Anti-Arthritic Effects of the Essential Oils of Turmeric (*Curcuma longa* L.)

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SUPPORTING INFORMATION

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Deleted: [†]Department of Medicine, University of Arizona, Tucson, AZ 85724, and [‡]Department of Medicinal Chemistry, School of Pharmacy, University of Kansas, Lawrence, KS 66045, USA¶ ¶ ¶ ¶ ¶ To whom correspondence should be addressed:¶ Janet L. Funk, MD¶ Tele: (520) 626-3242¶ Fax: (520) 626-3644¶ Email: ¶
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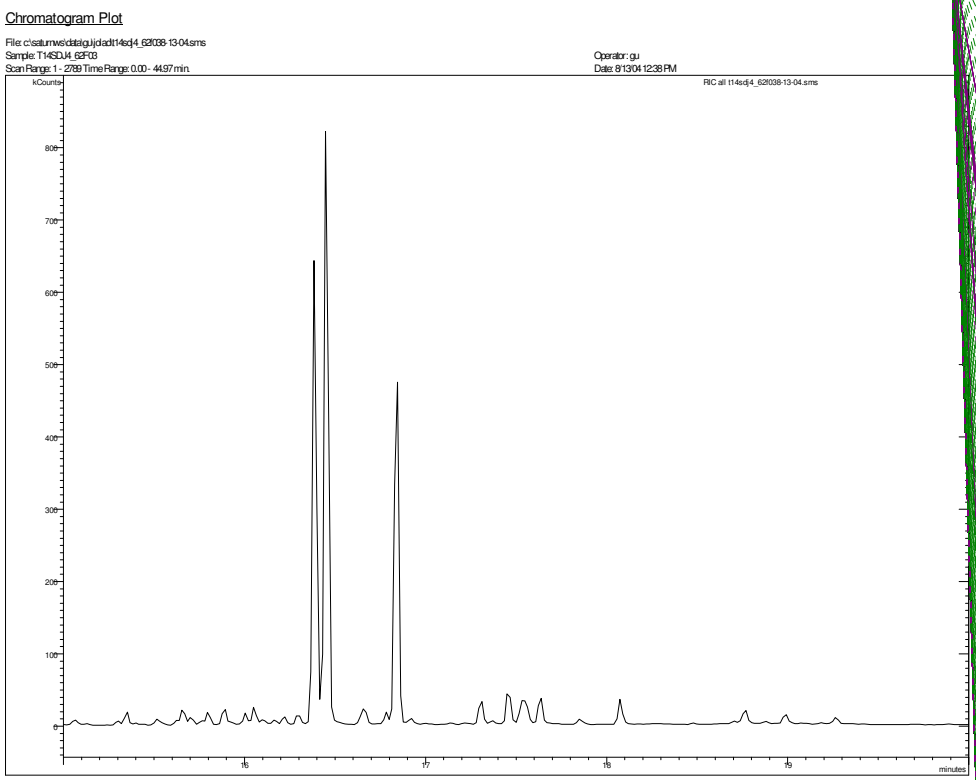
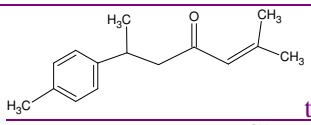
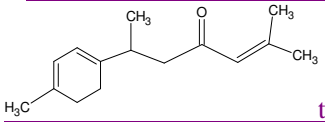
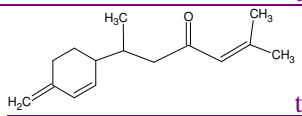




the first peak (R_t=16.384 min, MW 216), $C_{15}H_{20}O$, ar-turmerone



the second peak (R_t=16.448 min, MW 218), $C_{15}H_{22}O$, α-turmerone



the third peak (R_t=16.846 min, MW 218), $C_{15}H_{22}O$, β-turmerone

Figure S1 A GC profile of the refined turmeric oil extract, the three main peaks are identified as ar-turmerone, α-turmerone, and β-turmerone, respectively.

