Comparison of the Essential Oil Composition of Selected *Impatiens* Species and Its Antioxidant Activities

Katarzyna Szewczyk 1,*, Danuta Kalemba 2, Łukasz Komsta 3 and Renata Nowak 1

1 Department of Pharmaceutical Botany, Medical University of Lublin, Chodźki 1 St., 20-093 Lublin, Poland; ren101d@gmail.com
2 Institute of General Food Chemistry, Lodz University of Technology, Stefanowskiego 4/10 St., 90-924 Łódź, Poland; danuta.kalemba@p.lodz.pl
3 Department of Medicinal Chemistry, Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland; lukasz.komsta@umlub.pl

* Correspondence: k.szewczyk@umlub.pl; Tel./Fax: +48-81-448-7062

Abstract: The present paper describes the chemical composition of the essential oils obtained by hydrodistillation from four *Impatiens* species, *Impatiens glandulifera* Royle, *I. parviflora* DC., *I. balsamina* L. and *I. noli-tangere* L. The GC and GC-MS methods resulted in identification of 226 volatile compounds comprising from 61.7%–88.2% of the total amount. The essential oils differed significantly in their composition. Fifteen compounds were shared among the essential oils of all investigated *Impatiens* species. The majority of these constituents was linalool (0.7%–15.1%), hexanal (0.2%–5.3%) and benzaldehyde (0.1%–10.2%). Moreover, the antioxidant activity of the essential oils was investigated using different methods. The chemical composition of the essential oils and its antioxidant evaluation are reported for the first time from the investigated taxon.

Keywords: *Impatiens*; Balsaminaceae; herb; root; essential oils; antioxidants; PCA

1. Introduction

The genus *Impatiens* L. (Balsaminaceae) includes about 850 species, which occur mainly in tropical and subtropical climate zones, in particular in parts of the Old World, such as tropical Africa, India, southern China and the southwestern part of Asia. Some species also occur in Japan, Europe, Russia and North America [1,2]. Three species, *Impatiens glandulifera* Royle (Himalayan balsam), *I. noli-tangere* L. (touch-me-not balsam) and *I. parviflora* DC. (small balsam), occur in Central Europe, and they are perennial plants that grow in riparian zones along rivers on humid soils and in wet woodlands [3,4]. *I. glandulifera* and *I. parviflora* are among the invasive plants originally native to Asia that are rapidly spreading across Europe. In Poland, these are two of the top 20 invasive alien plants [2,5]. As *I. parviflora* is an extremely invasive plant in Europe, its relation with other plants [6] and with soil yeast complexes was recently investigated [7]. Furthermore, the different extracts from *I. glandulifera*, *I. noli-tangere* and *I. parviflora* showed a strong allelopathic effect on the seed germination of *Leucosinapis alba* and *Brassica napus* [4].

Many groups of active compounds have been isolated from different species of the genus *Impatiens* L. Phytochemical studies conducted on various organs of *Impatiens* have revealed the presence of quinones, flavonoids, phenolic acids, leucocyanidins, anthocyanins, tannins, coumarins, saponins, phytosteroids, peptides, alkaloids and essential oils [8–16]. However, only one report is available concerning the volatile constituents of *Impatiens* species. In the n-hexane extract of *I. bicolor* growing in Pakistan, fatty acid methyl esters were the major compounds [9].
Because of the rich and varied composition, numerous studies have been conducted to investigate the feasibility of a medicinal use of members of *Impatiens*. Among the representatives of the genus *Impatiens* L., some species have been used since a very long time in Asian and American medicine. In traditional therapeutic systems, *I. balsamina* L. has been the most popular species. Depending on the type of ailment, the dried herb has been used in the form of compresses, directly on the skin, or as a tea prepared by pouring hot water on dried leaves [14]. Moreover, it has been applied in Chinese traditional medicine to treat rheumatism, against fractures, swelling, contusions, beriberi disease and for its anticancer properties [16–18]. It has been also used to alleviate parturient and puerperal pain [14]. *I. balsamina* flowers have been used as a remedy against the effects of snake bites [19]. *I. parviflora* has been used in the treatment of warts [20]. Flowers of *I. glandulifera* are used in Bach flower remedies, which causes sedation, relaxes and helps to balance the emotional state, and they are recommended for psychological problems and pain [21]. The rhizomes of *Impatiens pritzellii* Hook. f. var. *hupehensis* Hook. f. [22] and whole plant of *Impatiens textori* MIQ [23] have been also used in Chinese medicine. In our previous study, we confirmed that the extracts from species of *Impatiens*, especially *I. balfourii* Hook. f., *I. glandulifera* and *I. parviflora*, contained significant amounts of phenols and flavonoids and have interesting multidirectional biological activity, such as antimicrobial and antioxidant abilities [11].

Based on the significance of these plants from an ecological perspective and no available reports on the essential oils of *Impatiens* species, the aim of the present study was to investigate the essential oil composition of herb and root of the two most invasive in Poland *Impatiens* species, *I. glandulifera* Royle and *I. parviflora* DC. For comparison, herb oils of two other species, *I. balsamina* L. and *I. noli-tangere* L., were included. The antioxidant activity of the essential oils of these species was also evaluated.

2. Results and Discussion

2.1. Chemical Composition of Essential Oils from *Impatiens* L.

Six *Impatiens* essential oils were obtained by steam distillation from air-dried herbs and roots. All of them were yellowish and fragrant. The highest yield of essential oil (w/w relative to dry material weight) was observed for the herb of *I. parviflora* (0.24%) and *I. glandulifera* (0.22%), while for the other materials, the yield amounted to 0.10%–0.16%. The chemical composition was analyzed by the GC-MS method, which resulted in identification of 54–94 volatile compounds comprising from 61.7%–88.2% of the total volume in individual oils. In total of 226 compounds was identified. All identified compounds in *Impatiens* oils are listed in Table 1.

The essential oils differed significantly in their chemical composition. What is more, all investigated oils contained specific constituents that distinguished the essential oils from each other. However, some similarities in the qualitative composition can be observed.

Seventy six compounds were identified in the herb oil of *I. glandulifera*, representing 82.5% of the total oil. The oil was dominated by oxygenated monoterpenes (28.2%), and α-terpinyl acetate (16.6%) was the major constituent, followed by phellandral (3.8%). Phthalides were the most characteristic constituents of this oil: (Z)-ligustilide (11.0%) and (Z)-butylidenphthalide (8.5%) were accompanied by small amounts of their (E)-isomers and butylphthalide. This oil was the only one that contain pronounced amounts of monoterpen hydrocarbons (9.9% in total), and β-phellandrene (7.4%) was the main one in this group.
Table 1. Composition of the essential oils of four *Impatiens* species. IGH, *I. glandulifera* herb; IGR, *I. glandulifera* roots; IPH, *I. parviflora* herb; IPR, *I. parviflora* roots; IBH, *I. balsamina* herb; INH, *I. noli-tangere* herb; RI<exp>, experimental retention index; RI<lit>, literature retention index; t, trace (percentage value less than 0.05%).

