrobial activity against *A. fumigatus* ATCC 90906, but no activity was seen against Gram (+) and Gram (-) bacteria or the yeasts used in this study; none of the tested extracts or the other compounds had important antimicrobial activities. Antimalarial activities of the same extracts and compounds were also tested to *Plasmodium falciparum* clone [Sierra Leone D6 (chloroquine-sensitive)]. The antimalarial agents chloroquine and artemisinin were used as positive controls. However, none of the tested extracts or the compounds showed antimalarial activities^{26,27}.

Antifungal screening of 16 compounds in a matrix format from two *Verbascum* species, namely *V. lasianthum* and *V. pterocalycinum* var. *mutense*, growing in Turkey, was conducted directly on thin-layer chromatographic (TLC) plates sprayed with a spore suspension. Compounds possessing a strong antifungal activity produced a clear zone of inhibition bounded by a sharp margin regardless of the size of the inhibition zone. Ilwensisaponin A and C from *V. 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*²⁸.

Combined extracts of *Verbascum* flower, *Mentha piperita* and *M. crispa* leaves as well as *Cynobata* fruits are bactericides and also agents that improve the defense of the human organism against diseases. The mixtures of the plant tissues were exhausted with ethanol and

water. The extract was bactericidal in vitro against *S. aureus*, *Sarcina lutea*, *Shigella flexneri* and other microorganisms²⁹.

The aqueous extract of the aerial parts of *V. fruticulosum* demonstrated a strong growth inhibition (26.9%) towards the malaria parasite *P. falciparum*³⁰.

Antioxidant activity

Antioxidant properties of various fractions of the methanolic extract obtained from the aerial parts of *V. macrurum* have been determined by monitoring their capacity to scavenge the stable free-radical diphenylpicrylhydrazyl (DPPH). They were also evaluated as natural preservatives against oxidative rancidity using the accelerated Rancimat method. Their activities, expressed as protection factor (PFR), indicated that the fractions rich in phenylpropanoid glycosides were more potent compared to α -tocopherol and of the same magnitude as butylated hydroxytoluene (BHT), which were used as references. Ten natural compounds were identified as the components of this methanolic extract. Assessment of their antioxidant activities established that acteoside was the most potent free radical scavenger and showed the highest protection factor against sunflower-oil-induced oxidative rancidity. Its activity is comparable to the synthetic antioxidant BHT and clearly superior to natural α -tocopherol. This compound, therefore, represents a very interesting candidate for use in food preservation as a natural protecting agent against oxidative rancidity³¹.

The methanolic extract of *V. wiedemannianum* Fisch. & Mey., which is endemic to Turkish flora, and its phenylethanoid glycosides, wiedemannioside A-C, acteoside, martynoside, echinacoside and leukoseptoside B, were screened for possible in vitro antioxidant activity by two complementary test systems, namely DPPH free radical-scavenging (by bioautography and spectrophotometry) as well as β -carotene/linoleic acid test system. In the first case, *V. wiedemannianum* extract exerted an insignificant antioxidant activity with an IC₅₀ value of 117 \pm 0.56 μ g/ml when compared to synthetic antioxidant BHT

(18.0 \pm 0.40 μ g/ml). The compounds demonstrated scavenging properties toward the DPPH radical in TLC autographic assays. In the β -carotene/linoleic acid test system, *V. wiedemannianum* exhibited antioxidant activity with 52.5 \pm 3.11% inhibition rate. Antioxidant activities of curcumin and ascorbic acid were also determined as positive controls in parallel experiments^{32,33}.

Natural compounds are receiving increasing attention as potential antioxidants. For this purpose, four flavonoid glucosides (apigenin-7-O- β -glucopyranoside, luteolin-7-O- β -glucopyranoside, luteolin-3'-O- β -glucopyranoside, and chrysoeriol-7-O- β -glucopyranoside), five phenylethanoid glycosides (verbascoside, b-hydroxyacteoside, forsythoside B, angoroside A, and martynoside) as well as two neolignan glycosides (dehydrodiconiferyl alcohol-9¹-O- β -D-glucopyranoside and dehydrodiconiferyl alcohol-9-O- β -D-glucopyranoside) were isolated from the aerial parts of *V. salviifolium*. Additionally, harpagoside, 6-O-vanilloylajugol, and poliumoside were isolated from the roots of *V. lasianthum* Boiss. ex Benth. These compounds demonstrated scavenging properties toward the DPPH radical in TLC autographic assays. They were found to have significant antioxidant properties, based on the experiments with DPPH, which indicated their ability to efficiently scavenge free radicals³⁴⁻³⁶.