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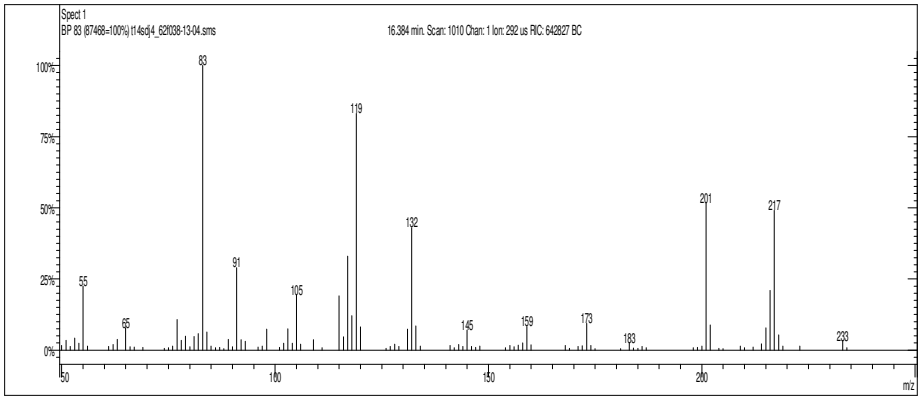

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Ion	Int	%BP	Ion	Int	%BP	Ion	Int	%BP	Ion	Int	%BP
50	1455	2	87	851	1	129	1104	1	175	510	1
51	2983	3	88	513	1	131	6490	7	181	513	1
52	1113	1	89	3338	4	132	37667	43	183	2217	3
53	3669	4	90	1027	1	133	7431	8	184	683	1
54	2136	2	91	25385	29	134	1276	1	185	593	1
55	19634	22	92	3248	4	141	1452	2	186	1107	1
56	1228	1	93	2718	3	142	768	1	187	767	1
61	1113	1	96	932	1	143	1704	2	198	769	1
62	1709	2	97	1269	1	144	1196	1	199	851	1
63	3332	4	98	6418	7	145	6151	7	200	1284	1
65	6828	8	101	855	1	146	1108	1	201	45283	52
66	1021	1	102	2140	2	147	846	1	202	7703	9
67	995	1	103	6585	8	148	1280	1	204	599	1
69	815	1	104	2135	2	154	770	1	205	512	1
74	593	1	105	17014	19	155	1455	2	209	1271	1
75	768	1	106	1791	2	156	1025	1	210	769	1
76	1278	1	109	3235	4	157	1536	2	212	940	1
77	9378	11	111	762	1	158	2225	3	214	1966	2
78	2985	3	115	16677	19	159	7255	8	215	6846	8
79	4256	5	116	4104	5	160	1625	2	216	18407	21
80	1024	1	117	28844	33	168	1453	2	217	42975	49
81	4167	5	118	10615	12	169	598	1	218	4707	5
82	5124	6	119	72756	83	171	1196	1	219	1284	1
83	87468	100	120	7188	8	172	1369	2	223	1198	1
84	5558	6	126	599	1	173	8215	9	233	2910	3
85	1272	1	127	1197	1	174	1454	2	234	770	1
86	767	1	128	1879	2						

Figure S1_B GC-MS fragments of the peak one with retention time 16.384 min

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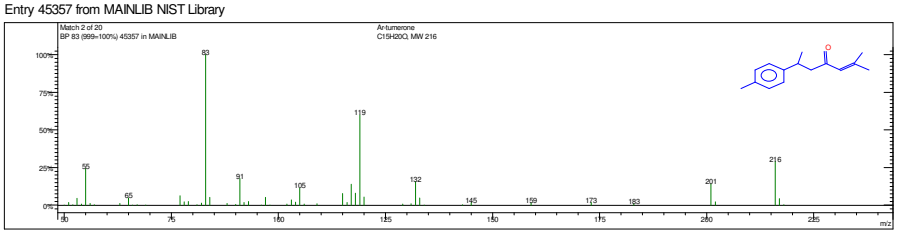
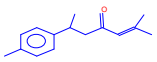
Entry 45357 from MAINLIB NIST Library



Spectrum 45357 from MAINLIB Library
Name: Ar-turmerone
Pair Count: 59 MW: 216 Formula: C15H20O CAS No: None Acquired Range: 39 - 218
NIST No: 292710
Contributor: R.I-Hiserodt ET AL. J.Chrom. 740,51(1996)
Synonyms: None
Other Databases: None
Replicates: None

