Traditional Uses and Biological Activities of Verbascum Species

I. İrem TATLI**, Zeliha Ş. AKDEMİR**

** Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, Sihhiye, Ankara, 06100, TURKEY
** Corresponding author e-mail: itatli@hacettepe.edu.tr

INTRODUCTION

There are many Mediterranean and Irano-Turanian Verbascum species in Turkish flora. 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 Islands. Verbascum species contain biologically active compounds, such as flavonoids, phenylethanoid and neolignan glycosides, saponins, and iridoid and monoterpenic glycosides.

The leaves and flowers of Verbascum are reported to have expectorant, mucolytic and demulcent properties, 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.

* Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Sihhiye, Ankara, 06100, TURKEY
** Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, Sihhiye, 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 Anatolia6,7.

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. thapsiforme 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 adamantamine 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
log.12

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 1.13

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. Hildegarde of Bingen, the great abbess of the early 12th century, mentions the plant in the first book of her ‘Physica’ under ‘De Wullena’, 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 active.16 In a similar study, the ethanolic extract of V. qulebrium 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. qulebrium (10 mm).17 V. sinaicicum 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 10231.19

The methanol extracts obtained from endemic V. gypsiscala Vural & Aydogdu, V. pseudoholotrichum Hub.-Mor., V. cymigerum Hub.-Mor., V. chlorostegium Bornm. & Murb., V. lingulifolium Hub.-Mor., V. pellitum Hub.-Mor., V. protractum Fenel ex Tchihat., V. bellum Hub.-Mor., V. dalamicum Hub.-Mor. V. chionophyllum Hub.-Mor., V. ciliicum Boiss., V. trapifolium (Stapf) Hub.-Mor., V. meinickeanum 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 study.20-23

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. roripolium were studied for their antimicrobial activities. The extracts had a strong
Barbour et al. 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. leptostychum* flowers (11.1% and 99.9%, respectively) and leaves (22.2% and 66.6%, respectively). The water extract of *V. leptostychum* did not result in inhibition of the test organisms.

The hydroalcoholic extract of *V. sinaicum*, 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 the ee 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*.

Turker and Camper 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* ex Bentham 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. Ilvensisapoin 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.

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. Ilvensisaponin 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*.

Combined extracts of *Verbascum* flower, *Mentha piperita* and *M. crispa* leaves as well as *Cynosbata* 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 microorganisms\textsuperscript{29}.

The aqueous extract of the aerial parts of V. fruiticulorum demonstrated a strong growth inhibition (26.9\%) towards the malaria parasite P. falciparum\textsuperscript{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 \(\alpha\)-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 \(\alpha\)-tocopherol. This compound, therefore, represents a very interesting candidate for use in food preservation as a natural protecting agent against oxidative rancidity\textsuperscript{31}.

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 leukosepitoside 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 \(\beta\)-carotene/linoleic acid test system. In the first case, V. wiedemannianum extract exerted an insignificant antioxidant activity with an \(\text{IC}_{50}\) value of 117\(\pm\)0.56 \(\mu\)g/ml when compared to synthetic antioxidant BHT (18.0\(\pm\)0.40 \(\mu\)g/ml). The compounds demonstrated scavenging properties toward the DPPH radical in TLC autographic assays. In the \(\beta\)-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\textsuperscript{32,33}.

Natural compounds are receiving increasing attention as potential antioxidants. For this purpose, four flavonoid glucosides (apigenin-7-O-\(\beta\)-glucopyranoside, luteolin-7-O-\(\beta\)-glucopyranoside, luteolin-3′-O-\(\beta\)-glucopyranoside, and chrysoeriol-7-O-\(\beta\)-glucopyranoside), five phenylethanoid glycosides (verbascoside, \(\beta\)-hydroxyacteoside, forsythiside B, angoroside A, and martynoside) as well as two neo-lignan glycosides (dehydrodiconiferyl alcohol-9\(^1\)-O-\(\beta\)-D-glucopyranoside and dehydrodiconiferyl alcohol-9-O-\(\beta\)-D-glucopyranoside) were isolated from the aerial parts of V. salviifolium. Additionally, harbaposide, 6-O-vanilloylajugol, and poliumoside were isolated from the roots of V. lasianthum Boiss. ex Bentham. 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 radicals\textsuperscript{34-36}.