| No. | Constituent | RI<exp> | RI<lit> | IGH | IGR | IPH | IPR | IBH | INH | Class of Compound |
|-----|-------------|---------|---------|-----|-----|-----|-----|-----|-----|------------------|
| 1   | Hexanal     | 776     | 771     | 0.2 | 1.7 | 5.3 | 1.6 | 0.7 | 3.6 | AO               |
| 2   | Furfural    | 803     | 795     | -   | 0.8 | 0.7 | 1.4 | -   | -   | O                |
| 3   | (E)-Hex-2-enal | 826 | 822     | 0.3 | -   | 2.1 | 1.4 | 2.9 | -   | AO               |
| 4   | (E)-Hex-3-en-1-ol | 838 | 838     | 0.6 | -   | -   | -   | -   | -   | AO               |
| 5   | (Z)-Hex-3-en-1-ol | 848 | 851     | 0.2 | -   | -   | -   | 0.2 | 9.5 | AO               |
| 6   | Hexanol     | 852     | 856     | 1.7 | -   | -   | -   | -   | -   | AO               |
| 7   | Heptan-2-one | 868     | 871     | 0.2 | 0.2 | 0.1 | 0.1 | 0.2 | -   | AO               |
| 8   | Heptanal    | 878     | 882     | 0.1 | 0.4 | 1.2 | 0.5 | 0.1 | 0.1 | AO               |
| 9   | 2-Butoxyethanol | 888 | 888     | -   | 0.2 | 0.1 | 0.6 | -   | -   | AO               |
| 10  | Heptan-3-ol | 893     | 884     | 0.1 | -   | -   | -   | -   | -   | AO               |
| 11  | Butyraldehyde diethyl acetal | 896 | 880     | 2.4 | -   | -   | -   | -   | -   | AO               |
| 12  | Nonane      | 900     | 900     | 0   | -   | 0.1 | -   | -   | -   | AH               |
| 13  | 1-Butoxypropan-2-ol | 926 | 936     | 0.4 | -   | -   | -   | -   | -   | AO               |
| 14  | Benzaldehyde | 929     | 928     | 0.1 | 1.8 | 10.2 | 2.3 | 0.7 | 4.7 | Ar               |
| 15  | Dimethyl trisulfide | 942 | 942     | 0.4 | -   | -   | -   | -   | -   | O                |
| 16  | Benzonitrile | 945     | 940     | 0   | 0.5 | -   | -   | -   | -   | Ar               |
| 17  | Isovaleraldehyde diethyl acetal | 946 | 930     | 0.7 | -   | -   | -   | -   | -   | AO               |
| 18  | Hept-1-en-3-one | 954 | 956     | 0.1 | -   | -   | -   | -   | -   | AO               |
| 19  | Heptanol    | 956     | 952     | 0.3 | 0.9 | 0.3 | 0.3 | -   | -   | AO               |
| 20  | Octane-2,3-dione | 961 | 959     | 0.3 | 0.4 | 0.5 | -   | -   | -   | AO               |
| 21  | Oct-1-en-3-ol | 964 | 962     | 1.3 | 0.9 | 0.7 | 0.3 | -   | -   | AO               |
| 22  | 6-Methylhept-5-en-2-one | 964 | 962     | 0.3 | -   | -   | -   | -   | -   | AO               |
| 23  | Octan-3-one | 965     | 963     | 1.2 | -   | -   | -   | -   | -   | AO               |
| 24  | β-Pinene    | 969     | 970     | 0.1 | -   | -   | -   | -   | -   | MH               |
| 25  | (E,E)-Hepta-2,4-dienal | 969 | 967     | 0.6 | 0.2 | -   | -   | -   | -   | AO               |
| 26  | 2-Pentylfurane | 979 | 981     | 0.3 | 1.2 | 0.8 | 0.4 | 1.4 | -   | O                |
| 27  | Octanal     | 980     | 982     | 0.3 | -   | -   | -   | -   | -   | AO               |
| 28  | Hexanoic acid | 981     | 973     | -   | -   | -   | -   | -   | -   | AO               |
| 29  | Hepta-2,4-dienal (isomer) | 981 | -   | -   | 4.2 | 1.2 | -   | 1.2 | -   | AO               |
| 30  | (E)-2-(Pent-2-enyl)furan | 986 | 984     | 0.5 | 0.2 | t   | -   | -   | -   | O                |
| 31  | α-Phellandrene | 997     | 997     | 0.5 | -   | -   | -   | -   | -   | MH               |
| 32  | Decane      | 1000    | 1000    | -   | -   | 0.1 | -   | -   | -   | AH               |
| 33  | Phenylacetaldehyde | 1009 | 1012 | t   | 2.0 | 2.7 | 3.3 | 0.8 | 3.4 | Ar               |
| 34  | α-Terpineol | 1009    | 1014    | 0.2 | -   | -   | -   | -   | -   | MH               |
| 35  | Salicylaldehyde | 1011    | 1013    | 1.0 | 0.1 | 0.4 | -   | -   | -   | Ar               |
| 36  | p-Cymene    | 1012    | 1015    | 0.7 | -   | -   | -   | -   | -   | MH               |
| 37  | 2,2,6-Trimethylcyclohexanone | 1013 | 1013 | -   | -   | t   | t   | -   | -   | O                |
| 38  | 2-Ethylhexan-1-ol | 1015 | 1011 | 0.1 | 0.3 | 0.5 | 0.1 | 0.1 | -   | AO               |
| 39  | β-Phellandrene | 1022    | 1023    | 7.4 | -   | -   | -   | -   | -   | MH               |
| 40  | Limonene    | 1025    | 1025    | 0.3 | -   | -   | -   | -   | -   | MH               |
### Table 1. Cont.