Ion	Int.	%BP	Ion	Int.	%BP	Ion	Int.	%BP
...	...		83	999	100	118	79	8
50	1	0	84	52	5	119	597	60
51	19	2	88	12	1	120	53	5
52	3	0	90	6	1	129	7	1
53	45	5	91	177	18	131	10	1
54	7	1	92	18	2	132	157	16
55	243	24	93	25	3	133	48	5
56	11	1	96	1	0	134	1	0
57	2	0	97	52	5	143	2	0
63	12	1	98	1	0	145	17	2
65	47	5	102	8	1	159	16	2
66	1	0	103	35	4	173	17	2
67	3	0	104	21	2	183	9	1
69	1	0	105	112	11	201	141	14
77	62	6	106	7	1	202	22	2
78	22	2	109	9	1	216	290	29
79	24	2	115	78	8	217	44	4
81	4	0	116	18	2	218	3	0
82	13	1	117	140	14			



GC-MS data of ar-turmerone from NIST08 library

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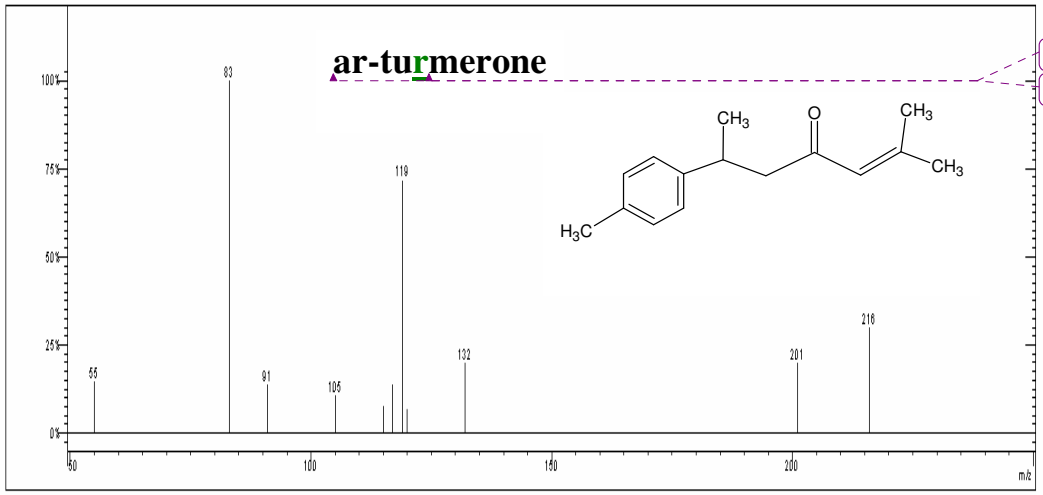

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GC-MS data of ar-turmerone from reference 23

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Figure S1 C GC-MS fragments of ar-turmerone in the NIST08 library and in reference 23

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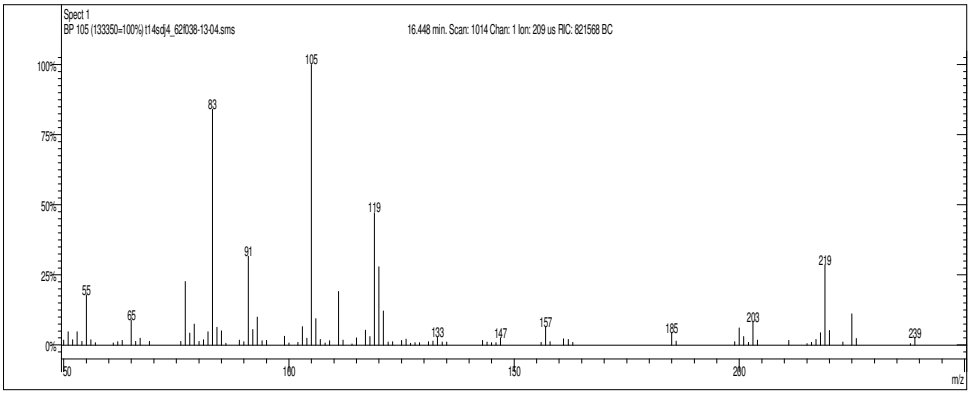

