

SUPPLEMENTARY MATERIAL

Chemical composition and antibacterial activity of essential oil from fruit of *Micromelum integerrimum* (Buch.-Ham. ex DC.) Wight & Arn. ex M. Roem

Short title: Essential oil from fruit of *Micromelum integerrimum* (Buch.-Ham. ex DC.) Wight & Arn. ex M. Roem

Rumi Kotoky,^{a*} Manobjyoti Bordoloi,^{b*} Archana Yadav,^c Kashyap J. Tamuli,^b Surovi Saikia,^b Partha P. Dutta,^b Prodip P. Khound^d and Siddhartha P. Saikia^a

^aMedicinal and Aromatic Plants Group, ^bNatural Products Chemistry Group, ^cBiotechnology Group and ^dAnalytical Chemistry Group; CSIR North East Institute of Science & Technology, Jorhat-785006, Assam, India

***Corresponding author's e-mails:** m.j.bordoloi.pub@gmail.com; mjb_rrljt@yahoo.co.in; kotoky15@yahoo.co.in; rumikotoky18@gmail.com

Experimental

Plant Material: The matured ripe orange or red colour fruits of *M. integerrimum* plants (family Rutaceae) were collected from Gibbon Wildlife Sanctuary, Jorhat district of Assam in the month of March-April, 2015. Identity of the species was confirmed at the Herbarium of Botanical Survey of India, (BSI) Shillong, Meghalaya, India followed by the deposition of its voucher specimen No. 4495 at the Herbarium of CSIR-North East Institute of Science & Technology, Jorhat, Assam, India.

Isolation of volatile components: 300 g of matured fruit were subjected to hydro-distillation in a Clevenger-type apparatus for 6 h to obtain about 0.75-0.85 ml of essential oil from it. The distilled oils were collected and dried over anhydrous Sodium sulphate and stored in refrigerator prior to analysis by Gas chromatography (GC) and Gas chromatography-mass spectroscopy (GC/MS).

Gas chromatography/Mass Spectroscopy: Analysis of the oil was carried out by a combination of capillary GC and GC-MS using Trace DSQ instrument of Thermo Fisher Scientific. The capillary column used for analysis was a Thermo Fisher Scientific TR-5MS 30m × 0.25 mmID × 0.25 μm. The initial oven temperature was held at 50⁰C for 1 min, with maximum temperature 350⁰C, ion source 200, split row 50 ml/min and split ratio 10; carrier gas helium at a flow rate of 30 cm³/s. The injector and detector (FID) temperature were maintained at 280⁰C. For GC/MS analysis, a quadruple mass analyser with electron impact ionization (EI) system was used. The mass spectra acquired was in the range m/z 50-650, with retention time 45 min. The percentage composition of the oil was calculated from electronic integration measurements using FID detection without response factor correction. Linear retention indices of the components were determined relative to n-alkanes. The constituents of the oil were identified by matching their mass spectra and retention indices using NIST library search facility available with the instrument along with Wiley library spectra and literature (Kovats E. 1965 and Adams RP. 1995). Herein, a total of 52 compounds were identified in the essential oil.

Evaluation of antibacterial activities of essential oil

Antibacterial activity of essential oil of *M. integerrimum* was determined by Agar well diffusion method (Balouiri *et al.* 2016). The Agar well diffusion methods was employed to determine the diameters of inhibition zones of essential oil of *M. integerrimum* (vide table **S2**) against the eight tested pathogenic microorganisms viz. *Staphylococcus aureus* (MTCC 96), *Escherichia coli*

(MTCC 739), *Pseudomonas aeruginosa* (MTCC 2453), *Bacillus subtilis* (MTCC 441), *Bacillus spizizenii* (ATCC 6633), *Corynebacterium diphtheria* (ATCC 13812), *Pseudomonas aeruginosa* (MTCC 2453), *Pseudomonas putida* (ATCC 49128). All test pathogens were grown overnight in Muller Hinton broth (Anonymous 1996). The Muller Hinton agar plate surface was inoculated spreading a volume of the test pathogen inoculums over the entire agar surface. Then holes were made in a diameter of 6mm punched aseptically with a sterilised borer in the seeded agar plates. Ten hundred microliters (100 μ L) of the extracted essential oil and same volume of negative control were filled to the wells with the help of micropipette. Neomycin was used as positive control for both gram positive and gram negative test pathogens. All culture plates were incubated for 18 to 24 hrs at 37⁰C. After incubation, the plates were observed for zone of inhibition, the diameters of the inhibition zones were measured in millimetres and recorded. Each test was carried out in triplicate.

