SHORT REPORT

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Flavonoids and Essential Oil of Bidens cernua of Polish Origin and in vitro Antimicrobial Activity of the Oil

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Abstract: Phytochemical studies of the aerial parts of *Bidens cernua* L. led to the isolation of six flavonoids: luteolin (**1**), flavanomarein (**2**), tilianin (**3**), maritimetin (**4**), cynaroside (**5**) and cosmosiin (**6**). The quantitative analysis of flavonoids exhibited 0.81% contents of flavonoids in flowers and 1.62% in the herb counted as a hyperoside equivalent. Total Tannins Content showed the occurrence of 7.87% in flowers and 5.83% in the herb. The chemical composition of the essential oil obtained from the herb of *B. cernua* was evaluated in 92.2%, which corresponds to 99 compounds. Antimicrobial activity of the essential oil was evaluated on Gram-positive, Gram-negative bacteria and fungi. The oil exhibited a strong antibacterial and antifungal activity, especially against Gram-positive bacterial strains. This is the first time the aforementioned compound (**3**) has been isolated from *B. cernua*.

Keywords: *Bidens cernua;* Asteraceae; phytochemical analysis; flavonoids; essential oil; antimicrobial activity.

1. Plant Source

The aerial parts of flowering plants of *Bidens cernua* L. (Asteraceae) were collected at the beginning of September 2009 south-east region of Poland, dried at room temperature and finely cutted. A voucher specimen (No. BC 03001) has been deposited at the Herbarium of the Department of Pharmacognosy, Medical University of Białystok, Poland.

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2. Previous Studies

Previous phytochemical reports on *B. cernua* have revealed the presence of secondary plant metabolites such as polyacetylenes, sesquiterpenes, essential oil and flavonoids [1-5]. In continuation of our interest in the phytochemistry of plants from genus *Bidens* growing in Poland we decided to carry out a phytochemical studies on the herb and flowers of *B. cernua* of Polish origin.

3. Present Study

To isolate the flavonoid compounds, the diethyl ether, ethyl acetate and butanolic extracts were subjected to subsequent column chromatography on polyamide as stationary phase with eluting solvent systems of increasing polarity. As a result of series isolation procedures and purification on Sephadex LH-20, compounds **1-6** were obtained. The known isolates were confirmed as luteolin (**1**) [6], flavanomarein (isookanin 7-O-β-D-glucopyranoside) (**2**) [7], maritimetin (**4**) [8], cynaroside (luteolin 7-O-β-D-glucopyranoside) (**5**) [6] and cosmosiin (apigenin 7-O-β-D-glucopyranoside) (**6**) [8] by the comparison of reported spectral and physical data. To the best of authors knowledge, this is the first report on the isolation of tilianin (acacetin 7-O-β-D-glucopyranoside) (**3**) from *B. cernua*.

Structure elucidation of compound 3: Compound 3 was isolated as a light yellow solid. The UV spectrum showed the characteristic flavonoid bands at 268 and 324 nm [9]. It showed the presence of free -OH group at C-5. On addition of NaOAc no apparent shift of the band at 268 nm was observed, suggesting the blocked -OH group at C-7 by a glycosidic linkage. The ^{1}H-NMR spectrum, indicated the presence of flavonoid moiety and a sugar group. In the ^{1}H-NMR (DMSO,400 MHz), the observed signals from protons were assigned as follow: δ: 6.96 (1H, s, H-3), 6.46 (1H, d, J=2.1, H-6), 6.86 (1H, d, J=2.1, H-8), 8.07 (2H, d, J=8.9, H-2′, H-6′), 7.13 (2H, d, J=8.9, H-3′, H-5′), 3.87 (3H, s, H-OCH_3-4′) to flavonoid skeleton. The anomeric proton signal H-1″(δ 5.07) of glucose (d, J=7.2 Hz) revealed the existence of β-glycosidic linkage between the aglycone and sugar. The results suggest that the isolated compound 3 exhibited a close resemblance with that of reported compound, tilianin [6]