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Pair Count: 95 MW: 0 Formula: None CAS No: None Acquired Range: 50 - 650

Ion	Int	%BP	Ion	Int	%BP	Ion	Int	%BP	Ion	Int	%BP
50	2272	2	85	6806	5	118	4065	3	161	2977	2
51	6326	5	86	834	1	119	62901	47	162	2628	2
52	2511	2	89	2271	2	120	37197	28	163	1313	1
53	6327	5	90	1555	1	121	16128	12	185	5616	4
54	1670	1	91	42301	32	122	1428	1	186	1907	1
55	23746	18	92	7411	6	123	1542	1	199	1550	1
56	2491	2	93	13376	10	125	2151	2	200	8133	6
57	1188	1	94	2024	2	126	2751	2	201	4058	3
61	956	1	95	2248	2	127	836	1	202	1313	1
62	1552	1	99	4186	3	128	1192	1	203	10884	8
63	2265	2	100	956	1	129	1067	1	204	2272	2
65	11701	9	102	1315	1	131	1539	1	211	2265	2
66	1668	1	103	8725	7	132	1909	1	215	714	1
67	3317	2	104	3224	2	133	3571	3	216	1315	1
69	1753	1	105	133350	100	134	1427	1	217	2627	2
76	1788	1	106	12553	9	135	1419	1	218	5860	4
77	30224	23	107	2614	2	143	2145	2	219	38157	29
78	5730	4	108	949	1	144	1433	1	220	6937	5
79	10023	8	109	2015	2	145	1183	1	223	1435	1
80	1671	1	111	25470	19	146	1071	1	225	14829	11
81	2483	2	112	2272	2	147	3339	3	226	3110	2
82	6327	5	114	597	0	156	1313	1	238	717	1
83	112168	84	115	3450	3	157	8368	6	239	3229	2
84	8485	6	117	7049	5	158	1554	1			

Figure S1 D GC-MS fragments of the peak two with retention time 16.448 min

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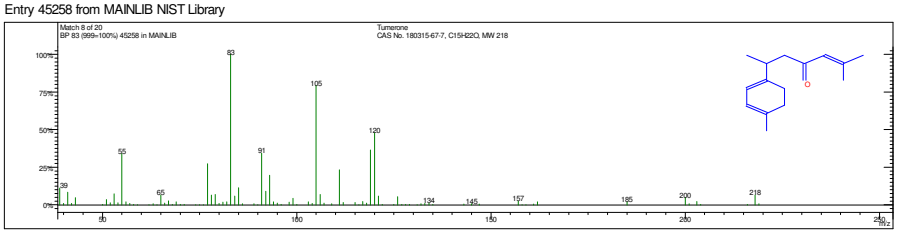
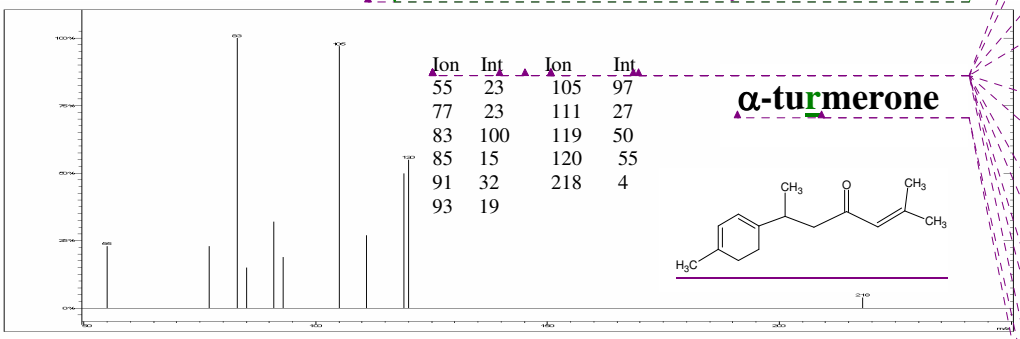
Entry 45258 from MAINLIB NIST Library



Spectrum 45258 from MAINLIB Library
Name: Turmerone
Pair Count: 91 MW: 218 Formula: C15H22O CAS No: 180315-67-7 Acquired Range: 39 - 219
NIST No: 292711
Contributor: R.I-Hisarcott ET AL. J.Chrom. 740,51(1996)
Synonyms: 1
2-Methyl-6-(4-methyl-1,3-cyclohexadien-1-yl)-2-hepten-4-one #
Other Databases: None
Replicates: None

