FABAD J. Pharm. Sci., 28, 71-75, 2003

RESEARCH ARTICLES

Antioxidant Flavonoids from *Verbascum salviifolium* Boiss.

Zeliha S. AKDEMİR*°, İ. İrem TATLI*, Erdal BEDİR, **,***, Ikhlas A. KHAN**

***Antioxidant Flavonoids from* Verbascum salviifolium Boiss.**

Summary : *Phenolic natural products such as flavonoids are known as potential antioxidant compounds. The aim of this study was the isolation, structure elucidation and determination of radical scavenging properties of flavonoids which were guided by DPPH autographic assay from the methanolic extract of the aerial parts of Verbascum salviifolium. From the titled plant, four flavonoid glucosides, apigenin-7-O-β-glucopyranoside* ***(1)****, luteolin-7-O-β-glucopyranoside* ***(2)****, luteolin-3'-O-β-glucopyranoside* ***(3)****, and chrysoeriol-7-O-β-glucopyranoside* ***(4)****, were isolated. The structures of the compounds were established on the basis of the spectroscopic evidence. Compounds* ***1-4*** *demonstrated scavenging properties toward the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical in TLC autographic assays*

Keywords: *Verbascum salviifolium, Scrophulariaceae, Flavonoid Glucosides, Apigenin-7-O-β-glucopyranoside, Luteolin-7-O-β-glucopyranoside, Luteolin-3'-O-β-glucopyranoside, Chrysoeriol-7-O-β-glucopyranoside, Radical Scavenging Activity.*

Verbascum salviifolium Boiss.'*in Antioksidan Flavonoitleri*

Özet: *Flavonoitler gibi fenolik doğal bileşiklerin potansiyel antioksidan maddeler oldukları bilinmektedir. Bu nedenle Verbascum salviifolium'un toprak üstü kısımlarının metanol ekstresinde, DPPH ile aktif olduğu tespit edilen flavonoitlerin, izolasyonlarının, yapı tayinlerinin ve serbest radikal süpürücü özelliklerinin tespit edilmesi amaçlanmıştır. Bitkiden dört flavonoit glukozidi, apigenin-7-O-β-glukopiranozit* ***(1)****, luteolin-7-O-β-glukopiranozit* ***(2)****, luteolin-3'-O-β-glukopiranozit* ***(3)*** *ve krizoeriyol-7-O-β-glukopiranozit* ***(4)*** *izole edilmiştir. Bileşiklerin yapıları spektroskopik yöntemlerle tayin edilmiştir.* ***1-4*** *nolu bileşiklerin radikal süpürücü özellikleri, İTK de, 1,1-difenil-2-pikrilhidrazil (DPPH) radikaline karşı test edilmiştir.*

Anahtar kelimeler: *Verbascum salviifolium, Scrophulariaceae, Flavonoit Glukozitleri, Apigenin-7-O-β-glukopiranozit, Luteolin-7-O-β- glukopiranozit, Luteolin-3'-O-β- glukopiranozit, Krizoeriyol-7-O-β-glukopiranozit, Radikal Süpürücü Özellik.*

INTRODUCTION

The genus *Verbascum,* commonly known as "Mullein", is represented by 228 species in the flora of Turkey[1]. Various preparations of some plants of this genus have been used as expectorant, mucolytic, sudorific, sedative, diuretic and constipate in traditional Turkish medicine[2]. *Verbascum* species are also used externally for desiccating wounds, anal fistula and pruritic conditions in urogenital organs[3]. In previous studies, flavonoids, phenylethanoids, lignans, saponins and iridoids have been reported from the *Verbascum* species, as secondary metabolites[4].