Free radical scavenging and cell-aggregation inhibitory activities of 36 secondary metabolites isolated from the methanolic extracts of *V. cilicicum* Boiss., *V. lasianthum* Boiss. ex Benth., *V. pterocalycinum* var. *mutense* Hub.-Mor., and *V. salviifolium* Boiss. (Scrophulariaceae) were investigated. The isolated compounds, 6-O-vanilloyl ajugol, ilwensisaponin A, ilwensisaponin C, verbascoside, b-hydroxyacteoside, martynoside, poliumoside, forsythoside B, angoroside A, dehydrodiconiferyl alcohol-9-O- β -D-glucopyranoside, dehydrodiconiferyl alcohol-9'-O- β -D-glucopyranoside, apigenin 7-O- β -glucopyranoside, luteolin 7-O- β -glucopyranoside, luteolin 3'-O- β -glucopyranoside and chrysoeriol 7-O- β -glucopyranoside exhibited a dose-dependent inhibition of bioautographic and spectrophotometric DPPH activities. Verbascoside

was the most active with IC₅₀ value of 4.0 µg/ml in comparison with vitamin C (IC₅₀ 4.4 µg/ml) to inhibit phorbol 12-myristate 13-acetate (PMA)-induced peroxide-catalyzed oxidation of 2', 7'-dichlorofluorescein (DCFH) by reactive oxygen species (ROS) within human promyelocytic HL-60 cells. Ilwensisaponin A (MIC 6.9 mg/ml) showed moderate *in vitro* activity on lymphocyte-associated antigen-1 (LFA-1)/intercellular adhesion molecule-1 (ICAM-1) mediated aggregation using HL-60 cell line, where positive control was cytochalasin B (MIC 2.3 µg/ml). Nevertheless, none of the other compounds possessed free radical scavenging or cell-aggregation inhibitory activities³⁷.

Pharmaceutical forms, such as capsules, tablets, a dried form as in a tea, a diluent or any delivery system prepared from the extract of *V. thapsus*, are used for the treatment of lung conditions or other degenerative conditions due to aging because of their essential antioxidant ingredients³⁸.

Antiinflammatory and antinociceptive activities

Infusions of *V. lasianthum* Boiss. ex Benth flowers have been used for hemorrhoids in Turkish folk medicine⁶. In order to evaluate the scientific basis for this practice, *in vivo* anti-inflammatory and antinociceptive activities of *V. lasianthum* flowers were investigated. The methanolic extract of the flowers was shown to possess significant inhibitory activity in the carrageenan-induced hind paw edema model and in p-benzoquinone-induced writhings in mice. Through bioassay-guided fractionation and isolation procedures, eight compounds (6-O-(4'''-O-trans-*p*-coumaroyl)- α -L-rhamnopyranosylaucubin, 6-O-(4'''-O-trans-*p*-methoxycinnamoyl)- α -L-rhamnopyranosylaucubin, sinuatol, aucubin, geniposidic acid, catalpol, ajugol and ilwensisaponin A) were isolated and their structures were elucidated by spectral techniques. An iridoid glucoside, aucubin, and a triterpenoid saponin, ilwensisaponin A, were found to possess significant antinociceptive and anti-inflammatory activities, *per os* without inducing any apparent acute toxicity or gastric damage. Results of the present study support continuous utilization of this species

employed in Turkish folk medicine³⁹.

A general correlation was suggested between the anti-inflammatory and antitumor-promoting activities of acylated saponins from Scrophulariaceae plants by Tokuda et al.⁴⁰. In addition to antitumor activity, songarosaponins and their acylated derivatives, which were isolated from *V. songaricum*, showed anti-inflammatory activity against the croton oil ear model.