Ion	Int	%BP	Ion	Int	%BP	Ion	Int	%BP
39	112	11	80	9	1	119	365	37
40	9	1	81	18	2	120	476	48
41	84	8	82	21	2	121	58	6
42	10	1	83	999	100	122	2	0
43	48	5	84	59	6	125	3	0
50	3	0	85	114	11	126	53	5
51	35	4	86	7	1	127	3	0
52	13	1	89	7	1	128	3	0
53	73	7	90	1	0	129	3	0
54	14	1	91	344	34	131	1	0
55	340	34	92	90	9	132	8	1
56	21	2	93	197	20	133	9	1
57	10	1	94	20	2	134	11	1
58	2	0	95	12	1	135	3	0
59	1	0	96	3	0	143	5	1
62	1	0	98	18	2	145	7	1
63	8	1	99	44	4	147	5	1
64	1	0	100	3	0	157	25	3
65	65	7	103	21	2	158	1	0
66	11	1	104	10	1	159	1	0
67	27	3	105	793	79	161	5	1
68	3	0	106	68	7	162	20	2
69	21	2	107	11	1	185	20	2
70	4	0	109	8	1	200	49	5
71	3	0	111	232	23	201	7	1
74	1	0	112	15	2	203	23	2
75	1	0	115	15	2	204	3	0
76	1	0	116	1	0	216	2	0
77	273	27	117	23	2	218	64	6
78	64	6	118	12	1	219	8	1
79	69	7						

GC-MS Data of α-turmerone from NIST08 library



GC-MS data of α-turmerone from reference 23

Figure S1 E GC-MS fragments of α-turmerone in the NIST08 library and in literarute

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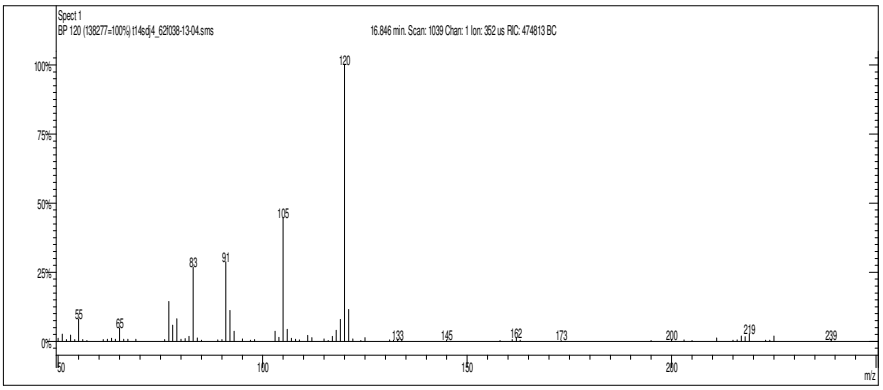

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Pair Count: 77 MW: 0 Formula: None CAS No: None Acquired Range: 50 - 650

Ion	Int.	%BP	Ion	Int.	%BP	Ion	Int.	%BP	Ion	Int.	%BP
50	1491	1	80	1132	1	108	1056	1	158	425	0
51	3680	3	81	1461	1	109	691	0	161	838	1
52	994	1	82	2473	2	111	3043	2	162	1488	1
53	3183	2	83	37109	27	112	1846	1	163	636	0
54	919	1	84	1768	1	115	1187	1	173	421	0
55	11163	8	85	556	0	116	491	0	195	424	0
56	855	1	89	709	1	117	2547	2	200	923	1
57	441	0	90	923	1	118	5538	4	203	780	1
61	852	1	91	39799	29	119	11061	8	205	425	0
62	1062	1	92	15547	11	120	138277	100	211	1767	1
63	1484	1	93	5020	4	121	15958	12	215	636	0
64	1136	1	95	1181	1	122	1199	1	216	781	1
65	6726	5	97	623	0	124	491	0	217	2693	2
66	1129	1	98	920	1	125	1843	1	218	2413	2
67	1103	1	103	5106	4	131	693	1	219	3764	3
69	1024	1	104	2053	1	132	563	0	223	568	0
76	988	1	105	61764	45	133	762	1	224	568	0
77	19916	14	106	6029	4	134	559	0	225	2624	2
78	8084	6	107	1544	1	145	697	1	239	852	1
79	11338	8									

