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Composition of the Essential Oil of *Bidens tripartita* L. Roots and Its Antibacterial and Antifungal Activities

Monika Tomczykowa,[1] Katarzyna Leszczyńska,[2] Michał Tomczyk,[1] Elżbieta Tryniszewska,[2] and Danuta Kalemba[3]

Departments of [1]Pharmacognosy and [2]Microbiological Diagnostics, Faculty of Pharmacy, Medical University of Białystok, Białystok; and [3]Institute of General Food Chemistry, Faculty of Biotechnology and Food Sciences, Technical University of Łódź, Łódź, Poland

ABSTRACT The chemical composition of the essential oil obtained from the roots of *Bidens tripartita* L. by hydrodistillation was investigated by gas chromatography–mass spectrometry. In total, 106 compounds were identified (97.1% of the total oil). The main components of the oil were α-pinene (15.0%), β-bisabolene (9.3%), *p*-cymene (6.0%), hexanal (5.7%), linalool (4.6%), *p*-cymene-9-ol (3.4%), β-elemene (2.6%), 2-pentylfuran (2.2%), and silphiperfol-6-ene (2.1%). The antibacterial and antifungal properties of the essential oil were evaluated against eight Gram-positive and 11 Gram-negative bacterial species and 10 fungal strains. The oil exhibited a strong antifungal activity.

KEY WORDS: • *antibacterial activity* • *antifungal activity* • *Asteraceae family* • **Bidens tripartita** • *essential oil*

INTRODUCTION

The genus *Bidens*, belonging to the Asteraceae family, is represented in Europe by about 10 species. *Bidens cernua* and *Bidens tripartita* are two main representatives of the genus from among six species occurring in Poland.[1–3] The herb of *B. tripartita*, commonly known as bur-marigold, is traditionally used in the treatment of a whole spectrum of diseases, such as kidney stones and other kidney and bladder disorders. Bur-marigold herb is used in folk medicine to treat fevers and bleeding of different description. It is very useful as an anti-inflammatory agent. The bur-marigold herb stimulates the immunological system. It is also used in the treatment of skin diseases and ruptured blood vessels.[4,5] High activity in the inhibition of cancer L1210 (mouse leukemia) cells, as well as against thrombin, by the methylene chloride extract of bur-marigold has been reported.[6]

Previous phytochemical studies on *B. tripartita* herb have proved the occurrence of flavonoids,[7–10] polysaccharides, carotenoids, amines, lactones, mineral elements,[9,11] coumarins,[12] and volatile oil.[13] The antioxidant properties of three flavonoids—flavanomarein, cynaroside, and luteolin—isolated from bur-marigold and various extracts obtained from herbs and flowers of this plant were evaluated using the electroparamagnetic resonance method.[14] The antimicrobial activities of 12 extracts and essential oils of *B. tripartita* flowers and herb were analyzed: the investigated extracts (with the exception for butanolic extracts) exhibited antibacterial activity, and none had an inhibitory effect against the tested fungi. In contrast to the extracts, both essential oils showed strong antifungal activity and an insignificant bacteriostatic effect.[15] Anti-inflammatory activity of aqueous extracts of *B. tripartita* herb against carrageenan-induced acute paw edema in rats was established.[16]

All previous phytochemical and microbiological investigations were focused on the aerial parts of *B. tripartita*. There are no phytochemical and microbiological data on roots. This prompted us to look at *B. tripartita* roots, especially at the essential oil,[17] with scientific interest.

MATERIALS AND METHODS

Plant material

B. tripartita roots were collected in the Białystok area in Poland in August 2008. A voucher specimen was deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy Medical University of Białystok, Białystok, Poland.