Table S1: Chemical composition of the essential oil of *M.integerrimum* fruit

Sl. No.	Constituents	^a RI	^b RT	Area %	^c Identification	Mol formula	Mol. Weight	M ⁺ m/z
1.	α -Thujene (1)	930	6.99	0.10	MS	C ₁₀ H ₁₆	136.23	136
2.	3-Methyl-cyclohexanone (2)	932	10.41	0.06	MS	C ₇ H ₁₂ O	112.17	112
3.	α -Pinene (3)	939	9.85	17.24	MS	C ₁₀ H ₁₆	136.23	136
4.	Camphene (4)	952	10.75	4.05	MS	C ₁₀ H ₁₆	136.23	136
5.	β -Pinene (5)	953	9.85	17.24	MS	C ₁₀ H ₁₆	136.23	136
6.	6-Methyl-heptan-2-ol (6)	955	18.96	0.75	MS	C ₈ H ₁₈ O	130.23	130
7.	β -Myrcene (7)	993	12.63	0.75	MS	C ₁₀ H ₁₆	136.26	136
8.	α -phelladrene (8)	1002	19.47	0.05	MS	C ₁₀ H ₁₆	136.23	136
9.	α -Terpinene (9)	1013	22.11	3.03	MS	C ₁₀ H ₁₆	136.23	136
10.	Limonene (10)	1029	10.75	2.05	MS	C ₁₀ H ₁₆	136	136
11.	1,8-Cineol (11)	1031	20.41	0.53	MS	C ₁₀ H ₁₈ O	154.25	154
12.	cis- β -Ocimene (12)	1035	16.38	3.20	MS	C ₁₀ H ₁₆	136.23	136
13.	β -Terpinene (13)	1038	16.65	0.29	MS	C ₁₀ H ₁₆	136.15	136

14.	Benzeneacetaldehyde (14)	1044	6.23	0.03	MS	C ₈ H ₈ O	120.15	120
15.	trans-β-Ocimene(15)	1048	11.26	0.16	MS	C ₁₀ H ₁₆	136.23	136
16.	Trans-Linalool oxide(furanoid) (16)	1074	16.65	0.29	MS	C ₁₀ H ₁₈ O ₂	170.30	170
17.	Terpinolene (17)	1086	12.71	32.21	MS	C ₁₀ H ₁₆	136.23	136
18.	Perillene (18)	1090	9.25	0.12	MS	C ₁₀ H ₁₄ O	150.22	150
19.	p-Cymene (19)	1091	19.88	0.21	MS	C ₁₀ H ₁₄	134.22	134
20.	cis-Sabinene hydrate (20)	1097	17.23	0.03	MS	C ₁₀ H ₁₈ O	154.25	154
21.	Linalool (21)	1098	8.26	0.20	MS	C ₁₀ H ₁₈ O	154.25	154
22.	trans-p-Mentha-2,8-dien-1-ol (22)	1118	8.26	0.40	MS	C ₁₀ H ₁₆ O	152.23	152
23.	cis-p-Mentha-2,8-dien-1-ol (23)	1123	12.15	0.55	MS	C ₁₀ H ₁₆ O	152.26	152
24.	Citronellal (24)	1158	17.54	0.06	MS	C ₁₀ H ₁₈ O	154.25	154
25.	Iso-Pulegyl acetate (25)	1166	15.06	1.09	MS	C ₁₂ H ₂₀ O ₂	196.29	196
26.	9-octadecenoic acid (26)	1192	2.77	0.77	MS	C ₁₈ H ₃₄ O ₂	282.5	282
27.	z-Cinnamaldehyde (27)	1210	18.47	0.12	MS	C ₉ H ₈ O	132.16	132
28.	trans-Carveol (28)	1217	18.47	0.12	MS	C ₁₀ H ₁₆ O	152.12	152
29.	Nerol (29)	1228	18.30	0.11	MS	C ₁₀ H ₁₈ O	154.25	154
30.	exo-Fenchyl acetate (30)	1230	17.90	0.13	MS	C ₁₀ H ₂₀ O ₂	196.29	196
31.	Neral (31)	1245	22.11	3.03	MS	C ₁₀ H ₁₆ O	152.23	152
32.	7-diethoxyethyl bicyclo[3.2.0]heptan-2-one (32)	1248	15.72	3.05	MS	C ₁₃ H ₂₂ O ₃	226.3	226
33.	Geraniol (33)	1255	19.47	0.05	MS	C ₁₀ H ₁₈ O	154.25	154
34.	Bornyl acetate (34)	1265	15.88	0.39	MS	C ₁₂ H ₂₀ O ₂	196.29	196
35.	Geranial (35)	1275	18.54	0.33	MS	C ₁₀ H ₁₆ O	152.23	152
36.	Methyl nerolate (36)	1279	15.19	0.44	MS	C ₁₁ H ₁₈ O ₂	182.26	182
37.	β-Elemene (37)	1280	22.49	0.13	MS	C ₁₅ H ₂₄	204.35	204
38.	Neo-iso-Isopulegyl acetate (38)	1321	12.42	0.74	MS	C ₁₂ H ₂₀ O ₂	196.29	196
39.	Methyl geranate (39)	1322	15.54	0.74	MS	C ₁₁ H ₁₈ O ₂	182.26	182

