Antinociceptive and Anti-Inflammatory Effects of Saponin and Iridoid Glycosides from Verbascum pterocalycinum var. mutense Hub.-Mor.

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The anti-inflammatory and antinociceptive properties of four major compounds from the flowers of Verbascum pterocalycinum var. mutense were investigated. Saponin glycosides called ilwensisaponin A and C and iridoid glycosides known as ajugol and picroside IV were isolated from the methanolic extract. A dose-related anti-inflammatory and antinociceptive response were obtained in this study at doses of 100 and 200 mg/kg. The results of the evaluation of the anti-inflammatory activity induced by carrageenan and PGE1 showed that this species possesses active constituents that could diminish the cyclooxygenase activity. No effects were observed in the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema model. Our results support the anti-inflammatory and analgesic effects of Verbascum pterocalycinum var. mutense. Ilwensisaponins A and C could explain in part the anti-inflammatory and analgesic activities of this species. Although antinociceptive and anti-inflammatory activities of ajugol and picroside IV were found insignificant in the statistical analysis, ilwensisaponin A and C showed notable activity without inducing any apparent acute toxicity as well as gastric damage.

Key words: Verbascum pterocalycinum var. mutense, Anti-Inflammatory Activity, Antinociceptive Activity

Introduction

The genus Verbascum L. (Scrophulariaceae), commonly known as “mullein”, is represented by 228 species, 185 of which are endemic in the flora of Turkey (Huber-Morath, 1978). Verbascum extracts, decoctions and infusions have been used in traditional medicine. The leaves and flowers of Verbascum are reported to have expectorant, mucolytic and demulcent properties and are used to treat respiratory problems such as bronchitis, dry coughs, tuberculosis and asthma in traditional Turkish medicine (Baytop, 1999; Turker and Camper, 2002). Verbascum flowers are boiled in milk and are applied externally for pruritic conditions affecting the urogenital organs. In addition, as a traditional Anatolian cure for anal fistulae, the flowers are boiled in a cauldron and then the anus is exposed to the vapours. These species are reported to be mildly diuretic and to have a soothing and anti-inflammatory effect on the urinary tract, as well as to act as a mild sedative. Oil made from the flowers is used to help soothing earache, and can be applied externally for eczema and other types of inflammatory skin conditions. These species are commonly used to treat hemorrhoids, rheumatic pain, superficial fungal infections, wounds and diarrhea. They are traditionally consumed as a tea to relieve abdominal pains (Tuzlaci and Erö, 1999; Sezik et al., 2001; Turker and Camper, 2002).

In previous studies, the aqueous extracts from the flowers of Verbascum thapsiforme demonstrated a strong inhibitory effect on the elongation step of protein biosynthesis. It was suggested that the saponin fraction was responsible for the activity (Paszkiewicz-Gadek et al., 1990). The protective activities of the saponins may be due to the activation of mucous membrane protective factors, inhibition of the gastric secretion volume and acid secretion. The water extract of V. cheiranthifolium Boiss. var. cheiranthifolium given orally was tested for gastric protection against an ethanol-induced
gastric ulcer model in rats, and the extract had a promising activity (Gurbuz et al., 2005).

Phytochemical studies of Verbascum pterocalycinum var. mutense have revealed the presence of oleanane-type triterpene saponins (Tatli et al., 2004). The pharmacological activities of saponins in plants, such as their anti-inflammatory, antitumour, antiexudative, antiulcer, analgesic, antipyretic and immunostimulant effects, have been known for many years, while new activities are continually being discovered (Hostettmann and Marston, 1995).

In the current study, anti-inflammatory and antinociceptive activities of the isolated compounds from Verbascum pterocalycinum var. mutense were discussed using carrageenan-induced hind paw edema, PGE1-induced hind paw edema and TPA-induced ear edema models, and p-benzoquinone-induced writhing in mice.

Material and Methods

Plant material

Verbascum pterocalycinum var. mutense Hub.-Mor. was collected from Icel, between Mut and Karaman, 930–1100 m, in July 2000. A voucher specimen has been authenticated by Prof. Dr. Hayri Duman (Gazi University, Faculty of Science, Etiler, Ankara, Turkey) and was deposited at the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 00184).