Essential oil preparation

Bur-marigold roots (254 g) were dried at room temperature, finely cut, and subjected to hydrodistillation (3 hours) using a Clevenger-type apparatus. The amount of essential oil has been determined according to the method described in the *Polish Pharmacopoeia*, 8th edition.[18]

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Address correspondence to: Monika Tomczykowa, Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Białystok, 15-230 Białystok, ul. Mickiewicza 2a, Poland, E-mail: monika.tomczyk@umwb.edu.pl

Gas chromatography and gas chromatography–mass spectrometry analysis

Gas chromatography (GC) and GC–mass spectrometry (MS) analysis were performed using a Trace GC Ultra apparatus (Thermo Electron Corp., Waltham, MA, USA) with a flame ionization detector (FID) and MS DSQ II detectors and FID-MS splitter (SGE Analytical Science, Ringwood, VIC, Australia). Operating conditions were as follows: apolar capillary column, Rtx-1ms (Restek Corp., Bellefonte, PA, USA), 60 m × 0.25 mm i.d., film thickness of 0.25 µm, temperature program of 50–300°C at 4°C/minute, SSL injector temperature of 280°C, FID temperature of 300°C, split ratio of 1:20, and carrier gas helium at a regular pressure of 200 kPa; polar capillary column. HP-Innowax (Agilent J&W, Palo Alto, CA, USA), 30 m × 0.25 mm i.d., film thickness of 0.25 µm, temperature program of 50–245°C at 4°C/minute (30 minutes), SSL injector temperature of 250°C, FID temperature of 260°C; carrier gas helium at a flow rate of 0.5 mL/minute, and split ratio of 1:20. Mass spectra were acquired over the mass range 30–400 Da, with an ionization voltage of 70 eV and an ion source temperature of 200°C.

Identification of components

Identification of components was based on the comparison of their retention indices on the apolar column (Rtx-1ms) and mass spectra with those of laboratory-made retention indices and mass spectrometry libraries, with commercial libraries (NIST 98.1, Wiley Registry of Mass Spectral Data, 8th ed., and MassFinder 4 obtained with the respective instruments from the manufacturers), and with literature data.[19,20] Retention indices were calculated by linear interpolation to retention times of a series of alkanes (C_8–C_{26}). A quantitative analysis (expressed as percentages of each component) was carried out by peak area normalization measurements without correction factors.

Microbiological material

Antimicrobial activity of the essential oil was estimated against Gram-positive and Gram-negative bacteria and against 10 fungal strains obtained from the American Type Culture Collection (Manassas, VA, USA) (designated ATCC) or isolated (designated with *) from the samples collected from selected patients of the University Clinical Hospital in Białystok. The microorganisms used for this assay were as follows:

1. Gram-positive: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Streptococcus pyogenes**, *Streptococcus pneumoniae**
2. Gram-negative: *Klebsiella pneumoniae* ATTC 700603 (extended-spectrum β-lactamase positive), *Klebsiella pneumoniae* (extended-spectrum β-lactamase positive)*, *Escherichia coli* ATCC 35218 (β-lactamase positive), *Escherichia coli* (extended-spectrum β-lactamase positive)*, *Proteus mirabilis**, *Pseudomonas aeruginosa* ATCC 27853, *Neisseria gonorrhoeae**, *Moraxella catarrhalis**
3. Fungi: *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Candida krusei**, *Candida glabrata**, *Geotrichum candidum**, *Cryptococcus neoformans**, *Rhodothorula rubra**

MacConkey's agar, mannitol salt agar, Sabouraud's dextrose agar with gentamicin and chloramphenicol, *Pseudomonas* agar, Columbia CNA agar + 5% SB, chocolate-II-agar with isovitalex and bacitracin, and CAN agar + 5% sheep blood (Becton Dickinson, Fair Lawn, NJ, USA) were used for isolation of bacteria and fungi from clinical samples. For identification purposes, the bacterial strains were cultured on Columbia agar + 5% sheep blood (Becton Dickinson) at a temperature of 35 ± 2°C for 18–24 hours in an oxygen atmosphere. In the case of *S. pneumoniae* and *N. gonorrhoeae*, a 5% CO_2 atmosphere was used (GENbag-CO_2, BioMérieux, Rhône-Alpes, France). Fungi were grown at 30–35°C for 24–72 hours on Sabouraud's dextrose agar plates (Becton Dickinson). Microorganisms were identified on an ATB apparatus (BioMérieux) using ID32 STAPH, IDrapidStrep, ID32GN, ID 32C, and apiNH (BioMérieux) sets as recommended by the manufacturer.