40.	α -Terpinyl acetate (40)	1328	11.63	0.03	MS	C ₁₂ H ₂₀ O ₂	196.29	196
41.	E-Hasmigone (41)	1334	19.10	0.44	MS	C ₁₁ H ₂₀ O	166.26	166
42.	Neryl acetate (42)	1345	16.08	0.96	MS	C ₁₂ H ₂₀ O ₂	196.29	196
43.	α -Copaene (43)	1376	14.12	0.46	MS	C ₁₅ H ₂₄	204.38	204
44.	Dodecanal (44)	1405	12.73	1.30	MS	C ₁₂ H ₂₄ O	184	185
45.	β -Caryophyllene (45)	1418	19.96	0.33	MS	C ₁₅ H ₂₄	204.35	204
46.	γ -Muurolene (46)	1449	33.19	0.76	MS	C ₁₅ H ₂₄	204.35	204
47.	α -Caryophyllene (47)	1454	17.90	0.13	MS	C ₁₅ H ₂₄	204.38	204
48.	α -Acoradiene (48)	1463	12.15	0.55	MS	C ₁₅ H ₂₄	204.35	204
49.	Trans-Cadina-1(6),4-diene (49)	1475	11.89	0.01	MS	C ₁₅ H ₂₄	204.35	204
50.	Germacrene D (50)	1480	13.76	0.10	MS	C ₁₅ H ₂₈	208.35	208
51.	cis- β -Guaiene (51)	1490	13.90	0.01	MS	C ₁₅ H ₂₄	204.35	204
52.	Caryophyllene oxide (52)	1581	17.54	0.06	MS	C ₁₅ H ₂₄ O	220.18	220
				99.98				

^aRI: Retention indices, ^bRT: Retention time determined on a Thermo Fisher Scientific TR-5MS 30m \times 0.25 mm ID \times 0.25 μ m with reference to n-alkanes, ^cMS: Identification by GC-mass spectrometry analysis, using NIST and Wiley library spectra and literature studies.

Table S2: The diameter of inhibition zones and MIC of *Micromelum* essential oil

Tested microorganism	Inhibition zones* (mm)		MIC of essential oil (µL)
	Essential oil	Neomycin	
<i>Bacillus subtilis</i> MTCC 441	16.3 ± 1.1	22.3 ± 1.1	50
<i>Bacillus spizizenii</i> ATCC6633	15.5 ± 0.9	22.1 ± 0.7	50
<i>Corynebacterium diphtheria</i> ATCC13812	10.2 ± 0.7	18.5 ± 0.4	60
<i>Escherichia coli</i> MTCC739	7.0 ± 0.2	20.6 ± 0.6	> 100
<i>Klebsiella pneumonia</i> ATCC13882	7.1 ± 0.4	21.2 ± 1.2	> 100
<i>Micrococcus luteus</i> ATCC 10240	9.2 ± 0.7	20.0 ± 0.1	80
<i>Pseudomonas aeruginosa</i> MTCC 2453	10.6 ± 0.4	16.5 ± 0.3	70
<i>Pseudomonas putida</i> ATCC 49128	9.4 ± 0.6	15.1 ± 1.5	80
<i>Staphylococcus aureus</i> 96	7.2 ± 0.2	18.7 ± 0.9	> 100

*Includes diameter of agar well (6mm). An inhibition zones 7mm means that the compounds are inactive against the investigated bacterium. For all tests 100µl of essential oil were applied. Neomycin was used in 2mg/ml.