Chromatographic separation and isolation of the constituents

The plant material was dried under shade and powdered to a fine grade by using a laboratory scale mill. Dried flower parts of the plant material (485.6 g) were extracted with methanol by maceration at room temperature for two times (2 × 2 L). The combined extract was evaporated to dryness in vacuo to give 43.5 g methanol extract. The methanol extract was fractionated by column chromatography (CC) on silica gel (500 g) using hexane, ethylacetate, chloroform, acetone and methanol (4 L each), respectively, to yield 5 fractions (Fractions A–E). Fraction E (11.5 g), eluted with methanol, was subjected to vacuum liquid chromatography (VLC) using reversed-phase material (Sephadex 40 µm, 750 g) and MeOH/H2O mixtures (0–100%) to give ilwensisaponin A (mimengoside A, 1) (283.1 mg), ilwensisaponin C (2) (260.1 mg), and fraction E2 (80.9 mg). Fraction E2 was rechromatographed on a silica gel column (65 g) and eluted with CHCl3/MeOH mixtures (90:10, 85:15, 80:20) to yield ajugol (3) (3.4 mg). Fraction D (1.6 g), eluted with acetone, was applied to VLC using reversed-phase material (Sephadex 40 µm, 150 g) and MeOH/H2O mixtures (0–100%) to give 3 fractions (Fractions D1–D3). Fraction D2 (945.6 mg) was subjected to a silica gel column (57 g) using CHCl3 and CHCl3/MeOH mixtures (95:5, 90:10, 85:15, 80:20, 70:30) to yield picroside IV (4) (6.3 mg).

Structure elucidation of compounds 1–4

Structure elucidation of the isolated compounds 1–4 was carried out by spectral techniques; UV, IR, 1D- and 2D-NMR, mass spectroscopy (HR-ESIMS) and other detailed data were recently published elsewhere (Tatli et al., 2004). The structures of compounds 1–4 were as follows (Fig. 1): ilwensisaponin A (1), ilwensisaponin C (2), ajugol (3) and picroside IV (4).

Animals

Male Swiss albino mice (20–25 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health (Ankara, Turkey). The animals left for 2 d for acclimatization to animal room conditions were maintained on standard pellet diet and water ad libitum. The food was withdrawn on the day before the experiment, but free access of water was allowed. A minimum of six animals was used in each group. Throughout the experiments, animals were processed according to the suggested ethical guidelines for the care of laboratory animals.

Preparation of test samples for bioassays

The methanolic extract and isolated compounds were administered in 100 and 200 mg/kg doses after suspending in 0.5% sodium carboxymethyl cellulose (CMC) suspension in distilled water. The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indomethacin (10 mg/kg and 0.5 mg/ear) or aspirin (acetyl salicylic acid; 100 and 200 mg/kg) in 0.5% CMC was used as reference drug.
Fig. 1. Chemical structures of isolated compounds 1–4.
Antinociceptive activity

*p*-Benzooquinone-induced abdominal constriction test (Okun et al., 1963) was performed on mice for the determination of the antinociceptive activity. According to the method, 60 min after the oral administration of test samples, the mice were intraperitoneally (i.p.) injected with 0.1 mL/10 g body weight of 2.5% (w/v) *p*-benzoquinone (PBQ; Merck) solution in distilled H₂O. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the 5th min after the PBQ injection. The data represent averages of the total number of writhes observed. The antinociceptive activity was expressed as percentage change from writhing controls. Aspirin at 100 and 200 mg/kg doses was used as the reference drug in this test.

Anti-inflammatory activity

Carrageenan-induced hind paw edema model

Carrageenan-induced hind paw edema model was used for the determination of the anti-inflammatory activity (Küpeli et al., 2005). The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. 60 min after the oral administration of a test sample or dosing vehicle, each mouse was injected with freshly prepared (5 μg/5 μL) suspension of PGE₁ (Fluka Chemie AG, Art. 82475) in Tyrode’s solution into the subplantar tissue of the right hind paw. As the control, 5 μL Tyrode’s solution were injected into the left hind paw. Paw edema was measured every 15 min during 75 min after induction of inflammation. The difference in footpad thickness was measured by a gauge calipers (Ozaki Co.). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. Indomethacin (10 mg/kg) was used as the reference drug.

TPA-induced mouse ear edema

Each mouse received 2.5 μg of TPA (12-O-tetradecanoylphorbol-13-acetate) dissolved in 20 μL of 70% EtOH (De Young et al., 1989). This was applied by an automatic pipette in 20 μL volumes to both anterior and posterior surfaces of the right ear. The left ear (control) received the same volume of solvent (70% EtOH) simultaneously with TPA. Indomethacin (0.5 mg/ear) was used as a standard drug. For the evaluation of the activity, two different ways were followed up:

1.) The thickness of each ear was measured 4 h after induction of inflammation using a gauge calipers (Ozaki Co.). The edema was expressed as the difference between the right and left ear due to TPA application and, consequently, inhibition percentage was expressed as a reduction thickness with respect to the control group.