Flavonoid glycosides are widely distributed in the plant kingdom. Phenolic natural products such as flavonoids are of particular interest because of their antioxidant activity through scavenging oxygen radicals. Antioxidants that scavenge free radicals play an important role in cardiovascular disease, aging, cancer and inflammatory disorders. Therefore, it is important to look for effective radical scavengers by using various screening methods. The model for scavenging stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals has been used to detect the free radical-scavenging activity in a relatively short time and is simpler than the other assay models. These

* Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, 06100 Ankara TURKEY.
** National Center For Natural Products Research Institute of Pharmaceutical Sciences, University of Mississippi, University, Mississippi 38677, USA
*** Present Address: Ege University, Faculty of Engineering, Department of Bioengineering, Bornova 35100 İzmir, TURKEY.
° Corresponding author • e-mail: zakdemir@hacettepe.edu.tr

observations have accelerated the search for potential antioxidant principles from traditional medicinal plants[5].

Our previous studies have resulted in the isolation of iridoid, phenylethanoid and monoterpene glycosides and saponins from *V. lasianthum*[6], *V. cilicicum*[7], *V. pterocalycinum* var. *mutense*[8] and *V. salviifolium*[9]. Further investigation on the aerial parts of *V. salviifolium* yielded four flavonoid glucosides, apigenin-7-*O*-*β*-glucopyranoside **(1)**, luteolin-7-*O*-*β*-glucopyranoside **(2)**, luteolin-3β-*O*-*β*-glucopyranoside **(3)**, and chrysoeriol-7-*O*-*β*-glucopyranoside **(4)**. The isolation of flavonoids was guided by using 0.2 % DPPH solution in MeOH as an antioxidant. The current study also describes the structure elucidation of the isolated flavonoids.

MATERIALS and METHODS

General Experimental Procedures

The UV spectra (λ_{max}) were recorded on a Hitachi HP 8452 A spectrophotometer. The IR spectra (ϑ_{max}) were determined on ATI Mattson Genesis Series FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were obtained on Bruker Avance DRX 500 spectrometer operating at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR spectra. The chemical shift values are reported as parts per million (ppm) relative to tetramethylsilane (TMS), and the coupling constants are in hertz (Hz, in parentheses). For the ^{13}C NMR spectra, multiplicities were determined by DEPT experiment. LC-ESIMS FT data were obtained using a Bruker BioApex FT-MS instrument in the ESI mode. Polyamide (ICN) was used for vacuum liquid chromatography (VLC) and open column chromatography. Pre-coated silica gel 60 F_{254} aluminum sheets (Merck) were used for thin-layer chromatography (TLC) with developing solvent-system, $CHCl_3$-MeOH-H_2O (61:32:7). Plates were examined by UV fluorescence and sprayed with Naturstoffe A (Sigma) and 1% vanillin in concentrated H_2SO_4, followed by heating at 105°C for 1-2 mins DPPH (1,1-diphenyl-2-picrylhydrazyl, Sigma) was used for radical scavenging assay.

Plant Material

Verbascum salviifolium Boiss. (Scrophulariaceae) was collected from Burdur, Yesilova, Southwest of Burdur Lake, 880 m, in June 2002. A voucher specimen was deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 02003).

Extraction and Isolation

The air-dried and powdered aerial parts of *Verbascum salviifolium* (339.08 g) were extracted twice with MeOH (2x2000 ml) at 40°C. After evaporation of the combined extract *in vacuo,* 40.84 g MeOH extract was obtained. The crude extract was dissolved in water and partitioned with $CHCl_3$. The isolation of compounds was guided on TLC autographic assay using 0.2% DPPH solution in MeOH to search for potential antioxidant molecules. The lyophilized H_2O phase (29.49 g) was fractionated by vacuum-liquid chromatography over polyamide column (VLC, 250 g), eluting with H_2O (400 ml) and gradient MeOH-H_2O mixtures (25-100%) to afford six main fractions (A-F) and pure **2** (59.5 mg) and **3** (20.4 mg). Fraction F (91.8 mg) was rechromatographed on a polyamide column eluting with $CHCl_3$-MeOH-ethylmethylketone-acetone mixture (3:2:0.5 : 0.5 v/v) to afford **4** (5.6 mg) and **1** (4.3 mg).

Reduction of DPPH Radical

Methanolic solutions (0.1%) of compounds **1-4** were chromatographed on a Si gel TLC plate using $CHCl_3$-MeOH-H_2O (61:32:7). After drying, TLC plates were sprayed with a 0.2% DPPH (Sigma) solution in MeOH. Compounds showing a yellow-on-purple spot were regarded as antioxidant[10, 11].