| No.  | Constituent                                | RI<sub>exp</sub> | RI<sub>lit</sub> | IGH | IGR | IPH | IPR | IBH | INH | Content (%) |
|------|-------------------------------------------|-----------------|-----------------|-----|-----|-----|-----|-----|-----|-------------|
| 41   | (Z)-β-Ocimene                             | 1028            | 1029            | 0.6 | -   | -   | -   | -   | -   | MH          |
| 42   | (E)-Oct-2-enal                            | 1032            | 1034            | -   | 0.1 | 0.2 | 0.3 | -   | 0.2 | AO          |
| 43   | Acetoephone                               | 1033            | 1030            | -   | 0.2 | -   | 0.2 | -   | 0.1 | Ar          |
| 44   | 2,6,6-Trimethylcyclohex-2-enone           | 1037            | 1045            | -   | 0.1 | -   | -   | -   | -   | O           |
| 45   | Octa-3,5-dien-2-one (isomer)              | 1043            | -               | -   | 0.3 | 0.4 | -   | -   | -   | AO          |
| 46   | γ-Terpinene                               | 1048            | 1059            | 0.1 | -   | -   | -   | -   | 0.1 | MH          |
| 47   | (E)-Oct-2-en-1-ol                         | 1052            | 1052            | -   | 0.2 | 0.6 | 0.1 | -   | -   | AO          |
| 48   | Octanol                                   | 1055            | 1054            | 0.2 | -   | -   | 0.1 | -   | -   | AO          |
| 49   | trans-Linalool oxide (F)                  | 1058            | 1058            | -   | 0.1 | 2.3 | 0.4 | -   | 1.0 | MO          |
| 50   | Guaiacol                                  | 1061            | 1059            | 0.1 | 0.1 | 0.1 | -   | -   | -   | Ar          |
| 51   | (E,E)-Octa-3,5-dien-2-one                 | 1065            | 1063            | -   | 0.4 | 0.4 | 0.1 | -   | -   | AO          |
| 52   | Nonan-2-one                               | 1070            | 1072            | -   | -   | 0.1 | -   | -   | -   | AO          |
| 53   | cis-Linalool oxide (F)                    | 1072            | 1072            | t   | -   | 1.0 | 0.1 | -   | 0.3 | MO          |
| 54   | 6-Methylhepta-3,5-dien-2-one              | 1077            | 1075            | -   | 0.1 | 0.3 | -   | -   | -   | MO          |
| 55   | Terpinolene                               | 1079            | 1082            | 0.1 | -   | -   | -   | -   | -   | MH          |
| 56   | Nonanal                                   | 1083            | 1086            | 0.1 | 1.1 | 1.9 | 1.5 | 0.5 | 1.2 | AO          |
| 57   | Linalool                                  | 1086            | 1087            | 0.7 | 5.3 | 4.9 | 1.6 | 6.5 | 1.6 | MO          |
| 58   | Isophorone                                | 1092            | 1094            | -   | 0.1 | -   | -   | -   | -   | MO          |
| 59   | cis-Rose oxide                            | 1096            | 1096            | -   | 0.2 | 0.5 | -   | 0.1 | -   | MO          |
| 60   | α-Campholenal                             | 1104            | 1105            | t   | -   | -   | -   | -   | -   | MO          |
| 61   | Oxoisophorone                             | 1105            | 1005            | -   | t   | 0.3 | t   | 0.2 | -   | MO          |
| 62   | (E)-But-1-enylbenzene                     | 1107            | 1110            | 0.5 | -   | -   | -   | -   | -   | Ar          |
| 63   | trans-Rose oxide                          | 1113            | 1114            | -   | 0.1 | 0.3 | -   | 0.1 | -   | MO          |
| 64   | trans-Pinocarvool                         | 1122            | 1126            | -   | 0.3 | -   | -   | -   | -   | MO          |
| 65   | trans-p-Menth-2-en-1-ol                   | 1123            | 1123            | -   | 0.7 | -   | -   | -   | -   | MO          |
| 66   | Citronellal                               | 1126            | 1129            | -   | 0.4 | 0.5 | 0.5 | -   | -   | MO          |
| 67   | α-Acetylphenol                            | 1129            | 1135            | -   | -   | 0.3 | -   | -   | -   | Ar          |
| 68   | Hexyl isobutyrate                         | 1133            | 1132            | 0.1 | -   | -   | -   | -   | -   | AO          |
| 69   | 4-Vinylisobenzal                          | 1134            | 1134            | -   | -   | 0.2 | -   | 0.2 | -   | Ar          |
| 70   | (E)-Non-2-enal                            | 1134            | 1139            | -   | -   | 0.5 | -   | -   | -   | AO          |
| 71   | (E,E)-Nona-3,6-dien-1-ol                  | 1136            | 1145            | -   | -   | -   | 0.2 | -   | -   | AO          |
| 72   | Pinocarvone                               | 1137            | 1137            | 0.1 | -   | -   | -   | -   | -   | MO          |
| 73   | Pentylenzene                              | 1143            | 1150            | 0.3 | -   | -   | -   | -   | -   | Ar          |
| 74   | Borneol                                   | 1149            | 1150            | 0.1 | 4.9 | -   | -   | -   | -   | MO          |
| 75   | cis- or trans-Linalool oxide (P)          | 1153            | 1148            | -   | 0.1 | -   | -   | -   | -   | MO          |
| 76   | Benzoic acid                              | 1156            | 1157            | -   | -   | -   | 2.0 | -   | -   | Ar          |
| 77   | p-Cymen-9-ol                              | 1156            | 1157            | -   | 0.4 | -   | -   | -   | -   | MO          |
| 78   | Nonanol                                   | 1157            | 1149            | -   | -   | 0.9 | -   | -   | -   | AO          |
| 79   | Cryptone                                  | 1157            | 1160            | 5.7 | -   | -   | -   | -   | -   | O           |
| 80   | Terpinen-4-ol                             | 1160            | 1164            | 1.5 | 1.7 | 0.3 | 0.2 | 0.5 | 0.5 | MO          |
Table 1. Cont.