Chemotaxonomic evaluation: From a chemotaxonomic point of view, the identification in the genus *Bidens* L. of derivatives of flavonoids confirms the general chemical homogeneity regarding this class of compounds inside the genus. The presence of flavonoid aglycones, in particular luteolin, has been reported in aerial parts of many species: *B. frondosa*, *B. parviflora*, *B. pilosa* and *B. tripartita* [7,10-13]. Similarly to flavonoid aglycones, the glycosylated derivatives have been used as chemotaxonomic markers in the genus *Bidens*. Glycosylation at C-7 of those compounds has been found to be the most frequent substitution. The presence of 7-O-glucosides of apigenin and luteolin in *Bidens* species such as *B. tripartita* or *B. frondosa* has been reported [11, 14]. The presence of hydroxyaurones and chalcones such as maritimetin and flavanomarein in *Bidens* species (*B. andina*, *B. ferulifolia*) has an important meaning and has been described as main polyphenolics in the genus [15, 16].

Total Flavonoid Content (TFC): The total content of flavonoids in flowers and herb of *B. cernua* was based on the Christ-Müller's method [17] with modifications according to Polish Pharmacopoeia 10th [18]. The results obtained for flowers showed the presence of 0.81% of flavonoids counted as hyperoside and 0.57% of flavonoid compounds expressed as quercetin. The results for the herb were 1.62% and 1.13%, respectively.

Total Tannins Content (TTC): The quantitative analysis of tannins was made according to hide powder weight method described in DAB10 [19]. TTC content (expressed in equivalents/100 g of dry weight) was higher by more than 2% in flowers (7.87%) than in the herb (5.83%).

Essential oil preparation: Dried at room temperature and finely cut herb (500 g) was hydrodistilled for 3h using Clevenger-type apparatus [18] to yield limpid essential oil, with light-yellow colour and an intensive, but unpleasant scent, in the quantity of 0.5 mL (0.10% v/w).

Identification of essential oil component: After detailed analyses of GC and GC-MS data, 99 constituents were totally identified (Table 1), which corresponds to 92.2% [20, 21]. The main components of the tested essential oil are oxygenated monoterpenes, sesquiterpene hydrocarbons and monoterpene hydrocarbons. According to previous studies *B. cernua* herb oil was rich in polyacetylenes and especially 1-phenylhepta-1,3,5-triyn [3, 4] that was absent in researched oil. The other compounds identified in the oil are in good accordance with data published for *B. cernua* [4]. The differences are based on quantitative, not qualitative content. The presence of β-caryophyllene, δ-cadinene, humulene epoxide II as main constituents has been confirmed for essential oils from flowers of *B. pilosa* and *B. tripartita* [22, 23]. Due to the heterogeneity of the compounds identified in the *Bidens* species, it is difficult to establish a characteristic pattern of compounds for the genus. Variability in the composition of essential oils derived from plants of different origin is common phenomenon and may be due to the varied climatic conditions of the regions as well as the method for drying and storing the raw material and the time elapsed from harvest to distillation of essential oil [24-27].