RESULTS AND DISCUSSION

Compounds **1-4** were isolated from the methanolic extract of the aerial parts of *V. salviifolium* by a combination of vacuum liquid chromatography (VLC) and open column chromatographic methods, with the following results (Fig.1):

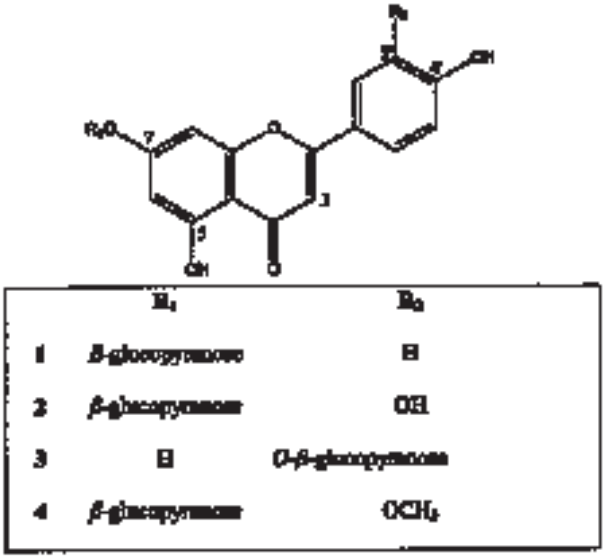

Figure 1. Flavonoids isolated from *Verbascum salviifolium.*

Apigenin-7-*O*-*β*-glucopyranoside **(1)** LC-ESIMS *m/z* 455 [M+Na]+ (positive mode) and *m/z* 431 [M-H]$^-$ (negative mode) (calc. for $C_{21}H_{20}O_{10}$). UV (MeOH) λ_{max} 268, 336 nm, IR (KBr) ϑ_{max} 3600 (OH), 1654 (C=C), 1529, 1363 (aromatic ring) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data (*see* Table 1).

Luteolin-7-*O*-*β*-glucopyranoside **(2)**: LC-ESIMS *m/z* 471 [M+Na]$^+$ (positive mode) and *m/z* 447 [M-H]$^-$ (negative mode) (calc. for $C_{21}H_{20}O_{11}$). UV (MeOH) λ_{max} 254, 339 nm, IR (KBr) ϑ_{max} 3600 (OH), 1654 (C=C), 1529, 1363 (aromatic ring) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data (*see* Table 1).

Luteolin-3′-*O*-*β*-glucopyranoside **(3)**: LC-ESIMS *m/z* 471 [M+Na]$^+$ (positive mode) and *m/z* 447 [M-H]$^-$ (negative mode) (calc. for $C_{21}H_{20}O_{11}$). UV (MeOH) λ_{max} 270, 329 nm, IR (KBr) ϑ_{max} 3600 (OH), 1654 (C=C), 1529, 1363 (aromatic ring) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) data (*see* Table 1).

Table 1. ^{1}H NMR (500 MHz, DMSO-d$_6$) and ^{13}C NMR (125 MHz, DMSO-d$_6$) data of compounds **1-3**.