| No. | Constituent                          | $R_I^{exp}$ | $R_I^{lit}$ | IGH | IGR | IPH | IPR | IBH | INH | Class of Compound | Content (%) |
|-----|-------------------------------------|-------------|-------------|-----|-----|-----|-----|-----|-----|-------------------|-------------|
| 81  | Octanoic acid                       | 1164        | 1160        | -   | -   | -   | -   | 0.1 | -    | AO                |             |
| 82  | $p$-Cymen-8-ol                      | 1166        | 1169        | -   | -   | -   | 0.3 | 0.5 | -    | MO                |             |
| 83  | Methyl salicylate                   | 1168        | 1171        | -   | -   | -   | 0.1 | -   | -    | Ar                |             |
| 84  | Myrtenal                            | 1168        | 1172        | 0.3 | -   | -   | -   | -   | -    | MO                |             |
| 85  | $\alpha$-Terpineol                  | 1174        | 1176        | 1.9 | 1.5 | 1.0 | 0.3 | 0.4 | 0.7  | MO                |             |
| 86  | Safranal                            | 1179        | 1182        | -   | 0.3 | -   | 0.5 | 0.5 | -    | MO                |             |
| 87  | cis-Piperitol                       | 1181        | 1181        | 0.1 | -   | -   | -   | -   | -    | MO                |             |
| 88  | Myrtenol                            | 1181        | 1184        | 0.6 | 4.2 | 1.2 | -   | -   | -    | MO                |             |
| 89  | Decanal                             | 1184        | 1184        | 0.8 | 0.1 | 1.6 | 0.4 | -   | -    | AO                |             |
| 90  | (E,E)-Nona-2,4-dienal              | 1186        | 1188        | -   | 0.1 | -   | -   | -   | -    | MO                |             |
| 91  | Benzothiazole                       | 1186        | 1186        | 0.1 | -   | -   | -   | -   | -    | AO                |             |
| 92  | 3,5,5-Trimethyl-4-methylenecyclohex-2-enone | 1190        | 1200        | -   | 0.3 | -   | -   | -   | -    | O                 |             |
| 93  | $\text{trans}$-Piperitol           | 1191        | 1193        | 0.3 | -   | -   | -   | -   | -    | MO                |             |
| 94  | Oxetyl acetate                      | 1193        | 1191        | 0.9 | -   | -   | -   | -   | -    | AO                |             |
| 95  | Carvotanacetol                     | 1193        | 1195        | -   | -   | -   | 0.2 | -   | -    | MO                |             |
| 96  | $\beta$-Cyclocitral                | 1195        | 1195        | 0.1 | 0.6 | 0.1 | 0.5 | 0.4 | 0.4  | MO                |             |
| 97  | $\text{trans}$-Carveol             | 1199        | 1200        | 0.1 | -   | -   | -   | -   | -    | MO                |             |
| 98  | $\text{p}$-Isopropylbenzaldehyde   | 1212        | 1214        | 0.9 | -   | -   | -   | -   | -    | MO                |             |
| 99  | Citronellol                         | 1214        | 1213        | 2.5 | 17.9| 1.9 | -   | -   | -    | AO                |             |
| 100 | Carvone                             | 1215        | 1214        | 0.6 | -   | -   | -   | -   | -    | MO                |             |
| 101 | Piperitone                          | 1226        | 1226        | 0.2 | -   | -   | -   | -   | -    | MO                |             |
| 102 | (2,6,6-Trimethylcyclohex-1-en-1-yl)acetaldehyde | 1235        | 1236        | -   | -   | -   | -   | 0.4 | -    | O                 |             |
| 103 | 2-Phenylbut-2-enal                 | 1235        | 1237        | 0.2 | -   | -   | -   | -   | -    | Ar                |             |
| 104 | Geranial                            | 1240        | 1240        | 0.7 | 12.8| -   | -   | -   | -    | MO                |             |
| 105 | Phellandral                         | 1251        | 1250        | 3.8 | -   | -   | -   | -   | -    | AO                |             |
| 106 | 4-Ethylguaiacol                    | 1253        | 1257        | 0.2 | -   | -   | -   | -   | -    | Ar                |             |
| 107 | Terpinen-7-al                      | 1257        | 1257        | 0.3 | -   | -   | -   | -   | -    | MO                |             |
| 108 | Nonanoic acid                      | 1258        | 1263        | -   | -   | -   | 0.2 | -   | -    | AO                |             |
| 109 | $p$-Cymen-7-ol                     | 1266        | 1266        | 0.9 | -   | -   | -   | -   | -    | MO                |             |
| 110 | Bornyl acetate                     | 1268        | 1270        | 0.3 | 4.3 | -   | -   | -   | -    | MO                |             |
| 111 | Thymol                             | 1271        | 1267        | -   | -   | -   | 0.2 | -   | -    | MO                |             |
| 112 | Undecan-2-one                      | 1273        | 1274        | 0.3 | 0.4 | -   | -   | -   | -    | AO                |             |
| 113 | Carvacrol                          | 1280        | 1278        | 0.2 | -   | -   | -   | -   | -    | MO                |             |
| 114 | 4-Vinylguaiacol                    | 1284        | 1282        | -   | 0.3 | 1.2 | 1.7 | -   | -    | Ar                |             |
| 115 | Undecanal                          | 1286        | 1286        | 0.5 | 0.6 | -   | 2.1 | -   | -    | AO                |             |
| 116 | (E,E)-Deca-2,4-dienal              | 1289        | 1288        | -   | 0.1 | 0.2 | 0.1 | 0.2 | -    | AO                |             |
| 117 | Theaspirane A                      | 1290        | 1293        | -   | 0.1 | -   | -   | -   | -    | O13               |             |
| 118 | Theaspirane B                      | 1304        | 1304        | -   | 0.1 | -   | -   | -   | -    | O13               |             |
| 119 | Eugenol                            | 1329        | 1331        | 1.0 | -   | -   | 0.1 | 1.1 | 0.4  | Ar                |             |
| 120 | 1,1,6-Trimethyl-1,2-dihydronaphthalene | 1335        | 1336        | -   | -   | -   | 0.1 | -   | -    | Ar                |             |
| No. | Constituent                              | R1exp | R1lit | IGH | IGR | IPH | IPR | IBH | INH | Class of Compound |
|-----|-----------------------------------------|-------|-------|-----|-----|-----|-----|-----|-----|------------------|
| 121 | α-Terpinyl acetate                       | 1336  | 1335  | 16.6 | -   | -   | -   | -   | -   | MO               |
| 122 | (E)-Undec-2-enal                        | 1339  | 1341  | -    | 0.2 | 1.6 | -   | -   | -   | AO               |
| 123 | Neryl acetate                           | 1341  | 1342  | -    | -   | -   | -   | -   | 0.2 | MO               |
| 124 | Decanoic acid                           | 1352  | 1350  | -    | -   | -   | -   | 0.3 | -   | AO               |
| 125 | α-Longipinepine                         | 1354  | 1360  | 0.3  | -   | -   | -   | -   | -   | SH               |
| 126 | Geranyl acetate                         | 1359  | 1362  | 0.6  | -   | -   | -   | -   | -   | MO               |
| 127 | (E)-β-Damascenone                       | 1361  | 1361  | -    | -   | t   | 0.3 | 3.6 | t   | O13              |
| 128 | Methyl eugenol                          | 1369  | 1369  | 0.1  | -   | -   | -   | -   | -   | Ar               |
| 129 | α-Copaene                                | 1375  | 1379  | 0.3  | -   | -   | -   | -   | -   | SH               |
| 130 | cis-β-Elemene                           | 1377  | 1381  | -    | 0.1 | -   | -   | -   | -   | SH               |
| 131 | Dodecan-2-one                           | 1385  | 1377  | -    | -   | -   | -   | 0.5 | -   | AO               |
| 132 | Dodecanal                               | 1385  | 1386  | -    | 0.7 | 0.2 | -   | -   | -   | AO               |
| 133 | β-Elemene                               | 1386  | 1389  | 0.3  | 0.9 | -   | -   | -   | 1.0 | O13              |
| 134 | (E)-β-Damascone                         | 1392  | 1398  | -    | -   | -   | -   | 0.7 | -   | O13              |
| 135 | 7,8-Dihydro-β-damascenone               | 1396  | 1424  | -    | -   | -   | -   | -   | -   | SH               |
| 136 | Tetradecane                             | 1400  | 1400  | 0.1  | 0.1 | 0.6 | 0.6 | 2.7 | 0.7 | O                |
| 137 | (E)-α-Ionone                            | 1404  | 1413  | t    | 0.1 | 0.1 | 0.1 | -   | -   | O13              |
| 138 | α-Barbatene                             | 1411  | 1414  | -    | 0.9 | -   | -   | -   | -   | SH               |
| 139 | cis-α-Bergamotene                       | 1412  | 1411  | 0.1  | -   | -   | -   | -   | -   | SH               |
| 140 | α-Santalene                             | 1416  | 1422  | 2.0  | -   | -   | -   | -   | -   | SH               |
| 141 | Geranylacetone                          | 1427  | 1428  | 0.1  | 0.6 | 0.1 | 0.6 | 2.7 | 0.7 | O                |
| 142 | γ-Elemene                               | 1427  | 1429  | 0.5  | -   | -   | -   | -   | -   | SH               |
| 143 | trans-α-Bergamotene                     | 1431  | 1434  | -    | 0.1 | -   | -   | -   | -   | SH               |
| 144 | Isobazzanene                            | 1439  | 1442  | -    | 0.5 | -   | -   | -   | -   | SH               |
| 145 | β-Barbatene                             | 1444  | 1445  | -    | 5.3 | -   | -   | -   | -   | SH               |
| 146 | Sesquisabinine B                        | 1445  | 1445  | 0.6  | -   | -   | -   | -   | -   | SH               |
| 147 | 4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one | 1456  | -    | -    | -   | -   | -   | 0.8 | -   | O13              |
| 150 | (E)-β-Ionone                             | 1462  | 1468  | t    | 0.6 | 0.8 | 1.1 | 5.7 | t   | O13              |
| 151 | 4,5-di-epi-Aristolochene                | 1465  | 1470  | -    | 0.1 | -   | -   | -   | -   | SH               |
| 152 | γ-Maurolene                             | 1470  | 1474  | 0.5  | -   | -   | -   | -   | -   | SH               |
| 153 | ar-Curcumene                            | 1471  | 1473  | -    | 0.2 | -   | -   | -   | -   | SH               |
| 154 | γ-Curcumene                             | 1472  | 1475  | -    | 0.1 | -   | -   | -   | -   | SH               |
| 155 | 5-epi-Aristolochene                     | 1473  | 1477  | 0.1  | -   | -   | -   | -   | -   | SH               |
| 156 | Tridecan-2-one                          | 1474  | 1476  | -    | 0.6 | 0.2 | -   | -   | -   | AO               |
| 157 | trans-β-Bergamotene                     | 1477  | 1480  | 0.1  | -   | -   | -   | -   | -   | SH               |
| 158 | 3,4-Dimethyl-5-pentyl-5H-furan-2-one   | 1480  | 1481  | -    | 1.1 | 0.1 | 2.4 | 1.5 | O               |
| 159 | Aristolochene                           | 1481  | 1486  | 0.7  | -   | -   | -   | 0.8 | -   | SH               |
| 160 | Dihydroactinidiolide                    | 1487  | 1487  | -    | -   | -   | -   | 0.8 | -   | O                |
Table 1. Cont.