Additionally, the minimum inhibitory concentration (MIC) of some antibacterial antibiotics (vancomycin and ciprofloxacin) against bacterial strains was tested. Amphotericin B was used against fungal strains. The efficacy of the tested antibiotics was estimated according to Clinical and Laboratory Standards Institute (formerly NCCLS) standards.[21]

The methicillin-resistant *Staphylococcus* strains and bacterial strains producing extended-spectrum β-lactamase were estimated according to previous recommendations.[21]

Broth microdilution method

The MICs of essential oil of *B. tripartita* roots for antibacterial and antifungal tests were determined using the broth microdilution method[22,23] with medium modifications. Five hundred milligrams of the tested essential oil was dissolved in 1 mL of dimethyl sulfoxide and further diluted to a 200 mg/mL concentration in three different types of medium. Mueller–Hinton broth was used to assign MIC values for most bacterial strains with the exception of *Streptococcus* and *Neisseria* strains, for which tryptone soya broth medium (Oxoid, Basingstoke, United Kingdom) was used. RPMI 1640 medium (Sigma, Poole, United Kingdom) was used to prepare the solutions of all tested fungi. Experiments were conducted using a final bacterial inoculum of 0.5×10^5 colony-forming units/mL and fungal suspensions of 10^3 colony-forming units/mL for inoculation. The MIC values were determined after incubation in 35 ± 2°C for 18–24 hours in ambient air and in a 5% CO_2 atmosphere for *S. pneumoniae* and *N. gonorrhoeae*.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The essential oil from the roots of *B. tripartita* was obtained with a yield of 0.098% (vol/wt). The tested oil was limpid and exhibited a yellow color and unpleasant, intense

scent. Using the GC and GC-MS methods 106 components were identified, representing 97.1% of total amount. The results of the qualitative and quantitative composition of the tested oil are listed in Table 1.

The major compounds of the tested oil represent the following groups of chemical compounds: monoterpene hydrocarbons, α-pinene (15.0%) and *p*-cymene (6.0%); sesquiterpene hydrocarbons, β-bisabolene (9.3%), β-elemene (2.6%), and silphiperfol-6-ene (2.1%); oxygenated monoterpenes, linalool (4.6%) and *p*-cymene-9-ol (3.4%); aliphatic compounds, hexanal (5.7%); and furan derivatives, 2-pentylfuran (2.2%). Eleven other components were present in the oil in quantities between 1% and 2%. They represent phenylpropane derivatives (eugenyl butyrate, 2.0%; methyleugenol, 1.7%; eugenol 1.1%), monoterpene hydrocarbons (camphene, 1.9%; α-phellandrene, 1.1%; (*Z*)-β-ocimene, 1.0%), oxygenated sesquiterpenes (eudesm-11-en-4α-ol, 1.9%; β-caryophyllene epoxide, 1.7%), sesquiterpene hydrocarbons (*trans*-β-bergamotene, 1.4%; α-selinene, 1.0%), and oxygenated monoterpenes (α-terpineol, 1.3%). The remaining chemical constituents occurred at a level below 1%. The content of hexadecanoic acid (3.3%) in the oil was noted.