2.) After 4 h, the animals were killed under deep ether anesthesia. Discs of 6 mm diameter were removed from each ear and weighed in balance. The swelling was estimated as the difference in weight between the punches from the right and left ear and expressed as an increase in ear thickness.

Acute toxicity

Animals employed in the carrageenan-induced paw edema experiment were observed during 48 h and morbidity or mortality was recorded, if happens, for each group at the end of the observation period.
**Gastric-ulcerogenic effect**

After the antinociceptive activity experiment, mice were killed under deep ether anesthesia and stomachs were removed. Then the abdomen of each mouse was opened through the greater curvature and examined under a dissecting microscope for lesions or bleedings. However, p-benzoquinone applied i. p. did not induce any irritation on the gastric mucosa, but anti-inflammatory agents of COX-1 inhibitors, *i.e.*, aspirin or indomethacin, orally, caused severe bleedings, without repeated administrations.

**Statistical analysis**

Data obtained from animal experiments were expressed as mean standard error (± SEM). Statistical differences between the treatments and the control were evaluated by ANOVA and Students-Newman-Keuls post-hoc tests. *p* < 0.05 was considered to be significant [*p* < 0.05; **p* < 0.01; ***p* < 0.001].

**Results and Discussion**

*Verbascum* species have been used since ancient times in traditional medicine for the therapy of scabies, eczema, tumours and various inflammatory affections (Turker and Camper, 2002). Therefore, the purpose of this paper was to establish the scientific basis for traditional uses of *Verbascum* species against inflammation and rheumatic pains. The results indicate that the activity of the isolated compounds from *V. pterocalycinum* var. *mutense* might contribute to the anti-inflammatory activity of *Verbascum* species.

In our previous studies, *in vivo* anti-inflammatory and antinociceptive activities of *Verbascum lasianthum* Boiss. ex Bentham flowers were investigated, which have been used in Turkish folk medicine to treat hemorrhoids. A 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, a triterpenoid saponin, ilwensisaponin A, was found to be the antinociceptive and anti-inflammatory principles, without inducing any apparent acute toxicity or gastric damage, while the iridoid glucoside ajugol had no activity (Küpeli *et al.*, 2007). Ilwensisaponin A (1) and ajugol (3) were also isolated from the flowers of *V. pterocalycinum* var. *mutense* (Tatli *et al.*, 2004); therefore these compounds were not tested for their antinociceptive and anti-inflammatory activities.

As shown in Table I, although the iridoid glucoside picroside IV did not show any antinociceptive activity, the saponin glycoside ilwensisaponin C (23.2%) significantly inhibited the abdominal constriction induced by p-benzoquinone in mice at a 200 mg/kg dose.

In addition, we have investigated the effects of pure compounds of *V. pterocalycinum* var. *mutense* in three models of acute inflammation, the carrageenan-induced hind paw edema, PGE1-induced hind paw edema and TPA-induced ear edema.

It was reported that the anti-inflammatory effects of several agents result in the partial inhibition of inflammation-mediator release (Amadio *et al.*, 1993). Subcutaneous injection of carrageenan into the rat paw produces plasma extravasation (Szolcsanyi *et al.*, 1998) and inflammation characterized by increased tissue water and plasma protein exudation with neutrophil extravasation and metabolism of arachidonic acid by both

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Dose [mg/kg]</th>
<th>Number of writhings ± SEM</th>
<th>Inhibition (%)</th>
<th>Ratio of ulceration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ilwensisaponin C (2)</td>
<td>100</td>
<td>33.6 ± 2.4</td>
<td>16.2</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>30.8 ± 2.1</td>
<td>23.2*</td>
<td>0/6</td>
</tr>
<tr>
<td>Picroside IV (4)</td>
<td>100</td>
<td>37.1 ± 3.2</td>
<td>7.5</td>
<td>1/6</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>36.3 ± 3.5</td>
<td>9.5</td>
<td>3/6</td>
</tr>
<tr>
<td>Aspirin</td>
<td>100</td>
<td>21.5 ± 2.6</td>
<td>46.4***</td>
<td>4/6</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>19.3 ± 2.1</td>
<td>51.9***</td>
<td>6/6</td>
</tr>
</tbody>
</table>

* *p* < 0.05; ** *p* < 0.01; *** *p* < 0.001; SEM, standard error mean.
cyclooxygenase and lipoxygenase enzyme pathways (Gamache et al., 1986). There are biphasic effects in carrageenan-induced edema. The first phase begins immediately after injection and diminishes within 1 h. The second phase begins at 1 h after injection and remains through 3 h (Garcia-Pastor et al., 1999). It is suggested that the early hyperemia of carrageenan-induced edema results from the release of histamine and serotonin (Kulkarni et al., 1986). On the other hand, the delayed phase of carrageenan-induced edema results mainly from the potentiating effect of prostaglandins on mediator release, especially of bradykinin. Hydrocortisone and some NSAIDs inhibit strongly the second phase of carrageenan-induced edema, but some others are effective against both phases (Kulkarni et al., 1986).

