GRASAS Y ACEITES 69 (3)
July–September 2018, e270
ISSN-L: 0017-3495
https://doi.org/10.3989/gya.0234181

Lipophilic components and evaluation of the cytotoxic and antioxidant activities of *Impatiens glandulifera* Royle and *Impatiens noli – tangere* L. (Balsaminaceae)

K. Szewczyk[a,✉], R. Bonikowski[b], A. Maciąg-Krajewska[b], J. Abramek[c] and A. Bogucka-Kocka[c]

[a]Department of Pharmaceutical Botany, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland
[b]Łódź University of Technology, Faculty of Biotechnology and Food Sciences,
Institute of General Food Chemistry, Stefanowskiego 4/10, 90-924 Lodz, Poland
[c]Department of Biology and Genetics, Medical University of Lublin, Chodźki 4a,20-093 Lublin, Poland

✉Corresponding author: k.szewczyk@umlub.pl

Submitted: 27 February 2018; Accepted: 10 May 2018

SUMMARY: The chemical composition of the lipophilic fractions of *Impatiens glandulifera* Royle and *I. noli-tangere* L. were analyzed by gas chromatography-mass spectrometry (GC-MS)., The study focused on the fatty acids, triterpenoids and sterols in the leaves, roots and seeds. Most of the identified compounds are new for these species. a-linolenic, oleic and palmitic acids were the most abundant in the fatty acid fractions, β-amyrin and 5α-lup-20(29)-en-3β-ol in the triterpenoid fractions, and β-sitosterol, spinasterol and chondrillasterol in the sterol fractions. The fatty acid and triterpenoid fractions showed strong antioxidant activity, similar to positive controls. Moreover, the triterpenoid fraction from *I. noli-tangere* seeds significantly inhibited HL-60 human leukemia cells. Other fractions showed moderate cytotoxicity. The present study suggests that *I. glandulifera* and *I. noli-tangere* are good source of omega-3 fatty acids, and they might be considered as antioxidant and chemopreventive agents.

KEYWORDS*: Antioxidant; Cytotoxicity; Fatty acids;* Impatiens*; Phytosterols; Triterpenoids*

RESUMEN: ***Componentes lipofílicos y evaluación de las actividades citotóxicas y antioxidantes de*** **Impatiens glandulifera** ***Royle e*** **Impantiet noli-tangere** ***L. (Balsaminaceae).*** La composición química de las fracciones lipofílicas, centrada en los ácidos grasos, triterpenoides y esteroles de las partes aéreas, raíces y semillas de *Impatiens glandulifera* Royle e *Impatient. noli-tangere* L. se analizaron por cromatografía de gases-espectrometría de masas (GC-MS). La mayoría de los compuestos identificados son nuevos para estas especies. Los ácidos α-linolénico, oleico y palmítico fueron los más abundantes en las fracciones de ácidos grasos, β-amirina y 5α-lup-20 (29)-en-3β-ol en las fracciones triterpenoides, y β-sitosterol, espinasterol y condriplasterol en las fracciones de esteroles. Las fracciones de ácidos grasos y triterpenos mostraron una fuerte actividad antioxidante, similar a los controles positivos. Además, la fracción triterpenoidea de las semillas de *I. noli-tangere* inhibió significativamente las células de leucemia humana HL-60. Otras fracciones mostraron citotoxicidad moderada. El presente estudio sugiere que *I. glandulifera* e *I. noli-tangere* son la buena fuente de ácidos grasos omega-3, y podrían considerarse antioxidantes y agentes quimiopreventivos.

PALABRAS CLAVE*: Ácidos grasos; Antioxidante; Cytotoxicidad; Fitoesteroless;* Impatiens*; Triterpenoides*

ORCID ID: Szewczyk K https://orcid.org/0000-0003-4884-9110, Bonikowski R https://orcid.org/0000-0003-3327-8984, Maciąg-Krajewska A https://orcid.org/0000-0001-9892-1219, Abramek J https://orcid.org/0000-0001-7925-4350, Bogucka-Kocka A https://orcid.org/0000-0001-8473-7429

Citation/Cómo citar este artículo: Szewczyk K, Bonikowski R, Maciąg-Krajewska A, Abramek J, Bogucka-Kocka A. 2018. Lipophilic components and evaluation of the cytotoxic and antioxidant activities of *Impatiens glandulifera* Royle and *Impatiens noli – tangere* L. (Balsaminaceae). *Grasas Aceites* **69** (3), e270. https://doi.org/10.3989/gya.0234181

1. INTRODUCTION

Impatiens glandulifera Royle and *Impatiens noli-tangere* L. are an annual herbaceous species which belongs to the Balsaminaceae family. These plants are native to North-Western, Central Europe and Asia (Tříska *et al*., 2013). In Poland, *I. noli-tangere* and *I. glandulifera* are two of the top 25 invasive alien plants (Tokarska-Guzik *et al*., 2010).

From a chemical point of view, *I. glandulifera* and *I. noli-tangere* have been the subject of few studies which have reported on the isolation or identification of flavonoids (Szewczyk *et al.*, 2016a), phenolic acids (Szewczyk and Olech, 2017), essential oils (Szewczyk *et al*., 2016b), triterpenoid saponins (Grabowska *et al*., 2017), glucosylated steroids (Cimmino *et al*., 2016), and naphthoquinones (Lobstein *et al*., 2001; Tříska *et al.,* 2013).

It has been reported that several *Impatiens* species have valuable biological properties. For example, the triterpenoid saponins isolated from the leaves of *I. parviflora* exhibited cytotoxic activity against human prostate and melanoma cancer cells (Grabowska *et al*., 2017), and the glucosylated steroids from *I. glandulifera* had cytostatic activity against U373 glioblastoma cells (Cimmino *et al*., 2016). Grabowska *et al.* (2016) noticed that fresh leaves of *I. parviflora* may be beneficial in inflammatory conditions. Moreover, *I. balsamina* has been used for a very long time in traditional Asian and American medicine. Depending on the type of ailment, it was applied by compression directly on the skin, or as a tea prepared by pouring hot water on the dried plant (Yang *et al*., 2001). The plant is also utilized in Chinese medicine for rheumatism therapy; in treating fractures, swellings and contusions, as well as beriberi disease and as a plant with anticancer properties (Fukumoto *et al*., 1996).

Due to the biological importance of *Impatiens* species and the fact that the current knowledge about the lipophilic components in these plants is negligible, the aim of the present study is to determine and to compare the fatty acids, triterpenoids and sterol contents in the leaves, roots and seeds of *I. glandulifera* and *I. noli-tangere*. Moreover, their antioxidant and cytotoxic potential were investigated.

2. MATERIALS AND METHODS

2.1. Plant material

The leaves, roots and seeds of two *Impatiens* species were collected in August 2015. *I. glandulifera* Royle (no. IG-0815) were collected in Józefów, near Biłgoraj (Poland) at an altitude of 240 m a.m.s.l. (coordinates N 50°29'06; E 23°02'12'') and *I. noli-tangere* L. (no. INT-0815) were gathered in Zalesie Górne near Warsaw (Poland) at an altitude of 115 m a.m.s.l. (coordinates N 52°2'16''; E 21°1'55''). Voucher specimens were deposited in the Department of Pharmaceutical Botany, Faculty of Pharmacy, Medical University of Lublin. The plants were identified by Prof. Tadeusz Krzaczek.

