

Isolation and Identification of Antibiotic Activity Containing Compounds from *Bupleurum Rotundifolium* L

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Abstract:

The composition of the essential oils obtained by hydrodistillation from different parts of *Bupleurum rotundifolium* L. including roots, flowers and fruits were investigated by GC and GC/MS systems, simultaneously. The antibacterial activity of the oils was assessed with micro-dilution assay. The results showed large variations in the composition of the oils. The main components of the plant were undecane (26.4%) and tridecane (12.3%) in the roots, hexadecanoic acid (12.2%) in the flowers and α -pinene (11.0%) in the fruits. The essential oils of *B. rotundifolium* obtained from flowers and fruits used in the study did not have any effect against bacteria. MIC values for the bacterial strains tested, which were sensitive to the essential oil of roots of *B. rotundifolium*, were in the ratio of 2 mg/mL. This investigation showed that the antibacterial activity of *B. rotundifolium* was attributed to the essential oil of roots.

Keywords: *Bupleurum rotundifolium*; essential oil composition; antibacterial activity; α -pinene;

INTRODUCTION

The genus *Bupleurum* L. of the family Apiaceae (Umbelliferae). Extracts and essential oils of *Bupleurum* genus plants have been largely used in traditional medicine for their anti-inflammatory and antiseptic activity [3]. *Radix Bupleuri* (dried roots of *B. chinense* DC. or *B. scorzonerifolium* Willd), known as 'Chai-Hu', is one of the most frequently prescribed crude herbs in the prescriptions of traditional Chinese medicines for the treatment of inflammatory diseases [4], nephrosis syndrome [5] and auto-immune diseases [6].

The previous studies on this species reported the occurrence of saponins and saikosaponin and antimicrobial activity of methanol extract of *B. rotundifolium* [7, 4, 8, and 9].

MATERIAL AND METHODS:

The essential oils from air-dried plant materials were isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus to produce a small amount of essential oil which was trapped in n-hexane. The obtained oils were dried over anhydrous sodium sulphate and stored at +4°C in the dark until analysed and tested.

GC and GC/MS: The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Inn wax FSC column (60 m x 0.25 mm, 0.25 μ m film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from m/z

35 to 450. The components of essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, Mass Finder Library and confirmed by comparison of their retention indices. Alkanes were used as reference points in the calculation of relative retention indices (RRI). Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Table 1. Chemical Compositions of the Essential Oils from Different Parts of *Bupleurum rotundifolium*

No	Compounds	RRI	Root %	Flower %	Fruit %
1	a-Pinene	1032	-	8.7	11
2	Hexanal	1093	1	-	-
3	Undecane	1100	26.4	4.9	10.4
4	b-Pinene	1118	-	0.4	1.1
5	Sabinene	1132	-	3.6	1.8
6	Myrcene	1174	-	0.9	1.9
7	a-Terpinene	1188	-	-	0.7
8	Limonene	1203	0.6	2.4	5.4
9	1,8-Cineole	1213	-	-	2.2
10	b-Phellandrene	1218	0.4	7.2	6.1
11	Amyl furan	1244	-	0.2	1.5
12	g-Terpinene	1255	-	0.3	2.3
13	p-Cymene	1280	-	Tr	1.6
14	Terpinolene	1290	-	Tr	0.5
15	Tridecane	1300	12.3	1.2	1.6
16	(Z)-3-Hexenyl-2-methyl butyrate	1482	-	1.1	0.7
17	(Z)-3-Hexenyl 3-methylbutyrate (=Z)-3-hexenyl isovalerate)	1494	-	1.3	0.5
18	a-Copaene	1497	-	1	1.3
19	Pentadecane	1500	1.3	0.7	1.1
20	Camphor	1532	-	-	0.5
21	b-Bourbonene	1535	-	0.6	1.9

No	Compounds	RRI	Root	Flower	Fruit
22	b-Cubebene	1549	-	0.4	-
23	b-Ylangene	1589	-	0.2	-
24	b-Copaene	1597	-	0.2	-
25	Terpinen-4-ol	1611	-	-	1.6
26	b-Caryophyllene	1612	-	0.9	-
27	Selina-5,11-diene	1620	1.1	-	-
28	(Z)-b-Farnesene	1668	-	1.6	2.4
29	Decyl acetate	1687	4.4	-	-
30	a-Humulene	1687	-	0.4	-
31	Heptadecane	1700	-	0.3	-
32	Germacrene D	1726	-	7.4	5
33	Chrysanthenyl isovalerate I	1743	-	-	0.8
34	Phellandral	1744	-	0.3	1
35	Bicyclgermacrene	1755	-	0.5	-
36	(E,E)-a-Farnesene	1758	-	-	-
37	Chrysanthenyl isovalerate II	1760	-	-	1
38	Decanol	1766	2	0.1	-
39	d-Cadinene	1773	-	0.5	-
40	Cumin aldehyde	1802	-	-	1
41	(E,E)-2,4-Decadienal	1827	0.3	0.2	-
42	(E)-Geranyl acetone	1868	-	0.2	0.8
43	Nonadecane	1900	0.9	0.2	-
44	Epi-Cubebo	1900	-	0.2	-
45	Tetradecanal	1933	0.5	-	-
46	a-Calacorene	1941	-	0.3	0.3
47	1,5-Epoxy-salvial(4)14-ene	1945	-	2	1.2
48	(E)-b-Ionone	1958	-	0.9	-
49	Dodecanol	1973	2	-	-
50	g-Calacorene	1984	-	0.3	-
51	2-Phenylethyl-2-methylbutyrate	1988	-	0.2	-
52	Isocaryophyllene oxide	2001	-	0.4	-
53	Caryophyllene oxide	2008	-	2.7	1.6
54	Salvial-4(14)-en-1-one	2037	-	0.7	1.6
55	Humulene epoxide-II	2071	-	0.5	-
56	Heneicosane	2100	0.9	0.2	-
57	Salviadienol	2130	-	1.4	-
58	Hexahydrofarnesyl acetone	2131	4.2	2.5	2.2
59	Spathulenol	2144	0.2	3.9	2.6q1
60	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	2179	-	0.4	-
61	Tetradecanol	2179	3.6	-	0.3
62	Torilenol	2278	-	-	1
63	Pentadecanol	2278	-	-	1.1
64	Tricosane	2300	4	-	-
65	Eudesma-4(15),7-dien-4b-ol	2369	-	2.4	-
66	Hexadecanol	2384	7.9	-	2.7
67	Pentacosane	2500	0.2	0.4	-
68	Phytol	2622	-	-	1.3
69	Tetradecanoic acid (= Myristic acid)	2670	-	tr	-
70	Heptacosane	2700	-	tr	-
71	Nonacosane	2900	-	1.8	-
72	Hexadecanoic acid	2931	-	12.2	-

