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CHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITY OF *n*-HEXANE EXTRACT OF *Impatiens bicolor*

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Plants belonging to the genus *Impatiens* (Balsaminaceae) are rich in organic acids, anthraquinones, and flavonoids [1–3]. It is well known that these classes of aromatic compounds possess widespread pharmacological activities. Previous investigations showed that various plant extracts of *Impatiens bicolor* Royle display antimicrobial and antifungal activity [4, 5], as well as acetylcholinesterase inhibition and antioxidant activities [6].

Thus, there is a considerable research interest in the assay of composition and/or antioxidant properties of various extracts of *I. bicolor*. This study presents the very first investigation on the chemical composition of plant extracts of *I. bicolor*, as well as their antioxidant activities, and it can provide a broad base for the possibility of further detailed pharmacological studies on this species.

The sub-extracted samples of the *n*-hexane fraction of *I. bicolor* were subjected to detailed GC and GC/MS analysis in order to determine the impact of chemical composition on their antioxidant activity. About 42 compounds were identified in eight samples, representing 56.64–93.85% in total. The percentage compositions of the samples were computed in each case from GC peak areas and are shown in Table 1. In general, all investigated samples can be characterized as mixtures of aliphatic compounds (20.11–93.85%), with oxygenated aliphatic compounds as the most abundant (5.97–89.38%).

The fatty acid esters (FAEs) were found to be major components of the investigated subfractions of the *n*-hexane extract, comprising *trans*-methyl 13-octadecenoate (2.44–47.29%), methyl heptadecanoate (8.76–18.82%), methyl octadecanoate (4.96–16.49%), methyl docosanoate (3.14–12.64%), methyl tetracosanoate (1.04–11.23%), and methyl eicosanoate (3.83–10.93%). These compounds were mainly found in samples eluted with solvents with relatively low polarity (MQ-1 to MQ-5), while samples eluted with higher polarity (MQ-6 to MQ-8) contained significant amounts of aromatic compounds and shorter-chain oxygenated aliphatic compounds.

The antioxidant activity of sub-extracted samples of the *n*-hexane fraction of *I. bicolor* were evaluated by DPPH radical-scavenging test. Assessed samples were able to reduce the stable violet DPPH radical to the yellow DPPH-H, reaching 50% of reduction with IC_{50} values ranging from 23.22 ± 0.75 μg/mL for sample MQ1 to 59.00 ± 2.01 μg/mL for sample MQ8 (Table 2). Those values are comparable to ascorbic acid ($IC_{50} = 7.80 \pm 0.14$ μg/mL), whose antioxidant properties are already known [7, 8].

Despite the fact that major natural antioxidants belong to the class of phenolic compounds, such as thymol and carvacrol [9, 10], the investigated samples, rich in FAEs, showed radical scavenging activity. The IC_{50} values presented in the Table 2 are comparable with the content of FAEs in the investigated sample. However, the percentage of these esters decreases from sample MQ1 to sample MQ8, and due to the polarity of eluent, the antioxidant activity decreases. Sample MQ1 was the exception, with a content of FAEs of 58.33%, which is much lower than in the next sample, MQ2, but sample MQ1 showed uniqueness in the content of 2-(1-naphthyl)acetophenone, a member of a class of already known natural antioxidants [11, 12].

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TABLE 1. Volatile Constituents of *I. bicolor*