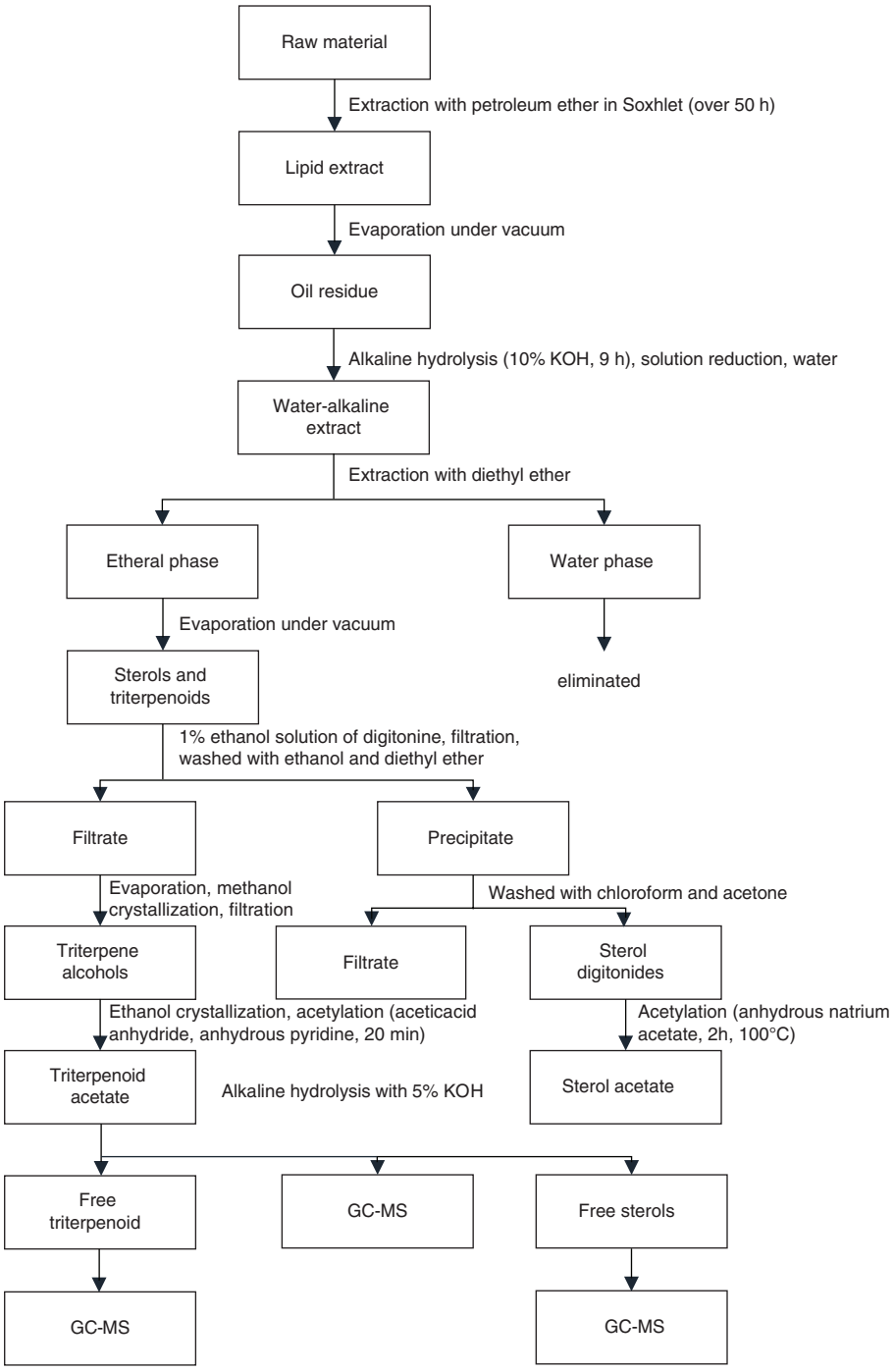
2.2. Chemicals and reagents

All chemical reagents used in the experiment were purchased from various commercial suppliers and were of the highest purity available. 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrozine (3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine), α-tocopherol, 2,6-di-tert-butyl-4-methylphenol (BHT), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphate-buffered saline (PBS) was purchased from Gibco (Carlsbad, CA, USA). Other chemicals used for the preparation of the extracts were of analytical grade, and were obtained from Polish Reagents (POCH, Gliwice, Poland).

2.3. Extraction and isolation procedure

The air-dried, ground leaves (100.0 g), roots (100.0 g) and seeds (20.0 g) of two plants were extracted with petroleum ether (bp 45–60 °C) in a Soxhlet apparatus for over 50 h. The obtained lipid extracts were evaporated under vacuum. Subsequently, yellowish oil residues were saponified with a 10% ethanol solution of potassium hydroxide for 9 h, and the solutions were reduced to about half of their volume under vacuum, diluted with water, and extracted with diethyl ether. The ether phases were washed with distilled water until neutral, dried, and concentrated to yield crude unsaponifiable fractions. The sterols were first separated from these fractions in the typical manner by precipitation with digitonine (Jerzmanowska, 1967). The obtained digitonides were filtered and washed with ethanol, and acetylation was performed (acetic acid anhydrous, with anhydrous sodium acetate, 2 h, 100 °C). Sterol acetates were crystallized from 50% ethanol (Jerzmanowska, 1967; Harrabi *et al*., 2016). Saponification of these mixtures yielded the free sterols which were identified by GC-MS. The total amount of the phytosterols was determined by means of the weight method (Jerzmanowska, 1967).

Triterpenoids were then isolated from the filtrates remaining after the crystallization of sterol digitonides. The eluates were reduced to about half of their volume under vacuum, and methanol was added for the crystallization of triterpenoids. The obtained precipitates were again crystallized from ethanol, acetylated (acetic acid anhydride, anhydrous pyridine, 20 min), and again crystallized from ethanol. Hydrolysis of the triterpenoid acetate mixtures (5% KOH in anhydrous ethanol, benzene, 7 h) yielded free triterpenoids (Jerzmanowska, 1967). Both free and triterpenoid acetates were analyzed



SCHEME 1. Schematic diagram of the isolation sterol and triterpenoid fractions from *Impatiens* species.

using GC-MS. A schematic diagram of the isolation sterol and triterpenoid fractions is presented in Scheme 1.

In the next stage, powdered plant materials were sonicated with n-hexane (3 × 30 min) at a controlled temperature (40 ± 2 °C). The supernatants were concentrated to dryness under vacuum at a controlled temperature. Then, 5 mL of methyl-tert-butyl ether were added to 0.1 g oil. The fatty acid methyl esters (FAMEs) were obtained by adding a trimethylsulfonium hydroxide solution (TMSH). The mixtures were then incubated (60 min, 60 °C), and analyzed using GC-MS.

For antioxidant and cytotoxic assays, 0.1 g of triterpenoid and fatty acid fractions were diluted in 80 and 95% ethanol (10 mL), respectively.

2.4. Chromatographic analysis

The analyses were performed on a Trace GC Ultra coupled with a DSQII mass spectrometer (Thermo Electron Corporation). GC-FID and MS analyses was performed using a MS-FID splitter (SGE Analytical Science). Mass range: 33-550 amu, ion source-heating: 200 °C, EI: 70eV, He (p: 300 kPa for phytosterols; 91 kPa for FAMEs). Operating conditions for derivatives of phytosterols: BPX5 (30 m×0.25 mm i.d., film thickness 0.25 μm), temperature program 100 °C (1 min, 10 °C/min) – 250 °C (15 min, 4 °C/min) – 300 °C (30 min). Injector temperature: 310 °C, and detector: 300 °C. Operating conditions for FAMEs: Stabilwax-DA, Restek (30m x 0.25 mm i.d., film thickness 0.25 μm), 50 °C (3 min) – 250 °C (40 min), 4 °C/min. Injector: 250 °C, detector 260 °C.