		1			**2**			**3**		
Position Aglycone	C	δ_C	δ_H	*J*	δ_C	δ_H	*J*	δ_C	δ_H	*J*
2	C	164.5	-	-	164.6	-	-	164.4	-	-
3	CH	103.3	6.86 s	-	103.7	6.70 s	-	104.1	6.54 s	-
4	C	182.0	-	-	181.9	-	-	181.8	-	-
5	C	161.3	-	-	161.0	-	-	161.6	-	-
6	CH	99.9	6.45 d	1.5	100.0	6.42 br s	-	99.3	6.20 br s	-
7	C	163.1	-	-	163.1	-	-	163.6	-	-
8	CH	95.3	6.84 br s	-	95.3	6.77 br s	-	94.7	6.80 br s	-
9	C	157.1	-	-	157.2	-	-	157.5	-	-
10	C	105.7	-	-	105.8	-	-	103.7	-	-
1'	C	120.9	-	-	121.8	-	-	122.3	-	-
2'	CH	128.9	7.95 d	9.0	114.0	7.40 br s	-	114.8	7.79 br s	-
3'	C*	116.5	6.93 d	8.5	146.0	-	-	145.9	-	-
4'	C	162.1	-	-	150.1	-	-	150.9	-	-
5'	C	116.5	6.93 d	8.5	116.4	6.87 d	8.5	116.8	6.98 d	7.5
6'	CH	128.9	7.95 d	9.0	119.6	7.41 d	9.0	122.1	7.64 d	6.0
β-Glucose										
1''	CH	100.3	5.07 d	7.0	100.4	5.05 d	7.0	102.3	4.91 brs	-
2''	CH	73.7	3.18-3.51†	-	73.7	3.11-3.46 †	-	73.9	3.01-3.87 †	-
3''	CH	77.0	3.18-3.51 †	-	76.9	3.11-3.46 †	-	76.5	3.01-3.87 †	-
4''	CH	70.1	3.20 t	9.0	70.1	3.29 t	9.0	70.6	3.01-3.87 †	-
5''	CH	77.7	3.00 m	-	77.7	3.00 m	-	77.9	3.01-3.87 †	-
6''a	CH	61.2	3.72 d	10.5	61.2	3.72 d	11.0	61.5	3.01-3.87 †	-
6''a	CH		3.48 †	-		3.48 d	12.0		3.01-3.87 †	-

† unclear due to overlapping

* CH for **1**

Chrysoeriol-7-*O*-*β*-glucopyranoside **(4)**: LC-ESIMS *m/z* 485 $[M+Na]^+$ (positive mode) and *m/z* 461 $[M-H]^-$ (negative mode) (calc. for $C_{22}H_{22}O_{11}$). UV (MeOH) λ_{max} 273, 345 nm, IR (KBr) ϑ_{max} 3600 (OH), 1654 (C=C), 1529, 1363 (aromatic ring) cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 6.96 (1H, s, H-3), 6.46 (1H, *br s*, H-6), 6.87 (1H, *br s*, H-8), 7.58 (1H, *br s*, H-2'), 6.95 (1H, *br s*, H-5'), 7.60 (1H, *br s*, H-6'), 5.05 (1H, d, *J* = 7.5 Hz, H-1''), 3.00-3.46 (each 1H, *overlapped,* H-2''/H-5''), 3.73 (1H, *br d, J* = 10.5 Hz, H-6'' a), 3.00-3.46 (1H, *overlapped,* H-6'' b) and 3.90 (3H, *s,* OCH_3).

Compound **1** was obtained as a yellow amorphous powder. Its molecular formula was determined as $C_{21}H_{20}O_{10}$ by LC-ESIMS, ^{13}C NMR and DEPT-135 data. The UV spectrum of **1** showed absorption maxima at 268 and 336 nm suggesting its flavonoid structure[12].

^1H NMR spectrum of **1** showed two meta-coupled protons at δ_H 6.45 (1H, *d, J* = 1.5 Hz) and 6.84 (1H, *br s*), which were assigned to characterize H-6 and H-8 protons, respectively, with a singlet at δ_H 6.86 for H-3. In addition, the methine carbon signal at δ_C 103.37 was attributed to C-3 in the ^{13}C NMR spectrum, indicating a 5,7 dihydroxy-flavone[13, 14]. Moreover, the ^1H NMR resonances typical for AB system of two *ortho*-coupled aromatic protons of ring B were observed at δ_H 6.93 (1H, *d, J* = 8.5 Hz, H-3'/5') and 7.95 (1H, *d, J* = 9.0 Hz, H-2'/6') which was verified from the ^{13}C NMR resonances at δ_C 116.5 (C-3',5') and 128.9 (C-2',6') [14, 15]. The anomeric proton appearing at δ_H 5.07 (1H, *d, J* = 7.0 Hz, H-1'', δ_C 100.3) together with the signals in the region of δ_H 3.00-3.72 and δ_C 61.2-77.7 suggested the presence of one glucopyranosyl moiety. The coupling constant of the anomeric proton indicated that sugar moiety was connected to the flavone *via* an *O*-linkage. Morever, the chemical shift value of H-1'' (δ_H 5.07) was typical of an attachment at C-7 position[14]. The downfield shifts of A ring proton signals (H-6/8, Δδ+ 0.2 and + 0.3 ppm, respectively) also supported this assumption, compared with that of apigenin[14]. The chemical shift value of H-1'' also confirmed that the β-glucose unit was connected to C-7 through an *O*-linkage[14-16].