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 Bentham, 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, \(\beta\)-hydroxyacteoside, martynoside, poliumoside, forsythiside B, angoroside A, dehydrodiconiferyl alcohol-9-O-\(\beta\)-D-glucopyranoside, dehydrodiconiferyl alcohol-9′-O-\(\beta\)-D-glucopyranoside, apigenin 7-O-\(\beta\)-glucopyranoside, luteolin 7-O-\(\beta\)-glucopyranoside, luteolin 3′-O-\(\beta\)-glucopyranoside and chrysoeriol 7-O-\(\beta\)-glucopyranoside exhibited a dose-dependent inhibition of bioautographic and spectrophotometric DPPH activities. Verbascoside

\textsuperscript{FABAD J. Pharm. Sci., 31, 85-96, 2006}
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 activities.

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 ingredients.

**Antiinflammatory and antinociceptive activities**

Infusions of *V. lasianthus* Boiss. ex Bentham flowers have been used for hemorrhoids in Turkish folk medicine. In order to evaluate the scientific basis for this practice, *in vivo* anti-inflammatory and antinociceptive activities of *V. lasianthus* 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 medicine.

A general correlation was suggested between the anti-inflammatory and antitumor-promoting activities of acylated saponins from Scrophulariaceae plants by Tokuda et al. 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.

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 glycyr rhizic 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 cells.
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 preparations.

Crude extracts from plants used in traditional medicine, including *V. pseudonobile*, and extracts from plant cell cultures have been screened for potential antitancer 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-intercalation. Ionkova and Alferman also found that there was correlation in DNA-intercalation and the hemolytic effect in plant extracts, which contained triterpenoid saponins. Hardman and his colleagues 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 $1.6 \times 10^{-5} \text{ M}$.

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 cells.

*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 lines.

**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. Verbasconsaponin, 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 $[^3]H$-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 tested.

**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 damage.

**Antihelatotoxic 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/VGH.

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 g.

**Antihyperlipidemic activity**

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

**Antitussive activity**

Antitussive activity of *V. thapsiforme* carbohydrate substances was tested on conscious cats by mechanical stimulation of the laryngopharyngeal and tracheobronchial 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 agents.

**Phytogrowth-inhibitory activity (antigermination activity)**

Pardo et al. 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. 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 matrix.

Krushkov and his colleagues 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
beneficial\(^5\).

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 \textit{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 observed\(^6\). Cosmetics, bath preparations, and detergents contain extracts of plants selected from \textit{Trifolium pretense}, \textit{Gerbera jamesonii}, \textit{Tulipa gesneriana}, \textit{Helianthus annuus}, \textit{Dianthus caryophyllus}, \textit{Trifolium repens}, \textit{Rosa}, \textit{Magnolia lilifolia}, \textit{V. thapsus} and \textit{V. thapsiforme}. A milky lotion containing these plant extracts showed skin moisturizing and conditioning effects\(^6\).

A composition for use as a tobacco substitute and as an aid in the cessation of tobacco use contains \textit{V. thapsus}, \textit{an algae}, \textit{Medicago sativa}, and \textit{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 tobacco\(^6\).

CONCLUSION

A wide range of biological activities have been determined from \textit{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 \textit{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-
REFERENCES

26. Akdemir ZS, Tatli II, Bedir E, Khan IA. Antimi-


43. Ionkova I, Aferman A. Use of DNA for detection and isolation of potential anticancer agents from plants, Farmatsiya, 47, 10-16, 2000.


48. Klimek B, Stepień H. Effect of some constituents of mullein (Verbascum sp.) on proliferation of rat