Antitumor, anticancer and cytotoxic activities

Common mullein (*V. thapsus* L.) is a herb with a long history of use in folk medicine and has been used for the treatment of inflammatory diseases, asthma, spasmodic coughs, diarrhea, and other pulmonary problems. The commercial popularity of this plant has been increasing for the past few years with the growing interest in herbs and preference for the 'greener' lifestyle. Today in health food stores in the United States, one can easily find dried leaves and flowers, swallow capsules, alcohol extracts and flower oil of mullein. Hence, the objective of the studies has been to assess the biological activity of common mullein extracts, which were prepared in water, ethanol, and methanol, and commercial Mullein products using selected bench top bioassays, antitumor and two toxicity assays (brine shrimp and radish seed). *Agrobacterium tumefaciens*-induced tumors in potato disc tissue were inhibited by all extracts. Toxicity to brine shrimp and radish seed germination and growth was observed at higher concentrations of the extracts^{4,5}.

The effect of the fractions isolated from the aqueous extract of the flowers of *V. thapsiforme* on protein biosynthesis was studied. A strong inhibitory effect of the aqueous extract on protein biosynthesis was demonstrated in isolated rat liver ribosomes. The saponin fraction was shown to be responsible for this activity and it was compared to commercial glycyrrhizic acid and its aglycon as the reference drug. It was found that these compounds strongly inhibited the incorporation of [¹⁴C]leucine into proteins *in vitro* and that the target site for inhibition was the ribosome fraction from rat liver cells⁴¹.

Some plants have long been used in folk medicine as sources of antitumor remedies. Their effects on protein biosynthesis *in vitro* have been examined and described. The separation features of the peptide elongation system, isolated from tumoral cells, have been demonstrated. Some elongation factors or ribosomes have been shown to be a target site for the inhibition of protein biosynthesis caused by the substances isolated from various sources. Saponin glycoside and its aglycon, isolated from *V. thapsiforme* flowers, as well as digoxin, emetine, and cephaline directly inactivated ribosomes. It may be supposed that the plant inhibitors of protein biosynthesis could be utilized for searching specific antitumoral preparations⁴².

Crude extracts from plants used in traditional medicine, including *V. pseudonobile*, and extracts from plant cell cultures have been screened for potential anticancer bioactive agents, using evaluation of DNA-interaction activity. Calf thymus DNA and pUC19 (ATCC 37254) *E. coli* plasmid was evaluated in order to optimize the employed test system. The results showed that of extracts and isolated compounds, 23% proved active in DNA-interaction. Ionkova and Alferman⁴³ also found that there was correlation in DNA-intercalation and the hemolytic effect in plant extracts, which contained triterpenoid saponins.

Hardman and his colleagues⁴⁴ tested the seeds of uncultivated plants for lectin activity. The alcoholic extract prepared from the seeds of *V. blattaria* was tested against human red cell samples. The extract agglutinated unmodified or enzyme-modified red cells.

3, 5-dihydroxy, 6, 7-dimethoxy flavone, useful as an antiasthmatic and antiallergic, isolated from *V. thapsus*, showed 24.8% inhibition of leukotriene biosynthesis in guinea pig ileum at 1.6×10^{-5} M⁴⁵.

Investigation of the leaves of *V. sinaiticum* has afforded two flavonolignans, hydrocarpin and the novel sinaiticin, as well as two flavones, chrysoeriol and luteolin. All compounds exhibited dose-dependent cytotoxicity when tested against cultured P-388 cells⁴⁶.

V. chionophyllum, *V. cilicicum*, *V. pterocalycinum* var. *mutense*, *V. pycnostachyum* and *V. splendidum* (Scrophulariaceae) were studied for their cytotoxic activities against SK-MEL, KB, BT-549, and SK-OV-3 cell lines. The results were evaluated by comparing cytotoxic activity in both their methanol and ethyl acetate extracts. The methanol extract of the flowers of *V. pterocalycinum* var. *mutense* showed a weak cytotoxic activity against SK-MEL cell line. Through bioassay-guided fractionation on the methanol extract of this species, seven fractions were obtained; however, none of the fractions had cytotoxic activity against the above-mentioned cancer cell lines⁴⁷.

Immunomodulatory activity

Chemical constituents of four species growing in Europe, *V. phlomoides*, *V. thapsiforme*, *V. lychnitis* and *V. nigrum*, were investigated, and individual compounds including flavonoids, saponins and phenylpropanoids were obtained. The influence of the five isolated compounds on spontaneous proliferation of rat spleen lymphocytes was studied *in vitro*. Verbascosaponin, luteolin 7-O-glucoside, verbascoside and forsythoside B showed antiproliferative effect at the concentration of 100 µg/ml (87, 63, 54 and 29% suppression of [³H]-thymidine uptake into DNA, respectively). At low concentration (0.1 mg/ml), verbascoside, forsythoside B and specioside revealed significant increase in proliferation (60, 64 and 53%, respectively). The results of that preliminary screening suggest immunomodulatory effects of the compounds tested⁴⁸.