| No. | Constituent                  | R\text{exp} | R\text{lit} | IGH | IGR | IPH | IPR | IBH | INH | Class of Compound |
|-----|------------------------------|-------------|-------------|-----|-----|-----|-----|-----|-----|------------------|
| 161 | Tridecanal                  | 1488        | 1490        | -   | 0.3 | 1.0 | -   | -   | -   | AO               |
| 162 | α-Selinene                  | 1490        | 1494        | 0.7 | -   | -   | -   | -   | -   | SH               |
| 163 | Cuparene                    | 1493        | 1498        | 1.3 | -   | -   | -   | -   | -   | SH               |
| 164 | Pentadecane                 | 1500        | 1500        | -   | -   | -   | -   | 0.3 | -   | AH               |
| 165 | Germacrene A                | 1500        | 1503        | -   | 0.7 | -   | -   | -   | -   | SH               |
| 166 | β-Bisabolene                | 1502        | 1503        | -   | 0.2 | -   | -   | -   | -   | SH               |
| 167 | Methyl dodecanoate          | 1504        | 1507        | -   | -   | -   | -   | 0.7 | -   | AO               |
| 168 | γ-Cadinene                  | 1505        | 1507        | 0.2 | -   | 0.3 | 0.3 | -   | -   | SH               |
| 169 | trans-Calamenene            | 1508        | 1517        | 0.1 | -   | -   | -   | -   | -   | SH               |
| 170 | Photosantol                 | 1509        | 1511        | -   | 0.2 | -   | -   | -   | -   | SO               |
| 171 | δ-Cadinene                  | 1518        | 1520        | 0.7 | 0.1 | -   | t   | -   | -   | SH               |
| 172 | Selina-4(15),7(11)-diene    | 1528        | 1534        | 0.4 | -   | -   | -   | -   | -   | SH               |
| 173 | Dodecanoic acid             | 1546        | 1554        | -   | -   | -   | 1.3 | 4.1 | -   | AO               |
| 174 | (E,E)-Pseudoisomeone        | 1555        | 1563        | -   | -   | -   | -   | 0.3 | -   | O13              |
| 175 | (2E)-2-Methyl-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-2-enal | 1568 | 1584 | -   | -   | -   | 0.4 | -   | -   | O13              |
| 176 | Maaliol                     | 1573        | 1565        | -   | 0.5 | -   | -   | -   | -   | SO               |
| 177 | Globulol                    | 1574        | 1578        | -   | -   | -   | 0.6 | 1.2 | 1.7 | SO               |
| 178 | Tetradecanol                | 1592        | 1592        | 1.0 | -   | 0.9 | -   | -   | -   | AO               |
| 179 | Hexadecane                  | 1600        | 1600        | -   | -   | -   | 0.1 | -   | -   | AH               |
| 180 | Butylphthalide              | 1610        | 1616        | 0.4 | -   | -   | -   | -   | -   | Ar               |
| 181 | α-Bisabolol                 | 1613        | 1619        | 0.1 | -   | -   | -   | -   | -   | SO               |
| 182 | T-Cadinol                   | 1624        | 1623        | -   | -   | -   | 0.1 | -   | -   | SO               |
| 183 | T-Muurolol                  | 1626        | 1633        | 0.2 | -   | -   | -   | -   | -   | SO               |
| 184 | Vulgarone B                 | 1630        | 1632        | -   | -   | 14.9| -   | -   | -   | SO               |
| 185 | α-Cadinol                   | 1639        | 1642        | -   | 0.2 | -   | -   | -   | -   | SO               |
| 186 | Pogostol                    | 1639        | 1647        | t   | -   | 0.3 | -   | -   | -   | SO               |
| 187 | (Z)-Butylidenphthalide      | 1641        | 1644        | 8.5 | -   | -   | -   | -   | -   | Ar               |
| 188 | α-Barbatenal                | 1652        | 1659        | 0.5 | -   | -   | -   | -   | -   | SO               |
| 189 | Hexahydrofarnesol           | 1659        | 1667        | -   | -   | -   | 0.8 | -   | -   | O                |
| 190 | Tetradecanil                | 1664        | 1670        | -   | -   | 0.5 | -   | -   | -   | AO               |
| 191 | α-Bisabolol                 | 1670        | 1673        | 0.3 | 0.3 | 1.0 | -   | -   | -   | SO               |
| 192 | Acorenone                   | 1671        | 1681        | 4.0 | -   | -   | -   | -   | -   | SO               |
| 193 | Pentadecan-2-one            | 1676        | 1677        | -   | 0.2 | 0.2 | -   | -   | -   | AO               |
| 194 | (E)-Butylidenphthalide      | 1681        | 1673        | 0.8 | -   | -   | -   | -   | -   | Ar               |
| 195 | Pentadecanil                | 1692        | 1693        | 5.8 | 0.6 | 1.6 | 1.7 | -   | -   | AO               |
| 196 | Heptadecane                 | 1700        | 1700        | -   | 0.1 | 0.4 | -   | -   | -   | AH               |
| 197 | (Z)-Ligustilide             | 1703        | 1732        | 11.0| -   | -   | -   | -   | -   | Ar               |
| 198 | Methyl myristate            | 1710        | 1713        | -   | 0.1 | 0.5 | -   | -   | -   | AO               |
| 199 | Phenanthrene                | 1746        | 1744        | 0.9 | -   | -   | -   | -   | -   | Ar               |
| 200 | Myristic acid               | 1747        | 1748        | 0.3 | -   | 0.9 | 3.3 | -   | -   | AO               |
| No. | Constituent                   | RI<sub>exp</sub> | RI<sub>lit</sub> | IGH | IGR | IPH | IPR | IBH | INH | Class of Compound | Content (%) |
|-----|-------------------------------|-----------------|-----------------|-----|-----|-----|-----|-----|-----|------------------|-------------|
| 201 | (E)-Ligustilide               | 1756            | 1782            | 0.1 | -   | -   | -   | -   | -   | Ar               |             |
| 202 | Hexadecanal                   | 1793            | 1782            | -   | 0.1 | -   | 0.3 | 0.2 | -   | AO               |             |
| 203 | Octadecane                    | 1800            | 1800            | -   | -   | -   | 0.3 | 0.5 | 0.3 | AH               |             |
| 204 | Hexahydrofarnesyl acetone     | 1829            | 1832            | 1.0 | -   | 0.4 | -   | 13.4| 0.3 | O                |             |
| 205 | Farnesylacetone               | 1894            | 1895            | -   | -   | -   | -   | 1.9 | -   | O                |             |
| 206 | Nonadecane                    | 1900            | 1900            | -   | 0.2 | -   | 0.5 | 0.7 | -   | AH               |             |
| 207 | Methyl palmitate              | 1904            | 1904            | 0.2 | 0.3 | 0.1 | 0.7 | 1.8 | 3.0 | AO               |             |
| 208 | Isophytol                     | 1939            | 1949            | -   | -   | -   | 0.4 | 0.9 | 0.3 | O                |             |
| 209 | Palmitic acid                 | 1945            | 1951            | 1.1 | -   | -   | 0.6 | 0.9 | -   | AO               |             |
| 210 | Ethyl palmitate               | 1974            | 1954            | -   | -   | -   | -   | 0.3 | -   | AO               |             |
| 211 | Eicosane                      | 2000            | 2000            | -   | -   | 0.2 | -   | 0.3 | -   | AH               |             |
| 212 | Fluoranthene                  | 2026            | 2020            | -   | 0.9 | -   | -   | -   | -   | Ar               |             |
| 213 | Methyl linoleate              | 2067            | 2046            | -   | 0.1 | -   | 0.3 | 0.4 | 0.2 | AO               |             |
| 214 | Methyl palmitate              | 2071            | 2102            | 0.1 | -   | -   | -   | -   | -   | AO               |             |
| 215 | Methyl linolenate             | 2072            | 2102            | 0.2 | -   | 0.7 | 1.3 | 0.3 | -   | AO               |             |
| 216 | Pyrene                        | 2076            | 2070            | 0.6 | -   | -   | -   | 0.2 | -   | AO               |             |
| 217 | Methyl oleate                 | 2087            | 2082            | -   | 0.9 | -   | -   | -   | -   | AO               |             |
| 218 | Henicosane                    | 2100            | 2100            | -   | 0.3 | -   | 0.3 | 0.3 | 0.2 | AH               |             |
| 219 | Phytol                        | 2107            | 2104            | 0.5 | 0.3 | 0.1 | 0.2 | 2.8 | 0.1 | O                |             |
| 220 | Docosane                      | 2200            | 2200            | -   | -   | -   | -   | 0.2 | 0.2 | AH               |             |
| 221 | Tricosane                     | 2300            | 2300            | 0.3 | 0.4 | 0.1 | 0.6 | 1.8 | 1.4 | AH               |             |
| 222 | Tetracosane                   | 2400            | 2400            | 0.1 | -   | -   | -   | 0.2 | 0.2 | AH               |             |
| 223 | Pentacosane                   | 2500            | 2500            | 0.5 | 0.3 | 0.1 | 0.4 | 1.7 | 1.7 | AH               |             |
| 224 | Hexacosane                    | 2600            | 2600            | -   | 0.1 | -   | -   | -   | -   | AH               |             |
| 225 | Heptacosane                   | 2700            | 2700            | 0.2 | -   | -   | -   | -   | -   | AH               |             |
| 226 | Nonacosane                    | 2900            | 2900            | 0.5 | -   | -   | -   | -   | -   | AH               |             |