2.5. Identification of compounds

Phytosterols were analyzed as TMS (trimethylsilyl ethers; Thanh *et al*., 2006) and fatty acids as FAME derivatives along with their mass spectra, compared with the MS of the NIST/EPA/NIH Mass Spectral Library 2009 and Wiley Registry of Mass Spectral Data, 8th edition. The retention times (Rt) were compared with a standard mixture Rt.

Area percent was obtained electronically from the GC-FID response without the use of an internal standard or correction factors. Quantitative determinations corresponded to the means of three replicates ± SD.

2.6. Cell lines and cell cultures

Human leukemia cell lines HL-60 and HL-60/MX2 were cell lines of AML (acute myeloid leukemia) origin and were obtained from the ECACC (the European Collection of Cell Cultures).

The HL-60 and HL-60/MX2 cells were maintained in RPMI 1640 medium (Biomed Lublin) supplemented with 20% and 10% of fetal bovine serum (FBS; PAA Laboratories, Linz, Austria), respectively and the antibiotics were 100 U/mL penicillin, 100 μg/mL streptomycin, and 2.5 μg/mL amphotericin B (Gibco, Carlsbad, USA), incubated at 37 ºC in a humidified atmosphere of 5% CO_2.

2.7. *In Vitro* cytotoxicity assay

The effect of the obtained triterpenoid and fatty acid fractions on HL-60 and HL-60/MX2 cell lines was measured using the trypan blue assay. These cells were seeded on 12-well plates (Sarstedt, Wiener, Austria) at a density of 2×10^5 and 3×10^5 cells per well, respectively. After 24 hours, the cell suspensions were treated with plant samples at concentrations ranging from 10 to 5000 μg/mL for triterpenoid and from 10 to 2500 μg/mL for fatty acid fractions, and incubated for 24 hours. Then, cell suspensions were centrifugated (800 rpm, 5 min), washed with PBS and centrifugated again. The cells were stained with a 0.4% solution of trypan blue (Bio-Rad) and counted with a TC10^{TM} Automated Cell Counter (Bio-Rad). Each experiment was repeated three times. Dose response curves were made and IC_{50} values were found.

2.8. Antioxidant activity

To determine the antioxidant activity of the obtained triterpenoid and fatty acid fractions, two methods were used. DPPH (2.2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was measured according to the Brand–Williams *et al*., (1995) method. The changes in color from deep-violet to light-yellow were measured at 515 nm in a UV/visible light spectrophotometer. The metal chelating power was determined using the Guo *et al*., (2001) method. Absorbance was measured at 562 nm and the percentage of inhibition of ferrozine- Fe^{2+} complex formation was calculated according to the formula:

$$\% \text{ inhibition} = [1-(A_s/A_b)] \times 100$$

where A_b - absorbance of the blank, A_s – absorbance in the presence of the test sample

Both antioxidant activities were expressed as an efficient concentration EC_{50}, the extract solution concentration provided 50% of the activity in a dose-dependent manner. α-Tocopherol and BHT were used as a positive control.

2.9. Statistical analysis

All extractions and determinations were done in triplicate. The results are presented as experimental means and ± SD. The obtained data were subjected to statistical analysis using Statistica 10.0. (StatSoft, Cracow).

3. RESULTS AND DISCUSSION

In continuation of our research on the genus *Impatiens* L., the present study deals with the determination of phytosterols, triterpenoids and fatty acids from the leaves, roots and seeds of *I. glandulifera* and *I. noli-tangere*, as well as the examination of their cytotoxicity and antioxidant activity.

3.1. Sterol composition

The mixtures of sterol acetates and free sterols were obtained in typical way, and then analyzed using the GC-MS method. The total amount of sterols was determined using the weight method and the results are given in Table 1. On the basis of GC-MS analysis,

TABLE 1. Sterol content (% of total fraction; mass %, GC) of *I. glandulifera* and *I. noli-tangere*.

Compound	IGL	IGR	IGS	INL	INR	INS
cholesterol	nd	2.00±0.15	1.04±0.02	1.21±0.01	1.55±0.08	3.79±0.13
campesterol	0.26±0.01	11.63±0.38	3.54±0.11	13.78±0.21	14.05±0.35	6.17±0.19
campestanol	nd	6.50±0.10	5.21±0.08	4.70±0.05	5.06±0.03	3.18±0.15
ergosta-7,22-dien-3-ol	0.10±0.01	0.10±0.01	nd	0.10±0.01	nd	nd
ergost-8(14)-en-3-ol	nd	0.70±0.02	0.85±0.05	0.81±0.01	1.00±0.03	0.59±0.01
stigmasterol	nd	0.21±0.03	0.42±0.03	0.19±0.01	0.36±0.04	nd
β-sitosterol	2.43±0.11	44.36±0.25	53.61±0.69	59.00±0.38	54.18±0.43	23.25±0.50
sitostanol	0.88±0.04	18.65±0.42	13.57±0.32	17.26±0.19	19.05±0.35	5.91±0.13
Δ5-avenasterol	nd	0.21±0.01	tr	0.91±0.10	1.72±0.05	0.68±0.08
Δ7-stigmastenol	nd	0.29±0.02	2.61±0.06	1.70±0.11	2.26±0.04	1.46±0.01
ergosterol	tr	0.18±0.02	nd	nd	nd	nd
3 β-hydroxy-5 α-cholestane-6-one	tr	0.19±0.01	nd	nd	nd	nd
5.Xi.-Ergost-7-ene-3β-ol	1.75±0.13	tr	nd	nd	0.08±0.02	1.12±0.02
Spinasterol + chondrillasterol	68.86±0.75	9.31±0.25	11.05±0.31	0.34±0.08	0.23±0.01	4.86±0.06
Δ^7-sitosterol	23.01±0.38	1.72±0.02	7.30±0.15	nd	nd	1.25±0.06
stigmasta-7,24(28)-dien-3β-ol (Δ^7-avenasterol)	2.20±0.02	nd	nd	nd	tr	0.37±0.01
Sum of identified [%]	99.49±0.01	96.05±0.05	99.20±0.03	100.00±0.03	99.54±0.01	52.63±0.01
Total amount of sterol [%]	0.14	0.28	0.30	0.23	0.16	0.07

Values are mean ± SD of three samples; nd – not detected; tr – traces; IGL, *I. glandulifera* leaves; IGR, *I. glandulifera* roots; IGS, *I. glandulifera* seeds; INL, *I. noli-tangere* leaves; INR, *I. noli-tangere* roots; INS, *I. noli-tangere* seeds.

in the sample from the roots of *I. Glandulifera* (IGR) fifteen phytosterols were identified. In the leaves of both species (IGL; INL) in the seeds of *I. noli-tangere* (INS), in the seeds of *I. Glandulifera* (IGS), and the roots of *I. noli-tangere* (INR),eleven and twelve compounds were observed, respectively.

The major components of the total sterol fractions obtained from the leaves of *I. glandulifera* were spinasterol + chondrillasterol and Δ^7-sitosterol. The co-eluted compounds were separated in the GCxGC analysis (Figure 1). Similarly, spinasterol was observed in the root cultures of *I. balsamina* (Panichayupakaranant *et al*., 1995). This compound is known as an antimutagen agent that was tested using the mouse skin tumor assay (Villaseñor and Domingo, 2000). α-Spinasterol, together with glanduliferins A and B (belonging to a cholestane subgroup), were isolated from *I. glandulifera* (Cimmino *et al.*, 2016). In the rhizomes of *I. pritzellii,* some sterols, such as α-spinasterol, spinasteryl-3-one, α-spinasteryl-3-*O*-β-D-glucopyranoside, and 3-*O*-[6'-*O*-palmitylo-β-D-glucosyl]-spinasterol were noticed previously (Zhou *et al*., 2007). Moreover, α-spinasterol was isolated from the seeds, roots, leaves and fruits of *I. balsamina* (Wang *et al*., 2011).