Anti-ulcerogenic activity

Several plants containing high amounts of saponins have been shown to possess anti-ulcerogenic activity in several experimental ulcer models. The protective activities of these saponins may be due to the activation of mucous membrane protective factors and inhibition of gastric secretion volume and acid secretion. Many phytochemical analyses led to the isolation of mucilages, flavonoids, phenylethanoids and saponins from the inflorescence of some *Verbascum* species. Consequently, data on the phytochemistry

of *Verbascum* sp. suggested that investigation of the anti-ulcerogenic activity of the flowers of *V. cheiranthifolium* Boiss var. *cheiranthifolium* could be a promising approach. The water extract of *V. cheiranthifolium* Boiss var. *cheiranthifolium* given orally was tested for gastric protection against ethanol-induced gastric ulcer model in rats. However, no rat was completely protected from any visible damage⁴⁹.

Antihepatotoxic activity

V. thapsus L., which is used as folk medicine in Canada, was evaluated for its antihepatoma activity on five human liver-cancer cell lines, i.e. HepG2/C3, SK-HEP-1, HA22T/VGH, Hep3B and PLC/PRF/5. The crude drug demonstrated in vitro growth inhibition (31.0, 69.9%, respectively) at 2000 µg/ml against HepG2/C3 and HA22T/VGH₅₀.

A biostimulating herbal mixture for use in hematopoietic, liver and respiratory disorders in humans comprises propolis extract (15% flavonoids as chrysin) 0.075, *Gratiola* extract, *Verbascum* extract 0.075, *G. officinalis* powder 0.005, and pollen 0.04 parts. Thus, a tablet formulation contained per tablet propolis 0.15, *G. officinalis* 0.005, pollen 0.040, starch 0.013, aerosol 0.010, talc 0.005, and lactose 0.55 g⁵¹.

Antihyperlipidemic activity

In rats with induced hyperlipidemia, polysaccharides obtained from the leaves of *V. thapsus* exhibited a significant decrease in cholesterol and triglyceride levels⁵².

Antitussive activity

Antitussive activity of *V. thapsiforme* carbohydrate substances was tested on conscious cats by mechanical stimulation of the laryngopharyngeal and tracheo-bronchial mucous areas of the airways through a surgically implanted endotracheal cannula. Parameters of the cough reflex were registered and statistically evaluated. Comparative tests with commonly used antitussive drugs from both narcotic (codeine) and non-narcotic (dropropizine) groups were also carried

out under the same conditions. Comparison of the cough suppressive ability of the classical drugs revealed that the antitussive effect of these carbohydrates was lower than of codeine but significantly higher than that of dropropizine. Expectoration parameters were reduced minimally after orally administrated herbal agents⁵³.

Phytogrowth-inhibitory activity (antigermination activity)

Pardo et al.^{54,55} investigated phytotoxins from an ethanolic extract of the dried aerial parts of *V. virgatum* and the roots of *V. thapsus*, which exhibited antigermination activity on the seeds of barley (*Hordeum vulgare*). The extracts led to isolation of iridoid glycosides, which are known to inhibit plant growth.

Other activities

Cunat et al.⁵⁶ screened plant species of the Spanish Mediterranean flora for juvenile hormone-mimic by alteration of *Tribolium castaneum* metamorphosis. *V. sinuatum* had important juvenile hormone activity, inducing severe metamorphical disturbances affecting over 75% of the treated insects.

The extract prepared from *V. thapsus* demonstrated its ability to inhibit one or more extracellular proteases, which degrade human tissue matrix⁵⁷.

Krushkov and his colleagues⁵⁸ established that an alkaloid (nobilin) obtained from *V. nobile* Vel. has ganglion-blocking and myotropic vasodilative effects, which explains their hypotensive action.

Otitis media is one of the most frequent diseases of early infancy and childhood and one of the most common reasons for children to visit a physician. Therefore, children were randomly assigned to receive treatment with Naturopathic Herbal Extract Ear Drops (NHED, including *V. thapsus*), with or without amoxicillin. This study suggests that in cases of ear pain caused by acute otitis media in children in whom active treatment, besides a simple 2- to 3-day waiting period, is needed, an herbal extract solution may be

beneficial⁵⁹.