Table 1. Chemical composition of essential oil of *B. cernua* herb

Peak No	Compound[1]	Content (%)	RI	RI lit.[2]
1.	Hexanal	0.4	777	769-775[a,b]
2.	Octane	0.1	800	800
3.	Furfural	0.1	809	794-800[a,b]
4.	(*E*)-Hex-2-enal	0.3	829	832-850[a,c]
5.	α-Thujene	0.1	925	926-932[a,c]
6.	α-Pinene	2.3	932	931-936[a-c]
7.	Benzaldehyde	0.7	935	927-941[a,b]
8.	Camphene	0.4	946	950[a,c]
9.	Thuja-2,4(10)-diene	0.1	949	946-957[a,b]
10.	6-Methylhept-5-en-2-one	0.2	965	963-978[a,b]
11.	Oct-1-en-3-ol	0.1	968	959-963[a,b]
12.	β-Pinene	t	973	970-978[a,b]
13.	2-Pentylfuran	0.5	981	977-981[a,b]
14.	Myrcene	0.4	983	979-987[a-c]
15.	*cis*-2-(Pent-2-enyl)furan	0.3	988	983[b]
16.	α-Phellandrene	0.6	999	997-1002[a-c]
17.	α-Terpinene	0.7	1011	1008-1013[a,b]
18.	p-Cymene	2.7	1014	1015[a,c]
19.	β-Phellandrene[3]	0.4	1024	1023-1025[a,b]
20.	Limonene[3]	0.2	1024	1020-1025[a,b]
21.	(*Z*)-β-Ocimene	2.5	1028	1024-1029[a,b]
22.	(*E*)-β-Ocimene	0.2	1040	1034-1041[a,b]
23.	γ-Terpinene	1.6	1052	1047-1051[a,b]
24.	*trans*-Sabinene hydrate	0.2	1057	1050-1053[a,b]
25.	*trans*-Linalol oxide (furanoid)	0.7	1061	1045-1068[a,b]
26.	*cis*-Linalol oxide (furanoid)	0.3	1075	1064-1072[a,b]
27.	p-Cymenene	0.3	1078	1061-1075[a,b]
28.	Nonanal	0.6	1084	1077-1091[a,b]
29.	Linalool	7.6	1086	1081-1086[a,b]
30.	Hotrienol	4.3	1088	1083-1089[a,b]
31.	Perillene	0.9	1090	1086-1090[a,b]
32.	α-Campholenal	0.1	1102	1105-1114[a,b]
33.	*cis*-p-Menth-2-en-1-ol	0.4	1107	1106-1011[a-c]
34.	*trans*-p-Mentha-2,8-dien-1-ol	0.2	1111	1113[a]
35.	*cis-allo*-Ocimene	0.9	1121	1117-1126[a,b]
36.	*cis*-p-Mentha-2,8-dien-1-ol	0.1	1128	1116-1123[a,b]
37.	*cis*-Verbenol	0.3	1131	1128-1132[a-c]
38.	*trans*-Verbenol	0.3	1134	1136-1144[a,b]
39.	(*E*)-Non-2-enal	0.1	1141	1139[a]