Antimicrobial and antifungal activities of the essential oil

The values of the MIC of the tested essential oil against Gram-positive (eight strains) and Gram-negative (11 strains) bacteria and 10 fungi strains are given in Table 2. The analyzed essential oil from the roots of *B. tripartita* moderately inhibited the growth of Gram-positive bacteria. The strongest antibacterial activity was observed for two Gram-negative bacterial strains: *N. gonorrhoeae* (MIC = 3.1 mg/mL) and *M. catarrhalis* (MIC = 3.12 ± 2.1 mg/mL). Low bacteriostatic effects for *P. aeruginosa* and *P. aeruginosa* ATCC 27853 (Gram-negative) were noted. The results obtained for other tested Gram-negative bacterial strains showed almost no activity (MIC > 100 mg/mL). The essential oil of *B. tripartita* showed a fungistatic effect. The highest inhibition effect of the oil was noted for *C. albicans* ATCC 10231 (MIC = 3.1 mg/mL), and the lowest one for *C. krusei* (MIC = 100 mg/mL). The growth of the remaining tested fungi was decreased by the oil with an MIC range of 6.2–25 mg/mL.

Most plants are traditionally used as remedies in folk medicine. *B. tripartita* is one of them. Only the aerial parts of *B. tripartita* have been the subject of scientific investigations until now. Thinking about new source of plant drugs we put attention to *B. tripartita* roots, especially to the essential oil. The qualitative and quantitative determination of the chemical composition of the essential oil and its antimicrobial and antifungal activities have been established. Differences in sensitivity between Gram-positive and Gram-negative bacteria, as well as among Gram-negative bacteria, were observed. The essential oil exhibited moderate antimicrobial activity against Gram-positive bacteria. The oil can be acknowledged as ineffective within the experimental concentration range against most tested Gram-negative bacteria. The essential oil from *B. tripartita* roots possesses high fungistatic activity, which makes it a potential remedy for phytophatogenic fungi.

The results obtained for *B. tripartita* root essential oil agree with those previously obtained for oils from flower heads and herbs of *B. tripartita*.[15] All three tested oils demonstrated good and approximately equivalent antifungal activity. Antimicrobial activity against Gram-positive bacteria was observed for three oils, with the highest efficacy for flower head oil and the lowest one for root oil. The three oils obtained from different parts of *B. tripartita* could be accepted as inactive against Gram-negative bacteria. However, there are some differences in the influence on Gram-negative organisms by the discussed essential oils: the essential oil from the roots slightly inhibited the growth of both *P. aeruginosa* strains, and it showed significant antimicrobial activity toward *N. gonorrhoeae* and *M. catarrhalis* (Gram-negative), whereas the oils from flower heads and from the herbs did not display activity toward *P. aeruginosa* but slightly inhibited the growth of *E. coli* strains. This is interesting because of differences in the chemical composition of the essential oils from different parts of the plant, which as a consequence may give various biological activities.[24] Usually, biological activity is assigned to major compounds.[25] The dominant group of compounds in *B. tripartita* root essential oil is the monoterpene hydrocarbons, with α-pinene as a main compound. Some studies have demonstrated good bacteriostatic and fungistatic properties of α-pinene, although some data show its low antimicrobial activity.[26] The reasons of those divergences are unknown, so far. There are hypotheses stating that the biological activity of the essential oil depends not only on major components, but also on minor constituents, and their synergistic interactions. Differences in bioactivity of α-pinene could be correlated with its solubility, which is low in water. This may cause low dispersion of α-pinene in water and limited physical contact with the tested organisms. The presence of many other chemical compounds possessing their own physical properties may have an influence on the bioactivity of a single compound and on the whole essential oil.[27,28]

The differences in resistance of Gram-positive and -negative bacteria caused by the essential oil are connected with their different cellular membranes. The lipopolysaccharide layer of Gram-negative cell walls is easily accessible for lipophilic monoterpene hydrocarbons (*e.g.*, α-pinene). The antifungal effect caused by essential oil appears to be connected with interaction of proteins of the fungal cell membrane and components of the oil.[15,29]

Comparison of our results with previous studies and literature data is very difficult because of differences in chemical composition of essential oils obtained from different part of the same plant, different fungal and bacterial strains, and their sensitivity. There is also no consensus as to how to correlate activity of essential oil with its composition.