In other samples in our research, the most important sterol was β-sitosterol (ranging from 23.25 to 59.00% of total sterols). High levels of campesterol and sitostanol were also observed in these fractions. Other compounds such as ergost-8(14)-en-3-ol, campestenol, Δ^7-stigmasterol, Δ^5- avenasterol, and cholesterol occurred in low concentrations.

3.2. Triterpenoid composition

The appropriate GC-MS procedure allowed for the identification of the triterpenoid acetate fraction from the leaves of *I. glandulifera* (IGLt). Thirteen compounds such as 5α-ergost-7-en-3β-ol, taraxasteryl, α-spinasterol, 13,27-cyclour-san-3-ol, (3β,13β,14β)-, β-amyrin, cycloeucalenyl, β-simiarenol, stigmast-7-en-3-ol, (3β,5a)-, 5α-lup-20(29)-en3β-ol, 9,19-cyclolanostan-3-ol, 24-methylene-, (3β)-, Ψ-taraxasteryl, lupan-3-ol, and olean-12-ene-3,28-diol, (3β)- were detected. The occurrence of eleven compounds was observed in the fraction from the roots of *I. glandulifera* (IGRt). In the roots of *I. noli-tangere* (INRt) and the seeds of *I. glandulifera* (IGSt), eight compounds were observed. In the leaves of *I. noli-tangere* (INLt), ten compounds were detected; and in the seeds of *I. noli-tangere* (INSt), seven compounds were found. As can be seen in Table 2, phytosterols were also present in these fractions, with the exception of triterpenoids. An exemplary GC-MS chromatogram of the triterpenoid acetates from the leaves of *I. Glandulifera* is shown in Figure 1.

In the GC-MS analysis of the triterpenoid acetate fraction from the leaves of *I. glandulifera*, peak 1 had an identical retention time to 5α-ergost-7-en-3β-ol

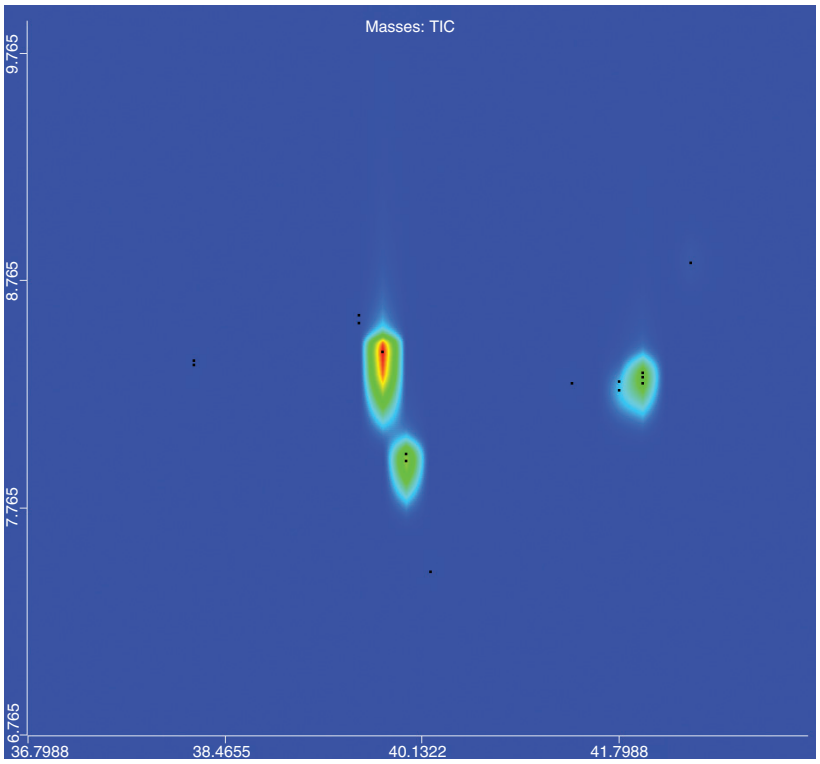


FIGURE 1. The GC x GC chromatogram (Rxi-5 x BPX50) of spinasterol and chondrillasterol (around 40 min) separation which were co-eluted (Rt=85.98 min) in GC analysis.

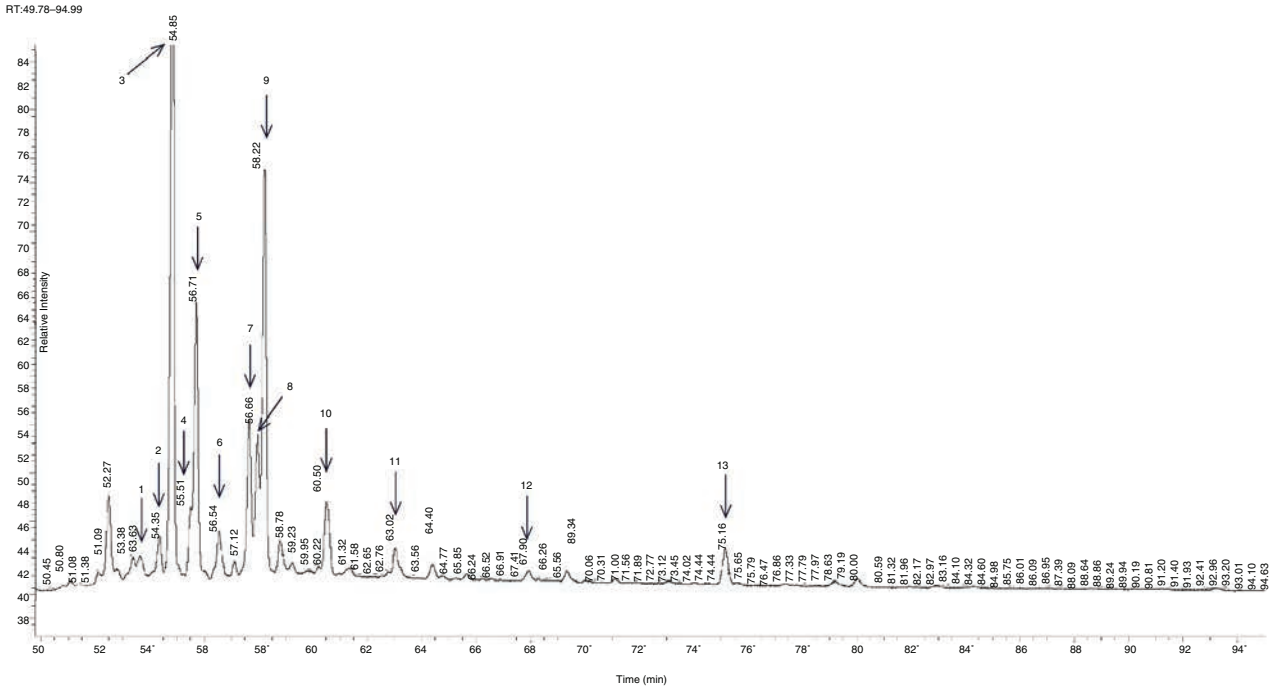


FIGURE 2. The GC-MS chromatogram of triterpenoid acetates from the leaves of *I. Glandulifera* with marked compounds. **1**-5a-ergost-7-en-3β-ol; **2**- taraxasteryl; **3**- a-spinasterol; **4**- 13,27-cycloursan-3-ol, (3β,13β,14β)-; **5**- β-amyrin; **6**- cycloeucalenyl; **7**- β-simiarenol; **8**- stigmast-7-en-3-ol, (3β,5a)-; **9**- 5a-lup-20(29)-en3β-ol; **10**- 9,19-cyclolanostan-3-ol,- 24-methylene-, (3β)-; **11**- Ψ-taraxasteryl; **12**- lupan-3-ol; **13** -olean-12-ene-3,28-diol, (3β)-.