Compound	RI	Content, %							
		MQ-1	MQ-2	MQ-3	MQ-4	MQ-5	MQ-6	MQ-7	MQ-8
Methyl octanoate	1057	–	–	–	2.96	7.45	–	–	–
1,3-Diethyl-5-methylbenzene	1084	–	–	–	–	–	–	–	7.05
Pentylbenzene	1099	–	–	–	–	–	–	–	3.18
1-Methylethylbenzene	1113	–	–	–	–	–	–	–	7.88
n-Dodecane	1200	–	–	–	–	–	–	–	5.97
1-Methyl-4-(pentan-3-yl)benzene	1205	–	–	–	–	–	–	–	4.81
n-Tridecane	1300	–	–	–	–	–	–	–	4.04
(2*E*, 4*Z*)-Decadienal	1303	–	–	–	0.46	–	–	–	–
3-Propoxy-1-propenylbenzene	1307	–	–	–	–	–	–	–	7.13
(2*E*, 4*E*)-Decadienal	1310	–	–	–	–	–	–	–	–
1-Methyl-2-hexenylbenzene	1366	–	–	–	–	–	–	–	6.48
(2*Z,* 6*E*)-Decadienal	1463	–	–	–	–	14.34	–	–	–
n-Pentadecane	1500	0.70	0.41	–	–	–	–	–	–
Cyclohexadecane	1530	1.43	–	–	–	–	–	–	1.45
n-Hexadecane	1600	1.58	0.56	–	–	1.12	–	–	0.67
Undecylbenzene	1690	1.50	–	–	–	–	–	–	–
Methyl tetradecanoate	1782	–	1.29	1.82	2.43	–	–	–	–
Dodecylbenzene	1804	1.35	–	–	–	–	–	–	–
Methyl pentadecanoate	1856	–	1.15	1.65	1.46	–	–	–	–
2-(1-Naphthyl)acetophenone	1868	2.00	–	–	–	–	–	–	–
Tridecylbenzene	1888	2.18	–	–	1.32	–	–	2.23	0.65
Methyl hexadecanoate	2001	12.35	12.69	8.76	18.82	16.09	–	–	–
Ethyl hexadecanoate	2056	0.54	–	–	–	–	–	–	–
n-Eicosane	2000	3.82	2.11	–	–	–	–	–	–
Methyl heptadecanoate	2088	2.88	4.30	6.66	–	–	–	–	–
trans-Methyl 13-octadecenoate	2158	2.44	8.78	–	20.81	19.42	47.29	29.87	–
Methyl octadecanoate	2190	9.63	13.14	9.26	16.49	4.96	–	–	–
Methyl nonadecanoate	2217	–	1.49	9.86	–	–	–	–	–
n-Docosane	2200	2.11	1.39	3.68	–	–	–	20.98	7.33
n-Tricosane	2300	1.94	–	2.41	3.04	–	7.82	–	–
Methyl eicosanoate	2360	5.45	10.93	8.89	3.83	–	–	–	–
n-Tetracosane	2400	3.25	–	–	–	–	–	–	–
Methyl heneicosanoate	2438	0.78	2.25	3.26	–	–	–	6.07	–
n-Pentacosane	2500	3.68	–	2.06	1.92	–	–	–	–
Methyl docosanoate	2521	8.95	12.64	10.02	3.14	–	–	–	–
n-Hexacosane	2600	2.48	–	–	–	–	–	–	–
Methyl tricosanoate	2595	4.37	5.32	6.56	0.70	–	–	–	–
n-Heptacosane	2700	2.85	–	–	–	–	–	–	–
Methyl tetracosanoate	2672	8.35	11.23	9.78	1.04	–	–	–	–
Ethyl tetracosanoate	2738	0.98	1.97	2.42	1.27	–	–	–	–
n-Octacosane	2800	1.44	–	1.32	–	–	7.91	–	–
Heptacosanoic acid	2808	1.61	2.20	2.36	–	–	–	7.07	–
Aliphatic hydrocarbons		28.96	4.47	9.47	6.28	15.46	15.73	23.21	14.14
Oxygenated aliphatic compounds		58.33	89.38	81.3	73.41	47.92	47.29	43.01	5.97
Aromatic hydrocarbons		1.35	–	–	–	–	–	–	29.4
Oxygenated aromatic compounds		2.00	–	–	–	–	–	–	7.13
Total identified		90.64	93.85	90.77	79.69	63.38	63.02	66.22	56.64

RI: retention index relative to *n*-alkanes on DB-5 capillary coated column; %: percentage composition; –: not detected; MQ-1 to MQ-8 = investigated samples.

TABLE 2. Antioxidant Activity of *I. bicolor*

Sample	IC_{50}, μg/mL	Sample	IC_{50}, μg/mL
Ascorbic acid	7.80 ± 0.14	MQ5	46.22 ± 2.00
MQ1	23.22 ± 0.75	MQ6	50.22 ± 1.56
MQ2	31.22 ± 0.98	MQ7	51.22 ± 0.41
MQ3	39.22 ± 1.27	MQ8	59.00 ± 2.01
MQ4	42.22 ± 0.33		

IC_{50}: the concentration required to inhibit radical formation by 50%; MQ-1 to MQ-8 are investigated samples.

Moreover, FAEs were not found in sample MQ8, and this sample showed the lowest antioxidant activity in comparison with other samples.

The fact that FAEs found in plant extracts show antioxidant activity is not usual, but literature survey shows that some plant extracts containing a high content of FAMEs could scavenge radicals [13, 14].

This paper presents the very first investigation on the chemical composition of plant extracts of *I. bicolor*, as well as their antioxidant activities, and it can provide a broad base for the possibility of further detailed pharmacological studies on this species.

Knowledge of the extent and mode of inhibition of specific compounds present in plant extracts may contribute to the successful application of such natural compounds for the treatment of infectious disorders like fungal and bacterial diseases. The present status of medicinal plants and their products provide the opportunity for the developing countries to benefit from the biodiversity of medicinal plants. The present study will provide researchers with basic data for future research in exploiting the hidden potential of this important plant, which has not been explored so far.