| Total identified constituents | 82.5 | 87.4 | 88.2 | 86.3 | 84.2 | 61.7 |
|------------------------------|------|------|------|------|------|------|
| Aliphatic hydrocarbons AH    | 1.5  | 1.5  | 0.3  | 2.7  | 6.0  | 5.0  |
| Monoterpane hydrocarbons MH  | 9.9  | 0.1  | 0.1  | 0.4  | -    | 0.1  |
| Oxygenated monoterpenes MO   | 28.2 | 20.3 | 24.7 | 42.8 | 5.4  | 12.1 |
| Sesquiterpene hydrocarbons SH| 5.1  | 12.6 | 1.1  | 0.3  | -    | -    |
| Oxygenated sesquiterpenes SO | 0.2  | 20.2 | -    | 1.2  | 1.5  | 3.0  |
| Other O + O13                | 7.3  | 0.1  | 3.4  | 1.2  | 7.0  | 14.4 |
| Identified compounds         | 76   | 94   | 70   | 88   | 80   | 54   |
| Oil yield                    | 0.22 | 0.19 | 0.24 | 0.16 | 0.10 | 0.14 |
Root oil of I. glandulifera (94 compounds, 87.4%) had a totally different composition than herb oil. Three major groups of the constituent were aliphatic, mono- and sesquiterpene oxygenated compounds, each amounting to ca. 20%. The main component was sesquiterpene ketone vulgarone B (14.9%). Linalool (5.3%), borneol (4.9%) and bornyl acetate (4.3%) were the major monoterpenes. Another important constituent was pentadecan-1-ol (5.8%). The main feature of this oil was the presence of sixteen sesquiterpene hydrocarbons (12.7%), with β-barbatene (5.3%) being the major one. Eighteen constituents were identified in both herb and root oils of this species, e.g., hexanal, heptanal, nonanal, benzaldehyde, linalool, borneol and bornyl acetate, terpinene-4-ol, α-terpineol, β-ionone and its epoxide. It is worth mentioning that among the twelve sesquiterpene hydrocarbons (5.1%) that were identified in the herb oil of this species, only one, δ-cadinene, was common for both oils.

In the essential oil of I. parviflora herb, seventy compounds were identified amounting to 88.2%, and among them, (E)-hex-3-en-1-ol (16.8%), linalool (15.1%) and benzaldehyde (10.2%) were the most prominent. The number of identified components in the oil of I. parviflora roots was eighty nine (86.3% of the total oil), and the major compounds were citronellol (17.9%), geranial (12.8%) and linalool (4.9%). Despite significant differences in the content of major constituents, both herb and root oils contained the same aliphatic saturated and unsaturated alcohols, aldehydes and ketones C6–C16, which were their common distinctive features and constituted 42.8% and 26.1%, respectively. Isomeric heptadienals and octadienones that were identified in these oils were only rarely identified in the remaining investigated oils.