TABLE 2. Composition of triterpene acetate fractions (% of total fraction; mass%, GC) of *I. glandulifera* and *I. noli-tangere*.

Compound	IGLt	IGRt	IGSt	INLt	INRt	INSt
5α-ergost-7-en-3β-ol acetate	0.96±0.05	tr	nd	nd	nd	nd
taraxasteryl acetate	1.90±0.17	0.20±0.01	2.64±0.16	0.93±0.06	tr	1.13±0.02
α-spinasterol acetate	33.91±0.20	29.06±0.51	21.63±0.19	14.89±0.09	18.02±0.39	2.42±0.11
13,27-cycloursan-3-ol, acetate, (3β,13β,14β)-	2.24±0.10	nd	nd	4.75±0.13	nd	nd
β-amyrin acetate	14.00±0.40	6.34±0.19	10.02±0.31	17.08±0.43	12.97±0.24	8.73±0.08
cycloeucalenyl acetate	2.45±0.18	0.91±0.12	nd	nd	nd	nd
β-simiarenol acetate	6.06±0.20	5.01±0.14	1.85±0.01	4.19±0.13	1.58±0.02	3.42±0.05
stigmast-7-en-3-ol, acetate, (3β,5α)-	4.78±0.08	3.96±0.02	tr	7.34±0.12	6.01±0.06	2.51±0.07
5α -lup-20(29)-en-3β-ol, acetate	20.17±0.56	16.04±0.30	5.73±0.10	22.91±0.38	9.75±0.29	13.08±0.69
9,19-cyclolanostan-3-ol, 24-methylene-, acetate, (3β)-	4.32±0.09	nd	2.71±0.02	1.04±0.10	nd	nd
Ψ-taraxasteryl acetate	1.12±0.01	1.79±0.02	nd	4.53±0.21	2.80±0.08	nd
lupan-3-ol acetate	tr	2.13±0.04	0.58±0.01	nd	nd	nd
olean-12-ene-3,28-diol, diacetate, (3β)-	1.85±0.03	0.70±0.01	nd	3.51±0.14	5.04±0.23	0.73±0.01
Sum of identified [%]	93.76±0.01	66.14±0.01	45.16±0.05	81.17±0.01	56.17±0.02	32.02±0.07
Total triterpene content[%]	0.61	0.37	0.59	0.42	0.48	0.29

Values are mean ± SD of three samples; nd – not detected; tr – traces; IGL_t, *I. glandulifera* leaves; IGR_t, *I. glandulifera* roots; IGS_t, *I. glandulifera* seeds; INL_t, *I. noli-tangere* leaves; INR_t, *I. noli-tangere* roots; INS_t, *I. noli-tangere* seeds.

acetate. The mass spectrum was similar to that obtained for authentic 5α-ergost-7-en-3β-ol and showed a molecular ion at m/e 426 and other important ions at m/e 411, 393, 355, 341, 302, 287, 269, 257, 218, and 204. This compound was only present in this sample. 13,27-cycloursan-3-ol, (3β,13β,14β)- was observed in the leaves of both species, and cycloeucalenyl was detected only in the leaves and roots of *I. glandulifera*. Ψ-Taraxasteryl acetate was absent in the analyzed seeds. The taraxasteryl, α-spinasterol, β-amyrin, β-simiarenol, stigmast-7-en-3-ol, (3β,5a)-, and 5α-lup-20(29)-en3β-ol acetates were identified in all the examined fractions from both *Impatiens* species.

The GC-MS method permitted an estimation of the contents of triterpenoid acetates determined in the leaves, roots and seeds of *I. glandulifera* and *I. noli-tangere*, based on the total fraction (Table 2). The quantitative analysis was done in triplicate. Amongst triterpenoid acetate fractions, the most abundant compounds in all samples were α-spinasterol (from 2.42 to 33.91%), 5α-lup-20(29)-en3β-ol (5.73 - 22.91%) and β-amyrin (6.34 – 17.08%).

3.3. Fatty acid composition

In the hexane extracts from the leaves, roots and seeds of *I. glandulifera* and *I. noli-tangere* ten fatty acids were identified (Table 3). The saturated fatty acids comprised from 12.2 (roots of *I. noli-tangere*; INRf) to 27.2% (leaves of *I. glandulifera*; IGLf), monounsaturated – 16.9 (IGLf) to 34.1% (leaves of *I. noli-tangere*; INLf), and polyunsaturated fatty acids comprised 40.3 (INRf) to 55.8% (IGLf). The unsaturated fatty acids α-linolenic and oleic acids were dominant compounds in all the examined samples. In *I. noli-tangere* γ-linolenic acid was also found at a high level, from 5.8% for the roots to 7.9% for the leaves. Trace fatty acids such as capric acid (C10:0) was detected only in the roots and seeds of *I. noli-tangere* (INSf). Small amounts of arachidonic acid (C20:4) were noticed in the leaves and seeds of *I. glandulifera* (IGLf, IGSf). The presence of azelaic acid in the studied plants is interesting, as this acid has antibacterial, anti-inflammatory, keratolytic and sebostatic and tyrosinase-inhibiting properties, which is quite rare among plants (Reszke and Szepietowski, 2016).

It was observed that the amounts of the saturated fatty acids from the leaves and seeds of both examined species were higher than in the roots (13.8% in *I. glandulifera* and 12.2% in *I. noli – tangere*). Differences in the values of the unsaturated / saturated ratios were observed from 2.7 for the leaves and seeds of *I. glandulifera* to 5.1 for the roots of *I. noli-tangere*. The polyunsaturated fatty acids were the most abundant of the unsaturated fatty acids in all organs of both species. The polyunsaturated (PUFAs) / monounsaturated (MUFAs) fatty acids ratios were from 1.2 for the leaves of *I. glandulifera* to 3.3 for the leaves of *I. noli-tangere*.

All the examined samples contained both ω-3 and ω-6 fatty acids. It has been reported that the ideal intake ratio of ω-6 to ω-3 fatty acids is

TABLE 3. Fatty acid composition (mass % of total fatty acids) in the hexane extracts of *I. glandulifera* and *I. noli-tangere*.