Plant Material and Reagents. The whole plant of *Impatiens bicolor* Royle was collected from Khwazakhela, Swat, N. W. F. P. Pakistan, during September 2008. A taxonomist, Dr. Hassan Sher, Jahan Zeb Post-Graduate College Saidu Sharif, Swat, Pakistan, identified the plant. A voucher specimen, No. 18NH-4-008, was deposited in the National Herbarium, Islamabad. All applied reagents were of the highest purity available and purchased from the Sigma-Aldrich.

Sample Preparation. Plant material (10 kg) was ground and extracted with methanol and water sequentially at room temperature. The combined methanol extract was filtered and evaporated under vacuum to obtain a thick greenish black gummy mass, which was fractionated with *n*-hexane. The *n*-hexane fraction was subjected to column chromatography on silica gel. The oily subfractions MQ-1 to MQ-8 were obtained using *n*-hexane as eluent, and then the polarity was increased gradually using dichloromethane gradient: MQ-1 (2.22 g, hexane), MQ-2 (1.28 g, hexane), MQ-3 (1.6 g, 1% CH_2Cl_2), MQ-4 (0.82 g, 3% CH_2Cl_2), MQ-5 (0.6 g, 5% CH_2Cl_2), MQ-6 (0.24 g, 7% CH_2Cl_2), MQ-7 (0.3 g, 8% CH_2Cl_2), MQ-8 (21.71 g, 10% CH_2Cl_2).

Gas Chromatography Analysis. Quantitative and qualitative data were determined by GC and GC-MS respectively. Plant extracts were injected onto a Shimadzu GC-17A system equipped with an AOC-20i autosampler and a split/splitless injector. The column used was a DB-5 (Optima-5), 30 m × 0.25 mm i.d., 0.25 μm df, coated with 5% diphenyl-95% polydimethylsiloxane and operated with the following oven temperature program: 50°C, held for 1 min, rising at 3°C/min to 250°C, held for 5 min, rising at 2°C/min to 280°C, held for 3 min; injection temperature and volume, 250°C and 1.0 μL, respectively; injection mode, split; split ratio, 30:1; carrier gas, nitrogen at 30 cm^3/s linear velocity and inlet pressure 99.8 kPa; detector temperature, 280°C; hydrogen flow rate, 50 mL/min; air flow rate, 400 mL/min; make-up (H_2/air), flow rate, 50 mL/min; sampling rate, 40 ms. Data were acquired by means of GC solution software (Shimadzu).

Gas Chromatography-Mass Spectrometry Analysis. An Agilent 6890N GC was interfaced with a VG Analytical 70-250s double-focusing mass spectrometer. Helium was used as the carrier gas. The MS operating conditions were: ionization voltage 70 eV and ion source 250°C. The GC was fitted with a 30 m × 0.32 mm fused capillary silica column coated with DB-5. The GC operating parameters were identical to those of the GC analysis.

The linear retention indices (RIs) for all compounds were determined by co-injection of *n*-hexane solution containing the homologous series of C_8-C_{26} *n*-alkanes [15]. Identification of the fixed oil constituents was accomplished by visual interpretation by comparing their RIs and mass spectra with literature data [16], from computer library search (NIST/EPA/NIH Mass Spectral Library 2.0, and Mass Finder 4 Computer Software and Terpenoids Library), and from the laboratory′s own database.

1,1-Diphenyl-2-picrylhydrazyl Radical-Scavenging Activity. The ability of the oils to donate a hydrogen atom or electron and scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by a spectrophotometric method based on the reduction of a methanol solution of DPPH using the method of Blois [17]. One milliliter of various concentrations of the fractions was added to 1 mL of a 0.2 mM methanol solution of DPPH. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. Then the absorbance was measured at 517 nm against a blank using a spectrophotometer (Shimadzu, Tokyo, Japan). Absolute methanol was used to zero the spectrophotometer. The DPPH solution was freshly prepared daily, stored in a flask covered with aluminum foil, and kept in the dark at 4°C between measurements. Inhibition of the free radical DPPH in percentage (I %) was calculated according to the formula $I\ (\%) = [(A_0 - A_t)/A_0] \times 100$ [18], where, A_0 is the absorbance of the control reaction (containing all reagents except the test fraction) and A_t is absorbance of the test fraction.

The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph after plotting inhibition percentage against extract concentration. A lower IC_{50} value indicates greater antioxidant activity. Tests were carried out in triplicate. Ascorbic acid was used as positive control.

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