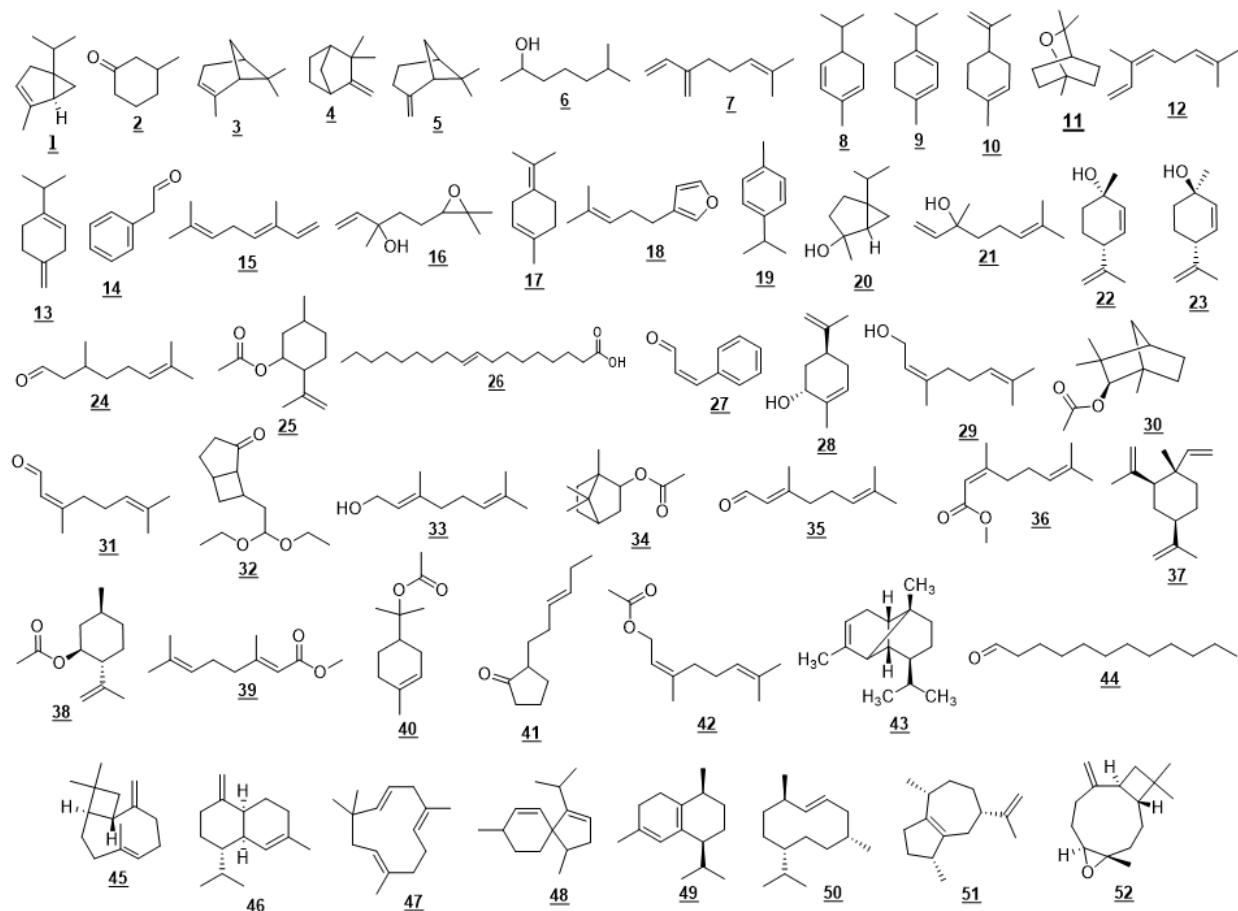


Figure S1: Chemical structures of fifty two molecules found from essential oil of *Micromelum integrimum*

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