Psoriasis is a skin disease that manifests itself as blotches, a silver-colored peeling and a hardening of the skin (paraketosis). In the framework of traditional medicine, the use of some plants (including *V. sinuatum* L.) typical of the flora of Sicily in the treatment of psoriasis has been reported. The aerial parts of the plants are collected in spring and used to make a decoction, which is prepared in 10% alcohol. Significant improvement in the pathology may be noted and in some cases complete recovery is observed⁶⁰.

Cosmetics, bath preparations, and detergents contain extracts of plants selected from *Trifolium pretense*, *Gerbera jamesonii*, *Tulipa gesneriana*, *Helianthus annuus*, *Dianthus caryophyllus*, *Trifolium repens*, *Rosa*, *Magnolia lilifolia*, *V. thapsus* and *V. thapsiforme*. A milky lotion containing these plant extracts showed skin moisturizing and conditioning effects⁶¹.

A composition for use as a tobacco substitute and as an aid in the cessation of tobacco use contains *V. thapsus*, an algae, *Medicago sativa*, and *Symphytum officinale*, together with other optional components. Use of the present invention as a tobacco substitute in cigarettes or pipes produces a diminished desire for tobacco⁶².

CONCLUSION

A wide range of biological activities have been determined from *Verbascum* extracts, including anti-inflammatory, antimicrobial, antioxidant, antitumor, immunomodulatory, and antiulcerogenic activities, which have been reviewed from our studies and the related literatures. Some of these effects may be attributed to the use of these species in folk medicine.

There are several isolated compounds from various *Verbascum* species, such as saponins and iridoids, which have aided in the healing of infections and were effective against ulcers in rats. Ilwensisaponin A and C have antimicrobial activity. Some flavonoids, phenylethanoid and neolignan glycosides, and saponins have antioxidative, anti-inflammatory, antitu-

mor, and immunostimulatory activities. Polysaccharides have been reported to be effective against hyperlipidemia.

In consideration of the claimed biological activities of *Verbascum* species, it is not necessarily a single compound that is responsible for these effects; they may be due to several compounds acting in a synergistic manner or to the regulation of one compound by another.

Due to the very long tradition of using *Verbascum* species in several types of inflammatory conditions and respiratory disorders, etc. and also in view of what is known today about their chemical constituents and biological activities, it seems worthwhile to explore these species further. For example, since ancient times, common mullein (*V. thapsus*) has been used as a medicinal herb. There are commercial products containing saponins, such as Mullein tea bag (Select Herb Tea Company), Com Mullein swallow capsule (Solaray), Com Mullein extract in alcohol (Gaia Herbs, Inc), and Com Mullein flower oil (Frontier™, Herbal Extracts), which are used to treat respiratory disorders and have a soothing and anti-inflammatory effect⁴. Therefore, other species growing in Turkey could be studied in detail like *V. thapsus*, leading to the suggestion of new commercial products in the drug industry.