Figure S1 F GC-MS fragments of the peak three with retention time 16.846 min

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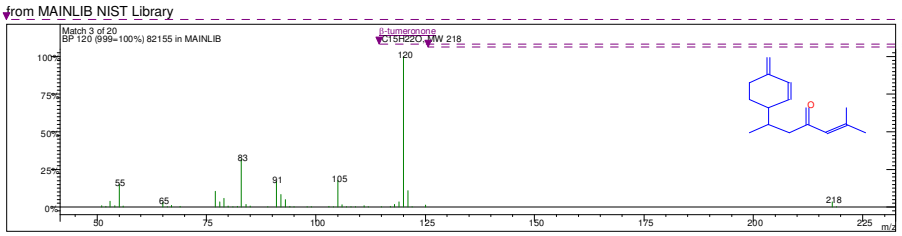
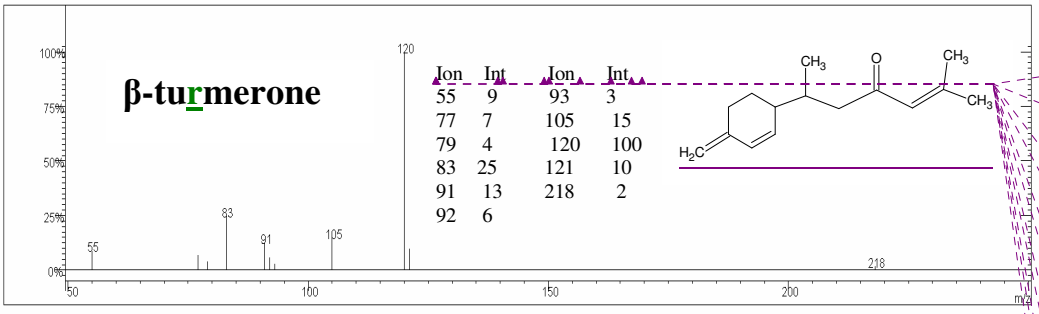


Name: β-turmerone
Pair Count: 48 MW: 218 Formula: C15H22O

Ion	Int	%BP	Ion	Int	%BP	Ion	Int	%BP
...	...		82	6	1	107	3	0
51	10	1	83	317	32	108	3	0
52	6	1	84	19	2	109	3	0
53	38	4	85	7	1	111	10	1
54	10	1	89	2	0	112	4	0
55	152	15	91	171	17	115	4	0
56	7	1	92	84	8	117	6	1
65	33	3	93	50	5	118	17	2
66	4	0	94	6	1	119	34	3
67	11	1	95	3	0	120	999	100
69	6	1	98	4	0	121	109	11
77	103	10	99	3	0	122	3	0
78	35	4	103	6	1	125	12	1
79	59	6	104	3	0	218	35	4
80	7	1	105	177	18	219	3	0
81	3	0	106	14	1			

GC-MS Data of β-turmerone from NIST08 library



GC-MS data of β-turmerone from reference 23

Figure S1 G GC-MS fragments of β-turmerone in the NIST08 library and in literature

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FIGURE S2

Figure S2. HPLC-UV profile of refined TEO. The three major compounds have strong absorption (>1250 mA in λ=250 nm), implying they are the main components and have conjugated functional groups such as  in turmerones. These three compounds are β-turmerone, α-turmerone, and ar-turmerone, respectively, consistent with the GC-MS results such as the ar-turmerone has strong absorption (280 nm). There are no curcuminoids in the refined TEO.

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July 20, 2009

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ABSTRACT

Turmeric (*Curcuma longa* L., Zingiberaceae) rhizomes contain two classes of secondary metabolites, curcuminoids and the less well-studied essential oils.