	Fatty acid composition (% of total fatty acids)					
Fatty acid	**IGLf**	**IGRf**	**IGSf**	**INLf**	**INRf**	**INSf**
Caprylic (C8:0)	0.3 ± 0.01	0.3 ± 0.00	0.4 ± 0.03	tr	0.3 ± 0.02	0.2 ± 0.10
Capric (C10:0)	nd	nd	nd	nd	0.1 ± 0.00	0.2 ± 0.01
Azelaic (C9:0)	2.2 ± 0.02	nd	2.0 ± 0.10	0.8 ± 0.02	nd	0.1 ± 0.10
Palmitic (C16:0)	21.1 ± 0.14	12.6 ± 0.19	21.0 ± 0.44	14.0 ± 0.05	7.2 ± 0.01	11.5 ± 0.13
Stearic (C18:0)	3.6 ± 0.02	0.9 ± 0.06	3.6 ± 0.11	9.4 ± 0.33	4.6 ± 0.18	7.6 ± 0.08
Oleic (C18:1)	16.9 ± 0.19	17.1 ± 0.11	18.3 ± 0.05	34.1 ± 0.66	21.4 ± 0.25	30.0 ± 0.50
Linoleic (C18:2) ω-6	12.9 ± 0.13	14.2 ± 0.10	12.3 ± 0.04	nd	2.5 ± 0.02	12.0 ± 0.03
α-Linolenic (C18:3) ω-3	40.5 ± 1.05	35.6 ± 0.66	40.3 ± 0.25	33.8 ± 0.14	32.0 ± 1.2	31.6 ± 0.90
γ-Linolenic (C18:3)	1.1 ± 0.10	tr	1.0 ± 0.01	7.9 ± 0.13	5.8 ± 0.08	6.6 ± 0.33
Arachidonic (C20:4)	1.3 ± 0.02	nd	1.1 ± 0.05	nd	nd	nd
Total [%]	99.9±0.01	80.7±0.03	100.0±0.01	100.0±0.00	73.9±0.02	99.8±0.01
Σ SAFAs	27.2±0.03	13.8±0.01	27.0±0.00	24.2±0.01	12.2±0.03	19.6±0.01
Σ UNSAFAs	72.7±0.01	66.9±0.05	73.0±0.02	75.8±0.01	61.7±0.06	80.2±0.02
UNSAFAs/SAFAs ratio	2.7	4.9	2.7	3.1	5.1	4.1
Σ MUFAs	16.9±0.01	17.1±0.02	18.3±0.01	34.1±0.03	21.4±0.01	30.0±0.01
Σ PUFAs	55.8±0.05	49.8±0.03	54.5±0.02	41.7±0.01	40.3±0.02	50.2±0.03
PUFAs/ MUFAs ratio	3.3	2.9	3.0	1.2	1.9	1.7
ω-6/ ω-3 ratio	1/3.1	1/2.5	1/3.3	-	1/12.8	1/2.6

Values are mean ± SD of three samples; nd – not detected; tr – traces; IGL_f, *I. glandulifera* leaves; IGR_f, *I. glandulifera* roots; IGS_f, *I. glandulifera* seeds; INL_f, *I. noli-tangere* leaves; INR_f, *I. noli-tangere* roots; INS_f, *I. noli-tangere* seeds; SAFAs, saturated fatty acids; UNSAFAs, unsaturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

between 1:1 and 4:1 (Simopoulos, 2006). According to Simopoulos (2006), increased levels of ω-3 fatty acids exert suppressive effects on many diseases such as cancer, cardiovascular disease, and inflammatory and autoimmune diseases. In our study, the ratio of ω-6 to ω-3 fatty acids is from 1/2.5 (roots of *I. glandulifera*; IGRf) to 1/12.8 (roots of *I. noli-tangere*; INRf). The obtained lower ratio of omega-6 to omega-3 fatty acids suggests that *I. glandulifera* and *I. noli–tangere* may be considered for the prevention and management of chronic diseases. The richer source of omega-3 fatty acids is *I. glandulifera*. A comparison of the present results with those of other authors has revealed that some of the identified compounds in the leaves, roots and seeds of *I. glandulifera* and *I. noli-tangere* have been reported in *I. glandulifera*. Ortin and Evans, (2013) noticed that the hydrophobic extracts from flower stalks with seed pods of *I. glandulifera* contained linolenic, palmitic, stearic, arachidic and *trans*-tetradec-2-enoic acids. According to Kaufmann and Keller, (1948), the *Impatiens* species contained oils with acetic and parinaric acid glycerides. They found that the seeds of *I. glandulifera* contain 50% and *I. noli-tangere* 55% of these types of oils. Saponification provided 13% glycerol, 10% acetic acid, 40% parinaric acid, about 3% palmitic acid, about 3% stearic acid and about 20% mixture of oleic, linoleic and linolenic acid. Nisar *et al*., (2012) analyzed a n-hexane extract of *I. bicolor*. Their study showed that the fatty acid esters, such as trans-methyl 13-octadecenoate, methyl heptadecanoate, methyl octadecanoate, methyl docosanoate, methyl tetracosanoate, and methyl eicosanoate are the major compounds of this extract.

3.4. *In vitro* cytotoxicity assay

Because plant triterpenoids and fatty acids have been reported to exhibit a variety of antioxidant, anti-inflammatory, antimicrobial, and antitumor promoting biological activities (Topçu, 2006; Dzubak *et al*., 2006), the antioxidant and cytotoxic properties of fractions obtained from the leaves, roots and seeds of *I. glandulifera* and *I. noli-tangere* were also evaluated.

In this study, the effect of the triterpenoid and fatty acid fractions in increasing concentrations on two types of cancer cell lines, HL-60 and HL-60/MX2, were investigated.

The cytotoxicity was estimated using trypan blue vital staining. The experiment was performed in triplicate and the mean values were calculated from the given values. The IC_{50} (Half Maximal Inhibitory Concentration, the inhibitor concentation when cell viability is 50%) values of the examined samples

were determined using MS Excel. The cells of both cancer lines exposed to these fractions presented diverse cytotoxicity depending on the dose of IC_{50}.

Based on the obtained results, it was found that the analyzed fractions from *I. glandulifera* and *I. noli-tangere* induce apoptosis of the cells of the both tested cell lines. The results, which are given in Table 4, showed that the triterpenoid fraction from *I. noli-tangere* seeds significantly inhibited HL-60 human leukemia cells, and was the most potent fraction with IC_{50} values of 11.69 μg/mL, followed by fatty acid fractions from the roots and leaves of *I. glandulifera* with IC_{50} of 41.54 and 61.81 μg/mL, respectively. Moreover, the fatty acids from the roots of *I. noli-tangere* and triterpenoids from the roots of *I. glandulifera* showed a moderate cytotoxicity against HL-60 with IC_{50} values of 65.37 and 65.56 μg/mL.

On the other hand, the triterpenoid fractions from the seeds of *I. glandulifera* and *I. noli-tangere* showed high inhibition activity against the HL-60/MX2 cell line with IC_{50} of 33.92 and 43.35 μg/mL, respectively. Relatively high cytotoxicity against HL-60/MX2, fatty acid fractions from the leaves of *I. noli-tangere* (IC_{50} = 50.82 μg/mL) and roots of *I. glandulifera* (IC_{50} = 52.81 μg/mL) was shown.

The weakest cytotoxic activity was found in the triterpenoid fraction from the leaves of *I. glandulifera*. The lowest IC_{50} doses against both cell lines used in the research was determined.

Based on the IC_{50} values, it can be concluded that the HL-60 cell line is more sensitive to the *Impatien* fractions studied. The highest IC_{50} value was observed after the exposure of HL-60 cells to leaf extract (IC_{50} = 963.69 μg/mL).

The presence of 5α-lup-20 (29)-en3β-ol and α-spinasterol may be responsible for the cytotoxic activity of the studied fractions. It has been reported that 5α-lup-20(29)-en3β-ol inhibits skin cancer in CD-1, and induces apoptosis in HL-60 human leukemia cells (Saleem *et al*., 2004; Zhang *et al*., 2009). Spinasterol has shown antitumor effect against ovarian, skin and breast cancer cells (Jeon *et al.,*2006).