The yield of essential oil obtained from I. balsamina herb was 0.1%. Eighty components were identified, representing 84.2% of this oil. The major constituent was hexahydrofarnesyl acetone (13.4%). Pronounced contents of ionones and damascones (15.8%), as well as fatty acids C6-C16 (9.5%) and alkanes (5.9%) were characteristic features of this oil. The main member of the first group was (E)-β-ionone (5.7%), and of the second group, dodecanoic acid (4.1%), ionones and damascones, which occur in a variety of essential oils, are degradation products of carotenoids and have the same C13 carbon skeleton, but differ in the site of oxygenation. (E)-β-Ionone and its epoxide were found in other investigated oils, however in smaller amounts. Among 54 identified constituents, which accounted for 61.7% of the total essential oil from I. noli-tangere herb, the main compounds were (Z)-hex-3-en-1-ol (9.5%), linalool (6.5%) and benzaldehyde (4.7%).

Fifteen compounds were shared among the essential oils of all investigated Impatiens species. The majority of these constituents was linalool (0.7%–15.1%), hexanal (0.2%–5.3%) and benzaldehyde (0.1%–10.2%). Linalool is a naturally occurring monoterpenic constituent found in more than 200 oils obtained from herbs, leaves, flowers and wood. This compound has many proven activities and is present in several remedies used in traditional medicine for sedative purposes. Moreover, linalool revealed antimicrobial, anti-inflammatory, antihyperalgesic and analgesic properties [24]. Chang and Shen investigated the cytotoxic activity of linalool. This study suggested good inhibitory effects against breast, colorectal and liver cancer cells [25].

According to the only available report on the composition of Impatiens volatiles, 42 components were characterized in the n-hexane extract of I. bicolor growing in Pakistan. The major ones were fatty acid methyl esters, such as trans-methyl 13-octadecenoate, methyl heptadecanoate, methyl octadecanoate, methyl docosanoate, methyl tetracosanoate, and methyl eicosanoate and aliphatic hydrocarbons [9].

Compounds of these two groups were identified in the essential oils of all investigated Impatiens species. However, their content was significantly lower.

The invasive ability of some vigorous nonnative plants was thought to be associated with the competitive ability of the invasive species or a release from natural enemies. The allelopathic activity of invasive species also has recently been reported as a significant factor that negatively influences species biodiversity and ecosystem succession [26]. Among the allelochemicals, essential oils and their individual components belong to the most investigated [27]. Oxygenated monoterpenes were proven to possess high phytotoxic activity that inhibits the seed germination and seedling growth of
many plants [28]. Terpinene-4-ol, which is a minor constituent of each investigated oil, appeared to be the most active of the 47 monoterpenes against *Lactuca sativa*, and the linalool, citronellol and geranial, major constituents of *I. glandulifera* and/or *I. parviflora*, revealed a pronounced phytotoxic effect [29].

### 2.2. Chemometric Analysis

The main constituents common for all tested essential oils (hexanal, heptanal, benzaldehyde, phenylacetaldehyde, nonanal, linalool, terpin-4-ol, α-terpineol, geranylacetone, β-ionone epoxide, (E)-β-ionone, methyl palmitate, phytol, tricosane, pentacosane) were compared with hierarchical cluster analysis with Euclidean distance as the similarity measure. The so-called “heatmap” with corresponding dendrograms is presented in Figure 1. Benzaldehyde and linalool form distinct cluster with different values than the rest of the analyzed main constituents. However, Euclidean distance does not uncover any distinct cluster among plant material samples, besides a distinct difference of *I. parviflora* herb (IPH) compared to the other samples.

![Figure 1. The heatmap analysis of the essential oil constituents.](image)

*Figure 1.* The heatmap analysis of the essential oil constituents. IBH, *I. balsamina* herb; IGH, *I. glandulifera* herb; IGR, *I. glandulifera* roots; INH, *I. noli-tangere* herb; IPH, *I. parviflora* herb; IPR, *I. parviflora* roots.
To the compare correlation between constituents (regardless of the absolute concentrations), scaled principal component analysis was carried out. Forty-eight-point-four percent and 32.4% of variance was explained by the first two PCs, respectively (Figure 2). Analyzing the loading vectors (Figure 3), it can be concluded that the compounds form three intercorrelated groups:

1. Terpinen-4-ol and α-terpineol, explained mainly with PC2 and weakly (reversely) correlated with other compounds;
2. Hexanal, nonanal, linalool, heptanal and benzaldehyde, located mainly in PC1, intercorrelated and reversely correlated with Group 3;
3. Geranyl-acetone, β-ionone-epoxide, methyl-palmitate, phytol, tricosane and pentacosane, explained by PC1 and negatively correlated with Group 2.

_I. glandulifera_ roots (IGR) and _I. glandulifera_ herb (IGH) contain the high concentration of group (1), whereas other material samples have smaller concentrations of them, and the differences are located mainly along the PC1 axis.

**Figure 2.** Scores of scaled principal component analysis: a comparison of the correlations between constituents among investigated materials. IBH, _I. balsamina_ herb; IGH, _I. glandulifera_ herb; IGR, _I. glandulifera_ roots; INH, _I. noli-tangere_ herb; IPH, _I. parviflora_ herb; IPR, _I. parviflora_ roots.
2.3. Antioxidant Activity

In the present study, the antioxidant activities of the essential oils from herb and roots of Impatiens species were determined using two different methods. The free radical scavenging activity of essential oils was evaluated by the DPPH method in comparison with that of ascorbic acid, at different concentrations. Radical scavenging activity was expressed as the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50% (EC$_{50}$). The highest antiradical activity was detected for herb oils of *I. glandulifera* (3.96 ± 0.03 µg/mL) and *I. noli-tangere* (4.76 ± 0.05 µg/mL), whereas the lowest was detected for herb oil of *I. balsamina* (Table 2). The EC$_{50}$ value of ascorbic acids to scavenge hydroxyl radicals was 2.05 ± 0.01 µg/mL. Our results are comparable to those obtained by Nisar and co-authors for the hexane extract of *I. bicolor*. The EC$_{50}$ values obtained for different fractions ranged from 23.22 ± 0.75 µg/mL–59.00 ± 2.01 µg/mL, while the value for ascorbic acid was 7.80 ± 0.14 µg/mL [9].