Consequently, compound **1** was determined to be apigenin-7-*O*-*β*-glucopyranoside[14-16].

Compounds **2** and **3** were isolated as yellow amorphous powders. Their UV and IR spectra were characteristic of a flavone system[12]. The molecular formula of compounds **2** and **3** were both determined as $C_{21}H_{20}O_{11}$ by LC-ESIMS, which exhibited a molecular ion at *m/z* 471 $[M+Na]^+$ (positive mode) and *m/z* 447 $[M-H]^-$ (negative mode). The ^1H and ^{13}C NMR data and DEPT-135 analysis of compounds **2** and **3** were almost identical with that of **1**. However, the presence of an AMX spin system at δ_H 6.87 (1H, *d, J* = 8.5 Hz, H-5'), 7.40 (1H, *br s,* H-2') and 7.41 (1H, *d, J* = 9.0 Hz, H-6') for **2** and at δ_H 6.98 (1H, *d, J* = 7.5 Hz, H-5'), 7.64 (1H, *d, J* = 6.0 Hz, H-6') and 7.79 (1H, *br s,* H-2') for **3**, instead of two *ortho*-coupled doublet signals, was indicative of a luteolin moiety as a basic skeleton[14]. The resonances of the anomeric protons observed in the low-field region at δ_H 5.05 (1H, *d, J* = 7.0 Hz) in **2** and at δ_H 4.91 (1H, *br s*) in **3**, implied that compounds **2** and **3** were both luteolin glucosides[14]. The major difference between **2** and **3** was concluded as the attachment site of the glucosyl unit on the aglycone moiety. The site of glycosylation in **2** was determined to be the C-7 position based on the chemical shifts of H-6 and H-8 (Δδ+ 0.2 and + 0.3 ppm, respectively) in comparison to that reported for luteolin[14]. When the ^1H NMR chemical shifts of B ring protons of **3** were compared with that of luteolin[14], the H-2' and H-6' signals were seen to be shifted downfield by 0.4 and 0.2 ppm, respectively. These assumptions were confirmed by ^{13}C NMR data (*see* Table 1). The chemical shift values of the anomeric protons and carbon atoms for compounds **2** and **3** as well as the C resonances of the hydroxyl group at indicated that the *β*-glucose units were connected at C-7 (for **2**) and C-3' (for **3**) through an *O*-linkage.

Consequently, compounds **2** and **3** were determined to be luteolin-7-*O*-*β*-glucopyranoside[14-16] and luteolin-3'-*O*-*β*-glucopyranoside[14-16], respectively.

Inspection of the ^1H and ^{13}C NMR data of compound **4** showed signals very similar to those of com-

pound **2** with additional signals arising from an aromatic methoxyl group at δ_H 3.90. This methoxyl group was found to be attached to C-3' atom, characteristic of a di-*ortho* substituted methoxyl group because methylation of a B-ring hydroxyl causes a downfield shift of about 0.15 ppm of the *ortho* and *para* position[14]. Comparison of the ^{1}H NMR spectroscopic data of **4** [δ_H 7.58 (1H, *br s*, H-2') and 7.60 (1H, *br s*, H-6')] with those of **2** [δ_H 7.40 (1H, *br, s* = 9.0 Hz, H-2') and 7.41 (1H, *d, J* = 9.0 Hz, H-6')] indicated that **4** was a 3'-methyleter derivative of **2**, based on the above knowledge. The full assignment of the proton signals of the aglycone and sugar moieties established that compound **4** was chrysoeriol-7-*O*-*β*-glucopyranosidel4.