In the light of these data and Table II, ilwensisaponin C (4.8–23.3%) from V. pterocalycinum var. mutense seems to be more effective in the second phase of acute inflammation than in the first phase. Therefore, V. pterocalycinum var. mutense may block the prostaglandin and/or bradykinin release better than that of the histamin and/or serotonin.

Ilwensisaponin C (4.3–21.5%) significantly inhibited hind paw edema induced by prostaglandin E₁ dose-dependent, which supports the carrageenan-induced hind paw edema hypothesis (Table III).

As shown in Table IV, the isolated compounds did not show any anti-inflammatory activity against ear edema induced by TPA. Although TPA model has limited selectivity in determining the

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Table II. Effect of the materials from Verbascum pterocalycinum var. mutense against carrageenan-induced hind paw edema in mice.

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Dose [mg/kg]</th>
<th>90 min</th>
<th>180 min</th>
<th>270 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>37.6 ± 4.2</td>
<td>41.3 ± 3.9</td>
<td>45.1 ± 5.2</td>
<td>49.7 ± 3.8</td>
</tr>
<tr>
<td>Ilwensisaponin C (2)</td>
<td>100</td>
<td>38.3 ± 2.9</td>
<td>44.1 ± 3.2</td>
<td>46.4 ± 2.5</td>
<td>51.6 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>35.8 ± 3.2</td>
<td>37.6 ± 3.6</td>
<td>35.3 ± 2.7</td>
<td>38.1 ± 3.1</td>
</tr>
<tr>
<td>Picroside IV (4)</td>
<td>100</td>
<td>39.2 ± 2.6</td>
<td>44.1 ± 3.2</td>
<td>47.8 ± 3.0</td>
<td>51.4 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>38.1 ± 4.3</td>
<td>42.7 ± 3.1</td>
<td>46.3 ± 4.3</td>
<td>47.4 ± 3.2</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>29.6 ± 2.9</td>
<td>28.1 ± 2.3</td>
<td>28.2 ± 1.7</td>
<td>30.6 ± 2.8</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01; *** p < 0.001; SEM, standard error mean.

Table III. Effect of the materials from Verbascum pterocalycinum var. mutense against PGE₁-induced hind paw edema in mice.

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Dose [mg/kg]</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>75 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1.5 ± 0.7</td>
<td>11.6 ± 1.2</td>
<td>19.8 ± 1.5</td>
<td>25.1 ± 1.2</td>
<td>27.6 ± 1.4</td>
<td>16.1 ± 1.1</td>
</tr>
<tr>
<td>Ilwensisaponin C (2)</td>
<td>100</td>
<td>1.6 ± 1.1</td>
<td>11.8 ± 1.7</td>
<td>18.1 ± 1.9</td>
<td>24.7 ± 1.5</td>
<td>28.2 ± 1.7</td>
<td>18.3 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1.5 ± 0.9</td>
<td>10.1 ± 1.3 (12.9)</td>
<td>16.2 ± 1.1 (18.2)</td>
<td>19.7 ± 1.2 (21.5)*</td>
<td>22.4 ± 1.3 (18.8)</td>
<td>15.4 ± 1.4 (4.3)</td>
</tr>
<tr>
<td>Picroside IV (4)</td>
<td>100</td>
<td>1.6 ± 0.3</td>
<td>12.1 ± 1.1</td>
<td>21.9 ± 1.2</td>
<td>26.8 ± 1.6</td>
<td>29.2 ± 1.2</td>
<td>25.2 ± 1.5 (6.2)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1.5 ± 0.5</td>
<td>13.4 ± 1.4</td>
<td>18.5 ± 1.7 (6.6)</td>
<td>24.7 ± 1.4 (1.6)</td>
<td>25.9 ± 1.5 (6.2)</td>
<td>17.1 ± 1.8</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>1.4 ± 0.4 (6.7)</td>
<td>10.1 ± 1.0 (12.9)</td>
<td>13.5 ± 0.9 (31.8)**</td>
<td>16.2 ± 1.1 (35.5)***</td>
<td>20.7 ± 1.3 (25.0)**</td>
<td>13.6 ± 1.4 (15.5)</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01; *** p < 0.001; SEM, standard error mean.
Table IV. Effect of the materials from *Verbascum pterocalycinum* var. *mutense* against TPA-induced ear edema in mice.