In conclusion, the chemical composition of the essential oil from *B. tripartita* roots and its antimicrobial and antifungal activities were established. To the best of our knowledge, this is the first report on the chemical composition and

Table 1. Percentage Chemical Composition of Essential Oil of *B. tripartita* L. Roots

Compound	*Content (%)*	*Retention index (minutes)*
Pentanol	0.5	750
Hexanal	5.7	777
Furfural	0.1	809
Hexanol	0.7	855
Heptan-2-on	0.2	871
2-Butylfuran	0.3	881
α-Thujene	0.1	925
α-Pinene	15.0	932
Benzaldehyde	0.5	935
Camphene	1.9	946
Verbenene	0.3	949
6-Methylhept-5-en-2-one	0.1	963
Oct-1-en-3-ol	0.6	966
Sabinene	0.4	971
β-Pinene	0.5	975
2-Pentylfuran	2.2	981
Myrcene	0.9	983
2-(Pent-2-enyl)furan	0.1	988
α-Phellandrene	1.1	999
2-Propionylfuran	0.1	1010
α-Terpinene	0.1	1011
p-Cymene	6.0	1014
β-Phellandrene[a]	0.2	1024
Limonene[a]	0.6	1024
(*Z*)-β-Ocimene	1.0	1028
(*E*)-Oct-2-enal	0.4	1037
(*E*)-β-Ocimene	0.3	1040
γ-Terpinene	0.3	1052
trans-Linalool oxide (furanoid)	0.3	1061
cis-Linalool oxide (furanoid)	0.1	1075
p-Cymenene	0.6	1078
Terpinolene	0.2	1081
Nonanal	0.3	1084
Linalool	4.6	1086
α-Campholenal	0.6	1102
trans-*p*-Mentha-2,8-dien-1-ol	0.1	1111
Nopinone	0.1	1114
(4*E*,6*Z*)-*allo*-Ocimene	0.3	1121
trans-Pinocarveol	0.5	1129
cis-Verbenol	0.4	1131
trans-Verbenol	0.2	1134
Pinocarvone	0.2	1145
Borneol	0.2	1154
p-Cymen-9-ol	3.4	1162
Terpinen-4-ol	0.7	1167
Dill ether	Tr	1173
α-Terpineol	1.3	1178
Myrtenol	0.6	1188
Verbenone	0.4	1188
α-Campholenol	0.3	1205
trans-Carveol	0.1	1216
Nerol	Tr	1216
Carvone	0.4	1223
Geraniol	0.8	1240
Granial	0.1	1247
Bornyl acetate	0.4	1274
Carvacrol	0.2	1283
(*E*,*E*)-Deca-2,4-dienal	0.4	1296
Eugenol	1.1	1337
7α*H*-Silphiperfol-5-ene	0.1	1354
7β*H*-Silphiperfol-5-ene	0.1	1360
Octyl butanoate	0.1	1362
Silphiperfol-6-ene	2.1	1369
β-Damascenone	Tr	1373
Methyleugenol	1.7	1375
α-Copaene	0.1	1383
cis-β-Elemene	0.1	1386
β-Elemene	2.6	1394
β-Caryophyllene	0.3	1428
Geranylacetone	Tr	1433
trans-α-Bergamotene	0.3	1439
(*E*)-β-Farnesene	0.1	1449
α-Humulene	0.2	1461
β-Ionone	Tr	1471
ar-Curcumene	0.5	1476
Selina-4,11-diene	0.3	1480
trans-β-Bergamotene	1.4	1484
(*Z*,*E*)-α-Farnesene	0.6	1486
β-Selinene	0.8	1493
Miristicine	Tr	1495
(*E*,*E*)-α-Farnesene	0.5	1499
α-Selinene	1.0	1502
β-Bisabolene	9.3	1507
Elemicin	0.4	1514
1β*H*-Presilphiperfolan-9α-ol	0.6	1523
Italicene ether	0.8	1526
(*Z*)-Nerolidol	0.1	1534
(*E*)-Nerolidol	0.1	1552
Caryolan-1-ol	0.3	1574
β-Caryophyllene epoxide	1.7	1584
Humulene epoxide II	0.4	1609
Humula-1,6-dien-3-ol	0.4	1614
Silphiperfol-6-en-5-one	0.3	1618
Alismol	0.1	1624
Eugenyl butyrate	2.0	1633
Eudesm-11-en-4α-ol	1.9	1653
α-Bisabolol	0.4	1675
Pentadecenal[b]	0.4	1698
Eudesma-4,11-dien-2-ol	0.5	1706
Eugenyl valerate	0.6	1733
Cyperenal	0.3	1734
Cyclocolorenone	0.3	1743
Methyldibenzothiophene[b]	Tr	1737
Methyldibenzothiophene[b]	0.1	1781
6,10,14-Trimethypentadecan-2-one	0.8	1833
Hexadecanoic acid[a]	3.3	1970
Total identified	97.1	