The inhibition of linoleic acid peroxidation revealed low capacities of the essential oils of *Impatiens* species in comparison to the DPPH test (Table 2). The most active essential oils from herb and roots of *I. glandulifera* showed even up to six-times higher IC$_{50}$ values than the lipophilic antioxidant BHT.
Table 2. Comparison of the antioxidant activity of Impatiens L. essential oils and standard antioxidants. Different superscripts in each column indicate significant differences in the means at \( p < 0.05 \).

| Essential Oils          | Radical Scavenging Activity DPPH (EC\(_{50}\), µg/mL) | Inhibition of Linoleic Acid Peroxidation (IC\(_{50}\), µg/mL) |
|-------------------------|------------------------------------------------------|------------------------------------------------------------|
| I. balsamina herb (IBH) | 16.14 ± 0.68 \(^a\)                                   | 468.06 ± 2.03 \(^f\)                                      |
| I. glandulifera herb (IGH) | 3.96 ± 0.03 \(^b\)                                   | 102.08 ± 0.71 \(^b\)                                      |
| I. glandulifera roots (IGR) | 5.84 ± 0.03 \(^d\)                                   | 116.98 ± 0.43 \(^c\)                                      |
| I. noli-tangere herb (INH) | 4.76 ± 0.05 \(^b,c\)                                | 123.18 ± 1.34 \(^c\)                                      |
| I. parviflora herb (IPH)  | 9.06 ± 0.07 \(^d\)                                   | 190.94 ± 0.76 \(^d\)                                      |
| I. parviflora roots (IPR) | 10.38 ± 0.17 \(^e\)                                  | 323.66 ± 0.24 \(^e\)                                      |
| Ascorbic acid            | 2.05 ± 0.01 \(^a\)                                   | -                                                         |
| BHT                      | -                                                    | 18.21 ± 0.11 \(^a\)                                       |

Means values followed by different superscripts (a–g) in a column are significantly different (\( p < 0.05 \)).

3. Experimental Section

3.1. Reagents and Materials

Aerial parts and roots of plants were collected during July–September 2014. I. balsamina L. (No. IB-0714) was collected in the Maria Curie-Sklodowska University (UMCS) Botanical Garden, which is a part of Maria Curie Sklodowska University in Lublin, Poland, at an altitude of 197 m a.m.s.l. (coordinates N 51°08'41"; E 22°18'17"). I. glandulifera Royle (No. IG-0814) and I. noli-tangere L. (No. INT-0914) were gathered 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 N 52°57'58"; E 23°04'46" respectively). I. parviflora DC. (No. IP-0914) was collected in Motycz Leśny near Lublin (Poland) at an altitude of 180 m a.m.s.l. (coordinates N 51°14'57"; E 22°20'36"). Voucher specimens were deposited in the Department of Pharmaceutical Botany, Faculty of Pharmacy, Medical University of Lublin. Plants were identified by Prof. Tadeusz Krzaczek.

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), ascorbic acid, 2,6-di-\( \text{tert}\)-butyl-4-methylphenol (BHT) and linoleic acid (LA) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

3.2. Isolation Procedure

The essential oils (EOs) of 100 g of dried herb or roots of Impatiens species were obtained by hydrodistillation for 3 h using the Clevenger-type apparatus, according to European Pharmacopoeia 5.0. Next, the EOs were trapped in 2 mL of freshly-rectified diethyl ether. After distillation, the organic layers were collected and dried over anhydrous magnesium sulfate. After filtration and additional washing of diethyl ether, the solvent was evaporated at room temperature, and the residues were weighed. The oil yields were calculated on the basis of the dry weight of plant material and according to the formula [30]:

\[
\text{EO (\%)} = \frac{W_1}{W_2} \times 100
\]

where \( W_1 \) is the net weight of oils (g) and \( W_2 \) is the total weight of dry samples (g).

3.3. GC-MS Analysis

The chemical composition of the essential oils was analyzed using GC-MS on a Trace GC Ultra apparatus (Thermo Electron Corporation, Milan, Italy) with FID and the MS DSQ II detector after dilution in diethyl ether (10 µL in 1 mL). A simultaneous GC-FID and MS analysis was performed using an MS-FID splitter (SGE, Analytical Science, Austin, TX, USA). Operating conditions: apolar capillary column Rtx-1ms (Restek), 60 m × 0.25 mm i.d., film thickness 0.25 µm; temperature program, 50–310 °C.
at 2 °C/min; SSL (splitless) injector temperature 280 °C; FID temperature 300 °C; split ratio 1:20; carrier gas helium at a regular pressure 300 kPa. Mass spectra were acquired over the mass range of 30–400 Da, ionization voltage 70 eV; ion source temperature 200 °C. Identification of the components was based on a comparison of their mass spectra and relative retention indices with data stored in computer libraries NIST 98.1, Wiley 8th Ed. and MassFinder 4.1. Retention indices (RI, apolar column) were determined with relation to a homologous series of alkanes (C8–C26) under the same conditions with linear interpolation. Percentages were calculated from the FID response without the use of correction factors.

3.4. Free Radical Scavenging Activity

To determine the antioxidant activity of essential oils from Impatiens species, the method based on the reduction of the methanolic solution of colored free radical DPPH• was used. The changes in color from deep-violet to light-yellow were measured at 515 nm in a UV/visible light spectrophotometer (Thermo Evolution 300, Madison, WI, USA). Radical scavenging activity was measured according to the Brand-Williams et al. [31] method with the use of six dilutions of the analytes in methanol. The activity of ascorbic acid was evaluated for comparison. Antioxidant activity was expressed as EC50 (efficient concentration): the amount of dry extract (µg of DW) needed to obtain 50% activity per 1.0 mL of the initial solution.

3.5. Inhibition of Linoleic Acid Peroxidation

The antioxidant activity was also determined as the degree of inhibition on the hemoglobin-catalyzed peroxidation of linoleic acid according to the method described in previous studies [32,33] with a slight modification. The hydroxyperoxide formed was assayed according to the ferric thiocyanate method with mixing with 0.02 M FeCl2 followed by 30% ammonium thiocyanate. The absorbance of the sample (A5) was measured at 480 nm. The absorbance of blank (A0) was obtained without hemoglobin to the reaction mixture; the absorbance of the control (A100) was determined without the sample added to the mixture. Thus, the antioxidative activity of the sample was calculated according to the formula:

\[
AA\% = \left(1 - \frac{(A_5 - A_0)}{(A_{100} - A_0)}\right) \times 100
\] (2)

Antioxidant activity was expressed as IC50 (inhibition concentration): the amount of antioxidant needed to decrease the linoleic acid peroxidation by 50%.

3.6. Data Analysis

All measurements were performed at least in triplicate and expressed as the means ± standard deviations (±SD). Statistical significance was estimated through Tukey’s test for the data obtained from three independent samples of each essential oil in three parallel experiments (n = 9). Besides the classical pairwise correlation check, we applied the scaled principal component analysis. Statistical tests were performed using Statistica 6.0 software (Stat-Soft, Inc., Tulsa, OK, USA).

4. Conclusions

It is well understood that invasive species produce specific compounds affecting native plants occurring in the same habitat. This phenomenon is known as allelopathy. Identification of chemical constituents produced and emitted by invasive species helps to understand their impact on the local environment.

Taking into account the chemical composition of I. glandulifera and I. parviflora essential oils and previous data on the allelopathic activity of monoterpenoids, it seemed possible that the emission of monoterpenes by herb and root of these two Impatiens species plays a role in their invasive ability. However, this hypothesis needs further research.
Author Contributions: K.S. conceived and designed the experiments, and wrote the paper; K.S. and D.K. performed the experiments and analyzed the data; Ł.K. performed the statistical analysis; R.N. contributed reagents/materials/analysis tools.

Conflicts of Interest: The authors declare no conflict of interest.

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