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Dose [mg/ear]</th>
<th>Swelling thickness ± SEM [μm]</th>
<th>Inhibition (%)</th>
<th>Weight edema ± SEM [mg]</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>202.7 ± 26.8</td>
<td>23.8 ± 4.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ilwensisaponin C (2)</td>
<td>0.5</td>
<td>205.4 ± 21.6</td>
<td>–</td>
<td>20.9 ± 5.2</td>
<td>12.2</td>
</tr>
<tr>
<td>Picroside IV (4)</td>
<td>0.5</td>
<td>213.9 ± 31.2</td>
<td>–</td>
<td>31.6 ± 5.7</td>
<td>–</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.5</td>
<td>60.1 ± 11.9</td>
<td>70.3***</td>
<td>8.6 ± 2.6</td>
<td>63.9***</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01; *** p < 0.001; SEM, standard error mean.

possible mechanism of action, it is appropriate for assessing the anti-inflammatory effects in a first stage trial. The phorbol ester (TPA) elicited an inflammatory response characterized by a delayed time of onset. It has been established that this agent exerts its inflammatory effect through protein kinase C activation with the subsequent cytosolic phospholipase A2 stimulation, AA mobilization, and biosynthesis of prostaglandins and leukotrienes (Nishizuka, 1988).

In contrast with the results obtained in the carrageenan-induced hind paw edema test, ilwensisaponin C failed to affect the ear edema. This could be due to the different mechanism of action of the phlogistic agents or to the fact that the systemic route of administration fails to achieve an adequate concentration of the compounds at the site of inflammation.

In a reference survey, some triterpenes have been reported to have anti-inflammatory, cytotoxic, antitumour activities and to be chemopreventive. Papillomas in mouse skin were initiated with 390 nmol of 7,12-dimethylbenz(a)anthracene and, 1 week later, were promoted 2 times a week with 4.1 nmol of 12-O-tetradecanoylphorbol-13-acetate (TPA). Five saponin-related compounds used as potential anti-inflammatory agents from *Verbascum songaricum* were applied in the same position at 82 nmol. The saponin-related compounds effectively inhibited tumour formation in the sensitive mouse stock even when these compounds were given 1 h prior to TPA treatment. These results suggested that there is a general correlation between the anti-inflammatory and antitumour-promoting activities of saponins (Tokuda et al., 1991). Songarosaponins and their acylated derivatives, which had been previously isolated from *Verbascum songaricum*, have also been tested for their anti-inflammatory activity using the croton oil ear model. Acylated derivatives showed excellent anti-inflammatory activity as compared to that of saponins (Anam, 2001). Giner et al. (2000) also demonstrated that verbascosaponin A and verbascosaponin, isolated from *Scrophularia auriculata*, significantly inhibited the mouse paw edema induced by carrageenan and ear edema induced by single and multiple doses of TPA.

To the best of our knowledge, this is the first report on ilwensisaponin C, an oleane-type triterpene saponin, exhibiting anti-inflammatory activity in the carrageenan-induced hind paw edema test (without apparent gastric lesion induction) and antinociceptive activity in the 12-O-tetradecanoylphorbol-13-acetate-induced abdominal constriction test. The mechanism of action, however, has not yet been established. It may be speculated that the glucose molecule in the sugar residue connected to the C-3 position of the genin is crucial for its acute anti-inflammatory effect (Hostettmann and Marston, 1995; Gepdiremen et al., 2005). Therefore, the anti-inflammatory and antinociceptive activity of *Verbascum pterocalycinum* var. *mutense* is contributed mainly to saponins. However, further studies are required in order to reveal the possible pharmacokinetic factors which contribute to the activity of these compounds and to evaluate the potential of the plant.

Results of the present study have clearly demonstrated that the flowers of *Verbascum pterocalycinum* var. *mutense* possess significant antinociceptive and anti-inflammatory activities which support the traditional utilization in Turkey. In connection with the role of ilwensisaponin A and C as the active principles of *V. pterocalycinum* var. *mutense*, it seems that they could be synergistic with other active substances in their fractions and extracts.


De Young L. M., Kheifets J. B., Ballaron S. J., and Young J. M. (1989), Edema and cell infiltration in the phor bol ester treated mouse ear are temporally separate and can be differently modulated by pharmacologic agents. Agents Activity 26, 335–341.