40.	Isoborneol	0.2	1151	1142-1146[a,b]
41.	Borneol	0.2	1154	1148-1150[a,b]
42.	p-Cymen-9-ol	t	1162	1158[a]
43.	Terpinen-4-ol	3.5	1167	1161-1164[a,b]
44.	Dill ether	0.1	1173	1170-1176[a,b]
45.	α-Terpineol	1.6	1178	1172-1176[a,b]
46.	Myrtenol	0.4	1181	1174-1178[a,b]
47.	Verbenone	0.4	1188	1185-1186[a,c]
48.	β-Cyclocitral	0.1	1203	1195[a]
49.	Nerol	0.3	1216	1209-1213[a,b]
50.	Neral	1.0	1219	1215-1218[a,c]
51.	Carvone	0.2	1223	1213-1218[a,b]
52.	Geraniol	1.4	1240	1235-1238[a,b]
53.	Geranial	1.2	1247	1244-1249[a-c]
54.	1,1,6-Trimethyl-1,2,3,4-tetrahydronaphthalene	0.2	1253	1255[b]
55.	Carvacrol	4.4	1283	1278-1282[a,c]
56.	Eugenol	0.3	1337	1331-1333[a,c]
57.	1,1,6-Trimethyl-1,2-dihydronaphthalene	0.3	1345	1339[b]
58.	7βH-Silphiperfol-5-ene	t	1354	1348-1352[a,b]
59.	Silphiperfol-6-ene	0.3	1369	1365-1379[a,b]
60.	β-Damascenone	t	1373	1363-1369[a,b]
61.	Methyleugenol	t	1375	1369-1371[a,b]
62.	Anastreptene	0.7	1376	1373[a]
63.	α-Copaene	0.1	1383	1376-1379[a,b]
64.	*cis*-β-Elemene	0.3	1386	1381[a]
65.	β-Elemene	7.3	1394	1389-1397[a,b]
66.	4-(2,6,6,-Trimethylcyclohexa-1,3-dienyl)butan-2-one	0.2	1404	1424[b]
67.	β-Caryophyllene	6.7	1428	1421-1424[a,b]
68.	Geranylacetone	0.6	1433	1426-1430[a,b]
69.	*trans*-α-Bergamotene	0.3	1439	1434[a]
70.	(*E*)-β-Farnesene	2.6	1449	1446-1448[a,b]
71.	α-Humulene	3.5	1461	1454-1456[a,b]
72.	4-(2,4,4-Trimethylcyclohexa-1,5-dienyl)but-3-en-2-one	0.3	1465	1460[b]
73.	β-Ionone	0.3	1471	1468[a]
74.	*ar*-Curcumene	0.3	1476	1468-1472[a,b]
75.	Selina-4,11-diene	0.4	1480	1475[a]
76.	*trans*-β-Bergamotene	0.8	1484	1480[a]
77.	(*Z*,*E*)-α-Farnesene	0.6	1486	1480[a]
78.	β-Selinene	1.0	1493	1486[a]
79.	(*E*,*E*)-α-Farnesene	0.4	1499	1496-1497[a,b]
80.	α-Selinene	1.4	1502	1494-1500[a,b]
81.	β-Bisabolene	2.1	1507	1503-1509[a,b]
82.	γ-Cadinene	0.1	1516	1507-1512[a,b]
83.	δ-Cadinene	0.2	1523	1520[a]
84.	(*Z*)-Nerolidol	t	1534	1522[a,b]
85.	(*E*)-Nerolidol	1.1	1552	1547-1553[a,b]
86.	β-Caryophyllene epoxide	1.8	1584	1576-1578[a,b]
87.	Humulene epoxide II	1.2	1609	1602[a]
88.	*neo*-Intermedeol	0.4	1633	1601[b]
89.	Intermedeol	0.2	1637	1630-1653[a,b]
90.	Eudesm-11-en-4α-ol	0.5	1653	1641-1649[a,b]
91.	α-Bisabolol	0.7	1675	1665-1674[a,b]
92.	Eugenyl valerate	t	1733	1728[b]
93.	Methyldibenzothiophene[4]	0.1	1737	1822[b]
94.	Drimenol	0.2	1772	1761[a,b]
95.	Methyldibenzothiophene[4]	1.3	1781	1842[b]
96.	6,10,14-Trimethypentadecan-2-one	0.9	1833	1838[b]

97.	Hexadecanol	0.4	1871	1864-1866[a,b]
98.	Hexadecanoic acid	4.0	1970	1972[b]
99.	Phytol	0.9	2107	2104-2123[a,b]
	Oxygenated monoterpenes	30.2		
	Sesquiterpene hydrocarbons	29.1		
	Monoterpene hydrocarbons	14.3		
	Oxygenated sesquiterpenes	6.1		
	Others	12.5		
	Total identified	**92.2**		

t – traces, <0.05%
[1] – compounds listed in order of elution from Rtx-1 column
[2] – Retention index of literature a) [28], b) [29], c) [30]
[3]– percentages from HP-Innowax column
[4] – correct isomer not identified

Antibacterial and antifungal activities of the essential oil: The biological activity of the essential oil was evaluated on Gram-positive, Gram-negative bacteria and fungi [31-33]. The MIC values received for the essential oil are listed in supporting materials (S3.). The growth of Gram-positive bacteria and fungi was very well inhibited by the oil. The strongest antibacterial influence of the tested oil was noted against *N. gonorrhoeae* (MIC = 1.56 mg/mL) and *M. catarrhalis* (MIC = 2.07+/-0.64 mg/mL) - two Gram-negative bacterial strains. Moderate inhibitory effect of essential oil was observed on growth of *E. faecalis* (Gram-positive) and *P. aeruginosa* (Gram-negative). The rest of tested Gram-negative bacterial strains were rather resistant to essential oil (MIC > 100 mg/mL). The highest fungistatic effect was observed for *G. candidum* (MIC = 3.1 mg/mL). The least impact was noted for *C. krusei* (MIC = 50.0 mg/mL).

To the best of our knowledge, this is the first report on chemical composition of the herb and on the analysis of volatile constituents and antimicrobial activities of the essential oil from of *B. cernua* gathered on territory of Poland.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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