	Root	Flower	Fruit
Monoterpene Hydrocarbons	1	23.8	32.4
Oxygenated Monoterpenes	-	0.1	8.1
Sesquiterpene Hydrocarbons	1.1	14.7	10.9
Oxygenated Sesquiterpenes	0.2	14.2	8.2
Fatty acid +esters		12.2	
Diterpenes			1.3
Alkanes	46	9.9	13.1
Others	25.9	7	9.8
Total	74.2	81.5	83.6

RRI Relative retention indices calculated against n-alkanes %
calculated from FID data tr Trace (< 0.1 %)

RESULTS AND DISCUSSION:

Antimicrobial Activity:

In microbiological tests, standard 11 bacteria strain that exists in human beings, animals and food are used. These are Staphylococcus aureus ATCC 6538, Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 3166 09:K35:K99, Escherichia coli ATCC 25922, Escherichia coli ATCC 25923, Escherichia coli ATCC 29988, Bacillus cereus ATCC11778, Streptococcus salivarius RSHE 606, Pseudomonas aeruginosa ATCC 29853, Pseudomonas aeruginosa ATCC 15442, Proteus mirabilis ATCC 43071. Bacterial strains were obtained from the Biotechnology Laboratory in Telangana University. Bacterial cultures were activated in Mueller Hinton Broth (MHB, Merck) for 24h at 37°C. At the end of the period of incubation, the cultures developed in the liquid medium were standardized at 108 cfu/mL (McFarland No: 0.5). The essential oils dissolved in 25% dimethylsulfoxide (DMSO) was first diluted to the highest concentration 4 mg/mL to be tested and then two-fold serial of dilutions were made in concentration range from 2 mg/mL to 3.906 µg/mL.

Antibacterial activity was assayed using the micro dilution technique [10, 11]. For antibacterial tests, pre-sterilized micro titration Petri dish (Brand) having 96 “U” type wells were used. Serial solutions of the essential oils were performed at micro titration petris. 100 µl of each microbial suspension were added to the wells. The eleventh well containing only serial dilutions of antibacterial agents without microorganisms was used as negative control. The last well contained only microorganisms as positive control. Solvent DMSO as negative control and Chloramphenicol (Sigma) as positive control were used. The minimum inhibitory concentration (MIC) values were determined as the last well doesn't include turbidity at the end of the incubation for 24 h at 37°C.

The essential oils were obtained by hydrodistillation from air-dried parts of B. rotundifolium and subsequently analyzed by GC and GC/MS systems, simultaneously. In total, 20 (root), 53 (flower) and 39 (fruit) constituents were identified and quantified in the various parts, respectively (Table 1).

In the root oil of B. rotundifolium, undecane (26.4%), tridecane (12.3%) and hexadecanol (7.9%) were the main constituents. The root essential oil comprised alkanes

(46%), 'others' (25.9%), sesquiterpene hydrocarbons (1.1%), monoterpene hydrocarbons (1.0%), and oxygenated sesquiterpenes (0.2%), respectively. As for the flower oil of *B. rotundifolium*, the main constituents were hexadecanoic acid (12.2%), α -pinene (8.7%), germacrene D (7.5%) and β -phellandrene (7.3%). Furthermore, flower essential oil was composed of monoterpene hydrocarbons (23.8%), sesquiterpene hydrocarbons (14.7%), oxygenated sesquiterpenes (14.2%), fatty acid+esters (12.2%), alkanes (9.9%), 'others' (7.0%) and oxygenated monoterpenes (0.1%), respectively. In the fruit oil of *B. rotundifolium*, α -pinene (11.0%), undecane (10.4%) and β -phellandrene (6.1%). The fruit essential oil comprised monoterpene hydrocarbons (32.4%), alkanes (13.1%), and sesquiterpene hydrocarbons (10.9%), others' (9.8%), oxygenated sesquiterpenes (8.2%), oxygenated monoterpenes (8.1%) and diterpenes (1.3%), respectively.

ACKNOWLEDGMENTS:

The authors are grateful to the Director, Indian Institute of Chemical Technology, Hyderabad, India, for providing spectral data.

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