[a]Results from the HP-Innowax column.
[b]Correct isomer not identified.
Tr, trace (< 0.05%).

biological activity of the essential oil of *B. tripartita* roots. However, it will be important to determine the most active component(s) responsible for antifungal and antibacterial activities, as well as to establish the mechanism of the action, especially against Gram-negative bacteria, and to explain the chemical and physical interactions of the individual compounds.

Table 2. Minimum Inhibitory Concentration of the Essential Oil of *B. tripartita* L. Roots Against Different Microorganisms

Microorganism (antibiotic used)	*MIC*	
	Essential oil (mg/mL)	*Antibiotic (μg/mL)*
Gram-positive bacteria (vancomycin)		
S. aureus ATCC 25923	100	0.75
S. aureus (MSSA)* (*n* = 4)	75 ± 25	0.45 ± 0.13
S. aureus (MRSA)* (*n* = 4)	87.5 ± 18.75	0.87 ± 0.38
E. faecalis ATCC 29212	100	3.1
*E. faecalis** (*n* = 4)	87.7 ± 18.75	1.13 ± 0.63
*S. pyogenes** (*n* = 3)	56.25 ± 21.88	0.29 ± 0.14
*S. pneumoniae** (*n* = 4)	62.5 ± 18.75	0.25 ± 0.13
*S. agalactiae** (*n* = 3)	66.67 ± 22.22	0.25 ± 0.17
Gram-negative bacteria (ciprofloxacin)		
K. pneumoniae ATTC 700603 (ESBL+)	> 100	0.75
*K. pneumoniae** (*n* = 2)	> 100	0.52 ± 0.19
K. pneumoniae (ESBL+)* (*n* = 2)	> 100	0.67 ± 0.11
E. coli ATCC 25922	> 100	0.16
E. coli ATCC 35218 (β-lactamase+)	> 100	0.75
E. coli (ESBL+)* (*n* = 3)	> 100	0.73 ± 0.18
*P. mirabilis** (*n* = 2)	> 100	0.42 ± 0.12
P. aeruginosa ATCC 27853	100	0.75
*P. aeruginosa** (*n* = 3)	66.67 ± 22.2	0.35 ± 0.1
*N. gonorrhoeae**	3.1	0.008
*M. catarrhalis** (*n* = 4)	3.12 ± 2.1	0.028 ± 0.02
Fungi (amphotericin B)		
C. albicans ATCC 10231	3.1	0.08
C. albicans ATCC 90028	6.2	0.5
*C. albicans** (*n* = 3)	11.46 ± 9.03	0.29 ± 0.14
C. parapsilosis ATCC 22019	12.5	0.25
*C. krusei**	100	0.5
*C. glabrata**	25	0.25
C. tropicalis	6.2	0.32
*G. candidum**	6.2	0.12
*C. neoformans**	25	0.5
*R. rubra**	6.2	0.5

Organisms were obtained from the American Type Culture Collection (designated ATCC) or isolated (designated with *) from the samples collected from selected patients of the University Clinical Hospital in Białystok.

ESBL+, extended-spectrum β-lactamase positive; MIC, arithmetical means and average deflection of minimum inhibitory concentration value from "*n*" numbers of tested strains, where *n* is the number of tested isolates; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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