REFERENCES

1. Huber-Morath A. *Verbascum* L., Davis PH (ed.), Flora of Turkey and the East Aegean Islands, University Press, Edinburgh, Vol. 6, 461-603, 1978.
2. Tatli II, Akdemir ZS. Chemical constituents of *Verbascum* L. species, *FABAD J. Pharm. Sci.*, 29, 93-107, 2004.
3. Baytop T. Therapy with Medicinal Plants in Turkey (Past and Present), 2nd ed., Nobel Tip Kitabevleri Ltd., Istanbul, 334, 1999.
4. Turker AU, Camper ND. Biological activity of common mullein, a medicinal plant, *J. Ethnopharmacol.*, 82, 117-125, 2002.
5. Turker AU, Gurel E. Common mullein (*Verbascum thapsus* L.): recent advances in research, *Phytother. Res.*, 19, 733-739, 2005.
6. Tuzlaci E, Erol MK. Turkish folk medicinal plants. Part II: Egirdir (Isparta), *Fitoterapia*, 70, 593-610, 1999.
7. Gurhan G, Ezer N. Plants which have been used for haemorrhoids in folk medicine-I, *Hacettepe Univ. J. Fac. Pharm.*, 24, 37-55, 2004.
8. Sezik E, Yesilada E, Honda G, Takaishi Y, Takeda Y, Tanaka T. Traditional medicine in Turkey X. Folk medicine in Central Anatolia, *J. Ethnopharmacol.*, 75, 95-115, 2001.
9. Grieve M. A Modern Herbal, Barnes and Noble Books, New York, 564-566, 1995.
10. Zgorniak-Nowosielska I, Grzybek J, Manolo N, Serkedjieva J, Zawilinska B. Antiviral activity of *Flos Verbasci* infusion against *Influenza* and *Herpes simplex* viruses, *Arch. Immunol. Ther. Exp. (Warsz)*, 39, 103-108, 1991.
11. Serkedjieva J. Combined antiinfluenza virus activity of *Flos Verbasci* infusion and amantadine derivatives, *Phytother. Res.*, 14, 571-574, 2000.
12. Zanon SM, Ceriatti FS, Rovera M, Sabini LJ, Ramos BA. Search for antiviral activity of certain medicinal plants from Cordoba, Argentina, *Rev. Latino. Microbiol.*, 41, 59-62, 1999.
13. McCutcheon AR, Roberts TE, Gibbons E, Ellis SM, Babiuk LA, Hancock REW, Towers GHN. Antiviral screening of British Columbian medicinal plants, *J. Ethnopharmacol.*, 49, 101-110, 1995.
14. Meurer-Grimes B, McBeth DL, Hallihan B, Delph S. Antimicrobial activity in medicinal plants of the Scrophulariaceae and Acanthaceae, *Pharm. Biol.*, 34, 243-248, 1996.
15. Hildegard von Bingen (12th century) Naturkunde. Translated (into High German) and commented by P. Riethe (ed.). 1980. Das Buch von dem inneren Wesen der verschiedenen Naturen der Schopfung, 3rd ed., Otto Muller Verlag, Salzburg, Austria.
16. Guarino C. Antimicrobial activity of *Verbascum macrurum* Ten. (Scrophulariaceae), *Boll. Chim. Farm.*, 141, 238-242, 2002.
17. Salim ML, Ammar HA, Abderrahman S, El-Remawy HA. Phytochemical screening and antimicrobial activity of wild Jordanian plants from Al-Balq'a, *Bull. Fac. Pharm. Cairo Univ.*, 34, 235-238, 1996.
18. Khafagi IK. Screening in vitro cultures of some Sinai medicinal plants for their antibiotic activity, *J. Microbiol.*, 34, 613-627, 2001.
19. Dulger B., Kirmizi S, Arslan H, Guleryuz G. Antimicrobial activity of three endemic *Verbascum* species, *Pharm. Biol.*, 40, 587-589, 2002.
20. Dulger B, Gonuz A. Antimicrobial activity of some endemic *Verbascum*, *Salvia* and *Stachys* species, *Pharm. Biol.*, 42, 301-304, 2004.
21. Dulger B, Ugurlu E. Evaluation of antimicrobial activity of some endemic Scrophulariaceae members from Turkey, *Pharm. Biol.*, 43, 275-279, 2005.
22. Dulger B, Ugurlu E, Aki C, Suerdem TB, Camdeviren A, Tazeler G. Evaluation of antimicrobial activity of some endemic *Verbascum*, *Sideritis* and *Stachys* species from Turkey, *Pharm. Biol.*, 43, 270-274, 2005.
23. Dulger B. Antimicrobial activity of some endemic Scrophulariaceae from Turkey, *Pharm. Biol.*, 44, 672-676, 2006.
24. Barbour EK, Sharif MA, Sagherian VK, Habre AN, Talhouk RS, Talhouk SN. Screening of selected indigenous plants of Lebanon for antimicrobial activity, *J. Ethnopharmacol.*, 93, 1-7, 2004.
25. Tadege H, Mohammed E, Asres K, Gebre-Mariam T. Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders, *J. Ethnopharmacol.*, 100, 168-175, 2005.
26. Akdemir ZS, Tatli II, Bedir E, Khan IA. Antimi-