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Having previously identified potent anti-arthritic effects of the curcuminoids in turmeric extracts in an animal model of rheumatoid arthritis (RA), studies were undertaken to determine whether the turmeric essential oils (TEO) were also joint protective using the same experimental model. Crude or refined TEO extracts dramatically inhibited joint swelling (90-100% inhibition) in female rats with streptococcal cell wall (SCW)-induced arthritis when extracts were administered via intraperitoneal injection to maximize uniform delivery. However, this anti-arthritic effect was accompanied by significant morbidity and mortality. Oral administration of a 20-fold higher dose TEO was non-toxic, but only mildly joint-protective (20% inhibition). These results do not support the isolated use of TEO for arthritis treatment, but, instead, identify potential safety concerns in vertebrates exposed to TEO.

Key Words: *Curcuma longa* L.; turmeric; essential oil; arthritis

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INTRODUCTION

Essential oils are complex mixtures of volatile terpenes selected throughout evolution to protect plants from external threats by various means including mimicking endogenous substrates in herbivores (*1*). While biologic and potentially medicinal effects of these essential oils are thus to be anticipated, these compounds are often less well studied than other classes of secondary plant metabolites, with polyphenols being a prominent example. Such is the case for turmeric (*Curcuma longa* L., Zingiberaceae), a medicinal botanical whose rhizome contains two major classes of secondary metabolites, the phenolic curcuminoids and the hydrophobic essential oils (*2*). Many laboratories, including our own (*3*, *4*), have demonstrated potent and physiologically important effects of the curcuminoids (*5*). For example, we have elucidated a profound anti-arthritic effect of curcuminoid-containing turmeric extracts due to their ability to inhibit

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nuclear factor-κB (NF-κB) activation in experimental rheumatoid arthritis, thus blocking multiple downstream signaling pathways critical to joint inflammation, including cyclooxygenase (COX)-stimulated prostaglandin-E_2 (PGE_2) production (*3*). Far less studied, and indeed, often discarded in the preparation of turmeric dietary supplements (AR), are turmeric's essential oils.

Reported biologic properties of the multi-component essential oils of turmeric include antifungal (*6*), mosquitocidal (*7*), antivenom (*8*), antibacterial and antioxidant properties (*9*, *10*).

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Medicinal effects of TEO in vertebrates have also been reported for stroke and diabetes: a single acute dose of TEO (250-500 mg/kg oral or ip) was neuroprotective in rats subjected to occlusive or embolic strokes (*11-13*), while chronic TEO dietary supplementation (≥620 mg/kg/d) normalized serum glucose in diabetic mice (*14*, *15*).

Major compounds present in turmeric essential oils (TEO) include ar-turmerone, α-turmerone and β-turmerone. The relative proportions of these terpenoids in TEO extracts vary depending on the rhizome's geographic origin and method of extraction (*9*,

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In isolation, these compounds have demonstrated bioactivity *in vitro*, including cytotoxic and apoptotic effects in various cells (*18*, *19*); binding to the nuclear receptor, peroxisome proliferator-activated receptor γ (PPAR-γ)

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(*14*); inhibition of inducible PGE_2 and NO production (*2*, *20*, *21*); and inhibition of

platelet aggregation (*22*).

Emerging *in vitro* evidence from our laboratories and others suggests that crude, multi-component hexane extracts of TEO may be more bioactive than any of their individual fractions or components (*2*, *9*, *11*).

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For example, when our laboratories isolated and compared the efficacy of 8 distinct fractions of a crude TEO extract vs. the extract itself in blocking COX-mediated PGE_2 production *in vitro*, the potency of the crude TEO extract exceeded that of each of its fractions (*2*).

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Moreover, the crude essential oils had the same potency as turmeric's other secondary metabolite, the curcuminoids, in this *in vitro* screening assay.

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Thus, we have postulated that the essential oils of turmeric may also have significant medicinal anti-arthritic effects *in vivo*--independent of those attributed to tumeric's polyphenols—that result from synergistic actions of the oil's various constituents.

To test this postulate, *in vivo* studies described herein were undertaken to determine whether the essential oils of turmeric are indeed effective anti-arthritic agents using an animal model of rheumatoid arthritis (RA), streptococcal cell wall (SCW)-induced arthritis.