Previous studies report the cytotoxic and antitumor activities of extracts and compounds isolated from *I. balsamina*. For example, the ethanol extract of *I. balsamina* was investigated for *in vitro* cytotoxic and *in vivo* antitumor activities against transplantable tumors and human cell lines. The obtained results showed significant antitumor and cytotoxic effects against Dalton's ascites lymphoma and human cancer cell lines (Baskar *et al*., 2012). Moreover, balsaminone C (dinaphthofuran-7,12-dione derivative) isolated from the seeds of *I. balsamina* exhibited cytotoxicity against A549, Bel-7402 and Hela cancer cell lines (Pei *et al*., 2012).

3.5. Antioxidant activity

The antioxidant activities of triterpenoid and fatty acid samples were evaluated using the DPPH radical-scavenging test and metal chelating power. As seen in Table 5, EC_{50} values for the analyzed fractions and standards on the DPPH radical were found in the range of 12.81 to 27.11 μg/mL and from 39.36 to 48.74 μg/mL for triterpenoid fractions from *I. glandulifera* and *I. noli-tangere*, respectively, from 9.43 to 18.18 μg/mL and from 11.61 to 22.04 μg/mL for fatty acid fractions from *I. glandulifera* and *I. noli-tangere*, respectively, 29.16 μg/mL for BHT and 1.50 μg/mL for α-tocopherol. Most of the samples tested showed better antioxidant activity than BHT (the lower EC_{50} values) but weaker than α-tocopherol. The fatty acid fraction from the leaves of *I. glandulifera* showed the strongest DPPH radical scavenging activity with EC_{50} at 9.43 μg/mL, followed by the fatty acid fraction from the seeds of *I. glandulifera* and the leaves of *I. noli-tangere* with EC_{50} of 10.56 and 11.61μg/mL, respectively. The strong antioxidant activity may be attributed to the presence of high amounts of omega-3 fatty acids in these fractions.

EC_{50} values for the Fe^{2+} chelating capacities of the analyzed fractions were found in the range from 9.62 to 15.13 μg/mL and from 16.00 to 19.83 μg/mL for triterpenoid fractions from *I. glandulifera* and *I. noli-tangere*, respectively. For fatty acid fractions the values of EC_{50} ranged from 5.49 to 11.86 μg/mL and from 6.01 to 13.62 μg/mL for *I. glandulifera* and *I. noli-tangere*, respectively. The positive controls BHT and α-tocopherol showed EC_{50} values of 5.30 and 13.04 μg/mL, respectively. The results obtained in this test showed that metal chelating power was similar to positive controls.

Similar findings were obtained for the n-hexane extract of *I. bicolor* in the DPPH assay. Nisar *et al*.,

TABLE 4. The IC_{50} values for HL-60 and HL-60/MX2 line cells.

	IC_{50} [μg/mL]											
	IGLt	**IGRt**	**IGSt**	**IGLf**	**IGRf**	**IGSf**	**INLt**	**INRt**	**INSt**	**INLf**	**INRf**	**INSf**
HL-60	963.69	65.56	88.07	61.81	41.54	246.54	145.69	92.21	11.69	74.46	65.37	71.20
HL-60/MX2	875.91	60.56	33.92	169.36	52.81	288.47	243.99	105.71	43.35	50.82	95.37	157.91

IGL, *I. glandulifera* leaves; IGR, *I. glandulifera* roots; IGS, *I. glandulifera* seeds; INL, *I. noli-tangere* leaves; INR, *I. noli-tangere* roots; INS, *I. noli-tangere* seeds; t, triterpenoid fractions; f, fatty acid fractions.

TABLE 5. The antioxidant activity, DPPH and metal chelating power (CHEL) of triterpenoid and fatty acid fractions of the *Impatiens* species.

	EC_{50} [µg/mL]											
	IGLf	**IGRt**	**IGSt**	**IGLf**	**IGRf**	**IGSf**	**INLt**	**INRt**	**INSt**	**INLf**	**INRf**	**INSf**	**BHT**	**VE**
DPPH	12.81±0.02	13.28±0.14	27.11±0.19	9.43±0.06	18.18±0.22	10.56±0.10	43.14±0.10	39.36±0.07	48.74±0.09	11.61±0.11	22.04±0.11	13.85±0.11	29.16±0.15	1.50±0.02
CHEL	9.62±0.01	10.93±0.06	15.13±0.06	5.49±0.01	11.86±0.07	5.67±0.03	18.82±0.03	16.00±0.11	19.83±0.06	6.01±0.04	13.62±0.05	11.13±0.06	5.30±0.04	13.04±0.06

The results are expressed as EC_{50} inµg/mL of DE (dry extract). BHT and α-tocopherol (VE) were used as the positive control. Each value is the mean ± SD (n=3). IGL, *I. glandulifera* leaves; IGR, *I. glandulifera* roots; IGS, *I. glandulifera* seeds; INL, *I. noli-tangere* leaves; INR, *I. noli-tangere* roots; INS, *I. noli-tangere* seeds; t, triterpenoid fractions; f, fatty acid fractions.

(2012) found that IC_{50} values ranging from 23.22 to 59.00 µg/mL were comparable to ascorbic acid. Moreover, the antioxidant activity was comparable with the content of the fatty acid esters in the tested samples.

In another study, it was found that ethanolic (Baskar *et al*., 2012) and water (Sha *et al*., 2013) extracts from *I. balsamina* showed antioxidant activity which was measured using different assays. On the other hand, our previously research showed that the essential oils obtained from *I. glandulifera*, *I. parviflora*, *I. balsamina*, and *I. noli-tangere* had moderate antioxidant activity (Szewczyk *et al*., 2016a). Furthermore, hydromethanolic extracts from the aerial parts of. *I. balfourii*, *I. glandulifera*, *I. parviflora*, *I. balsamina*, *I. noli-tangere*, and *I. walleriana* also possessed moderate antioxidant potential in the DPPH radical scavenging and reducing power assays (Szewczyk *et al*., 2016b).

4. CONCLUSIONS

In conclusion, evidence is given here for the occurrence of significant amounts of triterpenoids, fatty acids and sterols in the leaves, roots and seeds of *I. glandulifera* and *I. noli-tangere*. Among all the analyzed compounds, α-spinasterol, β-amyrin, 5a -lup-20(29)-en-3β-ol, β-sitosterol as well as α-linolenic, oleic and palmitic acidsare were largely predominant. We confirmed that the fatty acid and triterpenoid fractions have interesting multidirectional biological activity, such as antioxidant and cytotoxic abilities. The present research suggests that *I. glandulifera* and *I. noli-tangere* might be considered as novel sources of antioxidants and chemopreventive agents.

REFERENCES

Baskar N, Parimala DB, Jayakar B. 2012. Evaluation of antioxidant activity of ethanol extract of *Erythrina variegata* and *Impatiens balsamina* on chromium (VI) induced oxidative stress in Albino rats. *Int. J. Res. Pharm. Pharmacotherap*. **1**, 31–34.