- crobial and antimalarial activities of some endemic Turkish *Verbascum* species, *FABAD J. Pharm. Sci.*, 28, 131-135, 2003.
27. Tatli II, Akdemir ZS. Antimicrobial and antimalarial activities of secondary metabolites from some Turkish *Verbascum* species, *FABAD J. Pharm. Sci.*, 30, 84-92, 2005.
 28. Tatli II, Akdemir ZS, Bedir E, Khan IA. Search for antifungal compounds from some *Verbascum* species growing in Turkey, *FABAD J. Pharm. Sci.*, 28, 137-140, 2003.
 29. Toth L, Papay V, Toth L, Papay I, Torok A. Extract that improves the defense of the organism, Hung. Teljes HU 35,176 (Cl. A61K35/78), 28 Jun 1985, Appl. 83/2,741, 03 Aug 1983; 19 pp.
 30. Sathiyamoorthy P, Lugasi-Evgi H, Schlesinger P, Kedar I, Gopas J, Pollack Y, Golan-Goldhirsh A. Screening for cytotoxicity and antimalarial activities in desert plants of the Negev and Bedouin market plant products, *Pharm. Biol.*, 37, 188-195, 1999.
 31. Aligiannis N, Mitaku S, Tsitsa-Tsardis E, Harvala C, Tsaknis I, Lalas S, Haroutounian S. Methanolic extract of *Verbascum macrurum* as a source of natural preservatives against oxidative rancidity, *J. Agric. Food Chem.*, 51, 7308-7312, 2003.
 32. Abou Gazar H, Bedir E, Khan IA, Calis I. Wiedemanniosides A-E. New phenylethanoid glycosides from the roots of *Verbascum wiedemannianum*, *Planta Med.*, 69, 814-819, 2003.
 33. Tepe B, Sokmen M, Akpulut HA, Yumrutas O, Sokmen A. Screening of antioxidative properties of the methanolic extracts of *Pelargonium endlicherianum* Fenzl., *Verbascum wiedemannianum* Fisch. & Mey., *Sideritis libanotica* Labill. subsp. *linearis* (Benth) Borm., *Centaurea mucronifera* DC. and *Hieracium cappadocicum* Freyn from Turkish flora, *Food Chem.*, 98, 9-13, 2006.
 34. Akdemir ZS, Tatli II, Bedir E, Khan IA. Antioxidant flavonoids from *Verbascum salviifolium* Boiss., *FABAD J. Pharm. Sci.*, 28, 71-75, 2003.
 35. Akdemir ZS, Tatli II, Bedir E, Khan IA. Iridoid and phenylethanoid glycosides from *Verbascum lasianthum*, *Turk. J. Chem.*, 28, 227-234, 2004.
 36. Akdemir ZS, Tatli II, Bedir E, Khan IA. Neolignan and phenylethanoid glycosides from *Verbascum salviifolium* Boiss, *Turk. J. Chem.*, 28, 621-628, 2004.
 37. Tatli II, Takamatsu S, Khan IA, Akdemir ZS. Screening for free radical scavenging and cell-aggregation inhibitory activities on secondary metabolites from Turkish *Verbascum* species, *Z. Naturforsch. C.*, 62C, 673-678, 2007.
 38. Intelisano J. Food supplement/herbal composition for health enhancement, U.S. US 6,440,448 (Cl. 424-439; A61K47/00), 27 Aug 2002, Appl. 39,427, 16 Mar 1998; 5 pp.
 39. Kupeli E, Tatli II, Akdemir ZS, Yesilada E. Bioassay-guided isolation of anti-inflammatory and antinociceptive glycoterpenoids from the flowers of *Verbascum lasianthum* Boiss. ex Benth, *J. Ethnopharmacol.*, 110, 444-450, 2007.
 40. Tokuda H, Konoshima T, Kozuka M, Kimura R. Antitumour activities of triterpene saponins from *Verbascum songaricum*, *Oncology*, 48, 77-80, 1991.
 41. Paszkiewicz-Gadek A, Grochowska K, Galasinski W. Effect of the aqueous extract and saponin fraction from the flowers of *Verbascum thapsiforme* on protein biosynthesis in a rat liver ribosomal system, *Phytother. Res.*, 4, 177-181, 1990.
 42. Galasinski W, Chlabicz J, Paszkiewicz-Gadek A, Marcinkiewicz C, Gindzienski A. The substances of plant origin that inhibit protein biosynthesis, *Acta Pol. Pharm.*, 53, 311-318, 1996.
 43. Ionkova I, Alferman A. Use of DNA for detection and isolation of potential anticancer agents from plants, *Farmatsiya*, 47, 10-16, 2000.
 44. Hardman JT, Beck ML, Owensby CE. Range for lectins, *Transfusion*, 23, 519-522, 1983.
 45. Kawamo H, Hagiwara Y, Mizuno O, Kumakura M. Isolation of an antihistamine flavone from a plant, *Verbascum thapsus*, Jpn. Kokai Tokkyo Koho JP 63,227,584 (88,227,584) (Cl. C07D311/28), 21 Sep 1988, Appl. 87/61,304, 18 Mar 1987, 3 pp.
 46. Afifi MSA, Ahmed MM, Pezzuto JM, Kinghorn AD. Cytotoxic flavonolignans and flavones from *Verbascum sinaiticum* leaves, *Phytochemistry*, 34, 839-841, 1993.
 47. Tatli II, Akdemir ZS. Cytotoxic activity on some *Verbascum* species growing in Turkey, *Hacettepe Univ. J. Fac. Pharm.*, 26, 82-90, 2006.
 48. Klimek B, Stepień H. Effect of some constituents of mullein (*Verbascum* sp.) on proliferation of rat