Conclusion

To our knowledge, this study is the first report for luteolin-3'-*O*-*β*-glucopyranoside. (**3**) from the *Verbascum* spec.

Compounds **1-4** were found to have significant antioxidant properties, based on the experiments with 1,1-diphenyl-2-picrylhydrazyl (DPPH), which indicated their ability to efficiently scavenge free radicals. Definite structural features are important determinants for radical scavenging activity and/or antioxidant potential of flavonoids[17].

Acknowledgements

The authors are grateful to Prof. Dr. Hayri Duman (Gazi University, Faculty of Science, Etiler, Ankara, Turkey) for the authentification of the plant specimen and to Dr. Chuck Dunbar for conducting the LC-ESIMS analysis. This work was supported in part by the United States Department of Agriculture, ARS Specific Cooperative Research Agreement no. 58-6408-7-012.

References

1. Huber-Morath A. *Verbascum,* Davis, PH (ed.), Flora of Turkey and the East Aegean Islands, Edinburgh University Press, 6, 461-603, 1978.
2. Baytop T. Therapy with Medicinal Plants in Turkey (Past and Present), 2nd ed, Nobel Tıp Kitabevleri Ltd., İstanbul., 334-335, 1999.
3. Sezik E,Yeşilada E, Honda, G, Takaishi Y, Takeda Y, Tanaka T. Traditional medicine in Turkey X. Folk medicine in Central Anatolia, *J. Ethnopharm.,* 75, 95-115, 2001.
4. Abou Gazar H. Phytochemical studies on *Verbascum wiedenmannianum* Fisch. & Mey., Ph.D. Thesis, Institute of Health Sciences, Hacettepe University, Ankara, Turkey, 2001.
5. Pietta P-G. Flavonoids as antioxidants, *J. Nat. Prod.,* 63, 1035-1042, 2000.
6. Akdemir ZS, Tatli II, Bedir E, Khan IA. Acylated iridoid glycosides from *Verbascum lasianthum, Turk. J. Chem.,* 28, 101-109, 2004a.
7. Tatli II, Akdemir ZS, Bedir E, Khan IA. 6-*O*-*α*-L-Rhamnopyranosylcatalpol derivative iridoids from Verbascum cilicicum, *Turk. J. Chem.,* 27, 765-772, 2003.
8. Tatli II, Akdemir ZS, Bedir E, Khan IA. Saponin, iridoid, phenylethanoid and monoterpene glycoside from *Verbascum pterocalycinum* var. *mutense, Turk. J. Chem.,* 28, 111-122, 2004b.
9. Akdemir ZS, Tatli II, Bedir E, Khan IA. Two new iridoid glucosides from *Verbascum salviifolium, Z. Naturforsch.,* 2003 (submitted).
10. Cuendet M, Hostettman K, Potterat O, Dyatmiko W. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei, Helv. Chim. Acta,* 80, 1144-1152, 1997.
11. Takao T, Kitatani F, Watanabe N, Yagi A, Sakata K. A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish, *Biosci. Biotech. Biochem.,* 58, 1780-1783, 1994.
12. Mabry TJ, Markham KR, Thomas MB, The Systematic Identification of Flavonoids, Springer-Verlag, New York, 1970.
13. Tanaka T, Iunuma M, Mizumo M. Spectral properties of 2'-oxygenated flavones, *Chem. Pharm. Bull.,* 34, 1667-1671, 1986.
14. Markham KR, Geiger H. ^{1}H Nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulfoxide, Harborne JB (ed.), The Flavonoids, Advances in Research Since 1986, Chapmann and Hall, London, 1994.
15. Harborne JB, The Flavonoids, Advances in Research Since 1980, Chapmann and Hall, London, 1988.
16. Agrawal PK. Carbon-13 NMR of Flavonoids, Elsevier, NewYork, 1989.
17. Akdemir ZS, Tatli II, Saracoglu I, Ismailoglu UB, Sahin-Erdemli I, Calıs I. Polyphenolic compounds from *Geranium pratense* and their free radical scavenging activities, *Phytochemistry,* 56, 189-193, 2001.