Brand-Williams W, Cuvelier E, Berset CM. 1995. Use of free radical method to evaluate antioxidant activity. LWT-*Food Sci. Technol.* **28**, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Cimmino A, Mathieu V, Evidente M, Ferderin M, Banuls LMY, Masi M, De Carvalho A, Kiss R, Evidente A. 2016. Glanduliferins A and B, two new glucosylated steroids from *Impatiens glandulifera*, with *in vitro* growth inhibitory activity in human cancer cells. *Fitoterapia,* **109**, 138–145. https://doi.org/10.1016/j.fitote.2015.12.016

Dzubak P, Hajduch M, Vydra D, Hustova A, Kvasnica M, Biedermann D, Markova L, Urban M, Sarek J. 2006. Pharmacological activities of natural triterpenoids and their therapeutic implications. *Nat. Prod. Rep*. **23**, 394–411. https://doi.org/10.1039/b515312n

Fukumoto H, Yamaki M, Isoi I, Ishiguro K. 1996. Antianaphylactic effects of the principal compounds from the white petals of *Impatiens balsamina* L. *Phytother. Res*. **10**, 202–206. https://doi.org/10.1002/(SICI)1099-1573(199605)10:3<202::AID-PTR805>3.0.CO;2-0

Grabowska K, Podolak I, Galanty A, Załuski D, Makowska-Wąs J, Sobolewska D, Janeczko Z, Żmudzki P. 2016. *In vitro* anti-denaturation and anti-hyaluronidase activities of extracts and galactolipids from leaves of *Impatiens parviflora* DC. *Nat. Prod. Res*. **30**, 1219–1223. https://doi.org/10.1080/14786419.2015.1049175

Grabowska K, Podolak I, Galanty A, Żmudzki P, Koczurkiewicz P, Piska K, Pękala E, Janeczko Z. 2017. Two new triterpenoid saponins from the leaves of *Impatiens parviflora* DC. and their cytotoxic activity. *Ind. Crops Prod*. **96**, 71–79. https://doi.org/10.1016/j.indcrop.2016.11.022

Guo JT, Lee HL, Chiang SH, Lin HI, Chang CY. 2001. Antioxidant properties of the extracts from different parts of broccoli in Taiwan. *J. Food Drug Anal*. **9**, 96–101.

Harrabi S, Curtis S, Hayet F, Mayer PM. 2016. Changes in the sterol compositions of milk thistle oil (*Silybium marianum* L.) during seed maturation. *Grasas Aceites* **67**, https://doi.org/10.3989/gya.0495151

Jeon GC, Park MS, Yoon DY, Shin CH, Sin HS, Um SJ. 2006. Antitumor activity of spinasterol isolated from *Pueraria* roots. *Exp. Mol. Med*. **37**, 111–120. https://doi.org/10.1038/emm.2005.15

Jerzmanowska Z. 1967. Substancje roślinne. *Metody wyodrębniania*. PWN, Warszawa.

Kaufman HP, Keller M. 1948. Über das Vorkommen von Parinarsäure und Essigsäure in den Samenfetten der Balsaminaceae. *Chem. Ber*. **81**, 152–158. https://doi.org/10.1002/cber.19480810212

Lobstein A, Brenne X, Feist E, Metz N, Weniger B, Anton R. 2001. Quantitative determination of naphthoquinones of *Impatiens* species. *Phytochem. Anal*. **12**, 202–205. https://doi.org/10.1002/pca.574

Nisar M, Qayum M, Shah M, Zia-Ul-Haq M, Khan I, Ahmad K, Qayum Z. 2012. Chemical constituents and antioxidant activity of n-hexane extract of *Impatiens bicolor*. *Chem. Nat. Compd*. **48**, 143–146. https://doi.org/10.1007/s10600-012-0184-6

Ortin Y, Evans, P. 2013. *trans*-Tetradec-2-enoic acid in *Impatiens glandulifera*. *Synthetic Communications* **43**, 1404–1412. https://doi.org/10.1080/00397911.2011.635395

Panichayupakaranant P, Noguchi H, De-Eknamkul W, Sankawa U. 1995. Naphthoquinones and coumarins from *Impatiens balsamina* root cultures. *Phytochem*. **40**, 1141–1143. https://doi.org/10.1016/0031-9422(95)00418-7

Pei H, Lei J, Qian S H. 2012. A new cytotoxic dinaphthofuran-7,12dione derivatives from the seeds of *Impatiens balsamina*. *Zhong. Yao. Cai*. **35**, 407–410.

Reszke R, Szepietowski J. 2016. Azelaic acid in dermatological treatment – current state of knowledge. *Przegl. Dermatol*. **103**, 337–343. https://doi.org/10.5114/dr.2016.61785

Saleem M, Afaq F, Adhami VM, Mukhtar H. 2004. Lupeol modulates NF-κB and PI3K/Akt pathways and inhibits skin cancer in CD-1 mice. *Oncogene* **23**, 5203–5214. https://doi.org/10.1038/sj.onc.1207641

Simopoulos AP. 2006. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomed. Pharmacother*. **60**, 502–507. https://doi.org/10.1016/j.biopha.2006.07.080

Szewczyk K, Kalemba D, Komsta Ł, Nowak R. 2016a. Comparison of the essential oil composition of selected *Impatiens* species and its antioxidant activities. *Molecules* **21**, https://doi.org/10.3390/molecules21091162

Szewczyk K, Olech M. 2017. Optimization of extraction method for LC-MS based determination of phenolic acid profiles in different *Impatiens* species. *Phytochem. Lett*. **20**, 322–330. https://doi.org/10.1016/j.phytol.2017.02.005

Szewczyk K, Zidorn C, Biernasiuk A, Komsta Ł, Granica S. 2016b. Polyphenols from *Impatiens* (Balsaminaceae) and their antioxidant and antimicrobial activities. *Ind. Crop. Prod*. **86**, 262–272. https://doi.org/10.1016/j.indcrop.2016.03.053

Thanh T, Vergenes MF, Kaloustian J, El-Moselhy T, Amiot-Carlin M, Portugal H. 2006. Effect of storage and heating on phytosterol concentrations in vegetables oils determined by GC/MS. *J. Sci. Food Agric*. **86**, 220–225. https://doi.org/10.1002/jsfa.2322

Tokarska-Guzik B, Węgrzynek B, Urbisz A, Urbisz A, Nowak T, Bzdęga K. 2010. Alien vascular plants in the Silesian Upland of Poland: distribution, patterns, impacts and threats. *Biodivers. Res. Conserv*. **19**, 33–54. https://doi.org/10.2478/v10119-010-0019-x

Topçu G. Bioactive triterpenoids from *Salvia* species. 2006. *J. Nat. Prod*. **69**, 482–487. https://doi.org/10.1021/np0600402

Tříska J, Vrchotová N, Sýkora J, Moos M. 2013. Separation and identification of 1,2,4-trihydroxynaphthalene1-O-glucoside in *Impatiens glandulifera* Royle. *Molecules* **18**, 8429–8439. https://doi.org/10.3390/molecules18078429

Villaseñor IM, Domingo AP. 2000. Anticarcinogenicity potential of spinasterol isolated from squash flowers. *Teratog. Carcinog. Mutagen*. **20**, 99–105. https://doi.org/10.1002/(SICI)1520-6866(2000)20:3<99::AID-TCM1>3.0.CO;2-7

Yang X, Summerhurst DK, Koval SF, Ficker C, Smith ML, Bernards MA. 2001. Isolation of an antimicrobial compound from *Impatiens balsamina* using bioassay–guided fractionation. *Phytother. Res*. **15**, 676–680. https://doi.org/10.1002/ptr.906

Zhang L, Zhang Y, Zhang L, Yang X, Lv Z. 2009. Lupeol, a dietary triterpene, inhibited growth, and induced apoptosis through down-regulation of DR3 in SMMC7721 cells. *Cancer Invest*. **27**, 163–170. https://doi.org/10.1080/07357900802210745

Zhou XF, Zhao XY, Tang L, Ruan HL, Zhang YH, Pi HF, Xiao WL, Sun HD, Wu JZ. 2007. Three new triterpenoid saponins from the rhizomes of *Impatienspritzellii* var. *hupehensis*. *J. Asian Prod. Res*. **9**, 379–385. https://doi.org/10.1080/10286020600781019