- splenocytes in vitro, *European J. Pharm. Sci.*, 2, 123, 1994.
49. Gurbuz I, Ozkan AM, Yesilada E, Kutsal O. Anti-ulcerogenic activity of some plants used in folk medicine of Pinarbasi (Kayseri, Turkey), *J. Ethnopharmacol.*, 101, 313-318, 2005.
 50. Lin LT, Liu LT, Chiang LC, Lin CC. *In vitro* anti-hepatoma activity of fifteen natural medicines from Canada, *Phytother. Res.*, 16, 440-444, 2002.
 51. Paunescu T. Biostimulating composition for humans, Rom. RO 86,828 (Cl. Q61K35/78), 30 Apr 1985, Appl. 111,712, 25 Jul 1983; 2 pp.
 52. Aboutabl EA, Goneid MH, Soliman SN, Selim AA. Analysis of certain plant polysaccharides and study of their antihyperlipidemic activity, *Al-Azhar J. Pharm. Sci.*, 24, 187-195, 1999.
 53. Nosalova G, Sutovska M, Mokry J, Kardosova A, Capek P, Khan MTH. Efficacy of herbal substances according to cough reflex, *Minerva Biotech.*, 17, 141-152, 2005.
 54. Pardo F, Perich F, Torres R, Monache FD. Plant iridoid glycosides and phyto-growth-inhibitory activity of *Verbascum virgatum*, *Biochem. Syst. Ecol.*, 32, 367-370, 2004.
 55. Pardo F, Perich F, Torres R, Delle Monache F. Phytotoxic iridoid glucosides from the roots of *Verbascum thapsus*, *J. Chem. Ecol.*, 24, 645-653, 1998.
 56. Cunat P, Primo E, Sanz I, Garcera MD, March MC, Bowers WS, Martinez-Pardo R. Biocidal activity of some Spanish Mediterranean plants, *J. Agric. Food Chem.*, 38, 497-500, 1990.
 57. Cyr B. Plant extracts and composition containing extracellular protease inhibitors, PCT int. Appl, WO 02 69,992 (Cl. A61K35/78), 12 Sep 2002, CA Appl. 2,339,081, 2 Mar 2001; 132 pp.
 58. Krushkov I, Paskov D, Popivanov D. Ganglion-blocking effect of some alkaloids of the plant *Verbascum nobile* Vel., *Nauchni Tr. Vissh. Med. Inst.*, 49, 19-23, 1970.
 59. Sarrell EM, Cohen HA, Kahan E. Naturopathic treatment for ear pain in children, *Pediatrics*, 111 (5Pt1), 574-579, 2003.
 60. Amenta R, Camarda L, Di Stefano V, Lentini F, Venza F. Traditional medicine as a source of new therapeutic agents against psoriasis, *Fitoterapia*, 71, 13-20, 2000.
 61. Ohara M, Doi M, Kondo M. Cosmetics, bath preparations, and detergents containing plant extracts, Jpn. Kokai Tokkyo Koho JP 2001 122,730 (Cl. A61K7/00), 8 May 2001, Appl. 1999/303,537, 26 Oct 1999; 23 pp.
 62. Coy-Herbert P. Herbal composition as a substitute for tobacco products, U.S. US 6,497,234 (Cl. 131-352; A61K35/78), 24 Dec 2002, US Appl. PV167,056, 22 Nov 1999; 5 pp.