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to characterize anti-inflammatory properties of essential-oil free, curcuminoid-containing turmeric extracts (*3*, *4*), these experiments also allowed us to compare the relative *in vivo*

anti-inflammatory efficacy of each of turmeric's two major classes of secondary metabolites. Moreover,

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our isolation and *in vivo* testing of two complex TEO extracts, a crude hexane extract and a curcuminoid-stripped, refined fraction of the crude TEO extract, also allowed for clarification of the independent role of the essential oils (vs. curcuminoids) in preventing intra-articular inflammation.

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MATERIALS AND METHODS

Turmeric Essential Oil Preparation.

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Turmeric essential oil (TEO) extracts were isolated from dry turmeric rhizome powder as described in detail in United States Patent 7,205,011.

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In brief, a crude TEO extract was prepared from dry *Curcuma longa* L., Zingiberaceae rhizome powder (San Francisco Herb, SF, CA) by n-hexane extraction at room temperature for 24 hours.

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The resultant mixture was filtered, the filtrate was combined with an n-hexane wash of the marc, and the solvent was stripped under vacuum for 24 hours to obtain a crude turmeric oil extract (yield, 3.7%).

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HPLC analysis revealed minor curcuminoid contamination of this essential oil-enriched extract. The crude TEO was processed further by silca gel column chromatography.

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Elution was by the gravity method. The column was eluted with n-hexane (100%). Fractions (25-30 ml each) were collected until the color of the eluant changed from colorless to yellow to colorless again. The column was then eluted with a mixture of n-hexane

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and ethyl acetate (17:1 v/v) for a total of 15 L. The column was finally leached with 100% ethyl acetate (1 L) and elution was discontinued. The solvent from the 15 L fraction was stripped off under vacuum using a rotary evaporator, and the resulting refined TEO was then left under vacuum for a minimum of 24 hrs (66-74% yield, by weight).

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Chemical Analysis of Turmeric Essential Oil Extract

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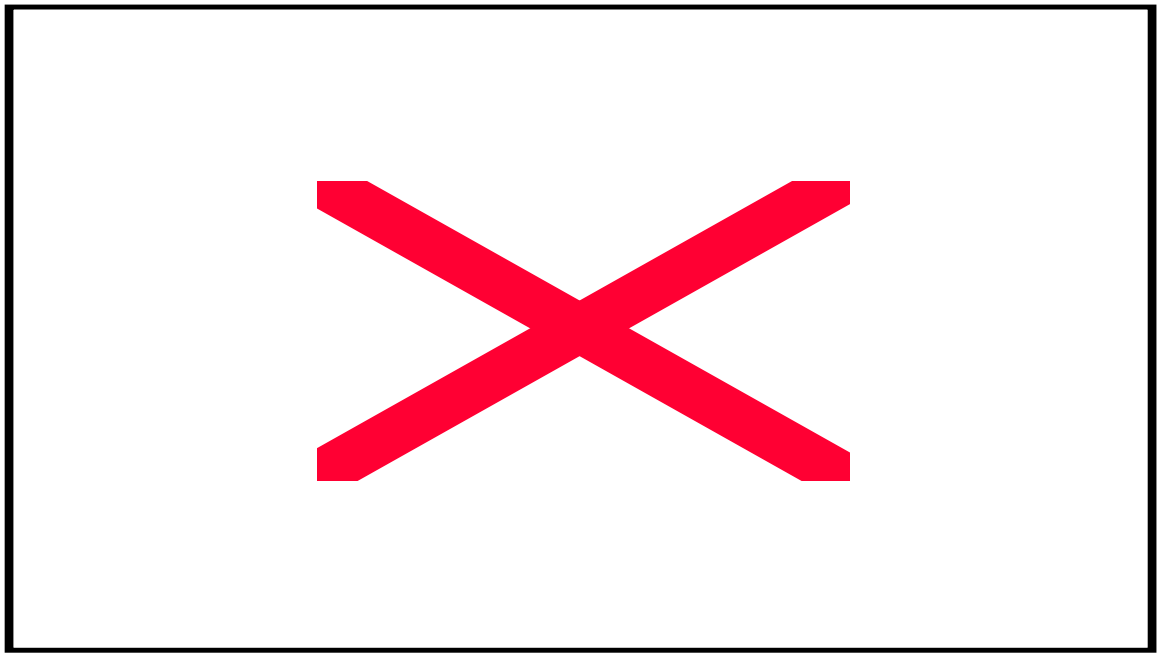

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