Chemical Composition of Turmeric Oil – A Byproduct from Turmeric Oleoresin Industry and Its Inhibitory Activity against Different Fungi

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Turmeric Oil, Aromatic Turmerone, Antifungal Activity

Curcumin, the yellow coloring pigment of turmeric is produced industrially from turmeric oleoresin. The mother liquor after isolation of curcumin from oleoresin known as curcumin removed turmeric oleoresin (CRTO) was extracted three times with *n*-hexane at room temperature for 30 min to obtain turmeric oil. The turmeric oil was subjected to fractional distillation under vacuum to get two fractions. These fractions were tested for antifugal activity against *Aspergillus flavus, A. parasiticus, Fusarium moniliforme* and *Penicillium digitatum* by spore germination method. Fraction II was found to be more active. The chemical constituents of turmeric oil, fraction I and fraction II were determined by GC and identified by GC-MS. Aromatic turmerone, turmerone and curlone were major compounds present in fraction II along with other oxygenated compounds.

Introduction

Turmeric is an essential coloring spice of Indian and other cuisines. Its medicinal value has been known since long and its use as a cure for hypercholesterolemia, arthritis, indigestion and liver problems is common (Srimal, 1997). Aromatic turmerone (20–30%) was reported as the major compound present in *Curcuma longa* volatile oil (Govindarajan, 1980). Synthetic turmerone is reported to act as neoplasm inhibitor and anticarcinogenic (Baik *et al.,* 1993) and synthetic turmeronol A as soybean lipoxygenase inhibitor (Kitahara *et al.,* 1993). Antivenom activity of turmerone isolated from *Curcuma longa* has been also reported. (Ferreira *et al.,* 1992). Turmerone is also used in insect repellent coatings (Whalon *et al.,* 1998; Helen *et al.,* 1982). Recently, antifungal and mosquitocidal activities of turmeric leaf extracts were reported (Roth *et al.,* 1998). Curcumin, the yellow coloring pigment of turmeric is industrially produced using oleoresin of turmeric as the starting material. The mother liquor (approximately 70–80%) after isolation of curcumin from oleoresin is known as "curcumin removed turmeric oleoresin" (CRTO). It has a composition of oil, resin and unisolable curcumin and this has no commercial value at present. In this communication, we report the isolation and identification of antifungal fractions from this byproduct and their antifungal activity.

Materials and Methods

Materials

All chemicals and solvents used were of AR grade. CRTO was obtained from M/S Flavour and Essences (P) Ltd, Mysore.

Extraction of turmeric oil

Fifty grams of CRTO was extracted three times with *n*-hexane (200 ml each) for 30 min at room temperature. The extracts were pooled and the solvent was removed under vacuum (Büchi, Switzerland) which gave 20 g of turmeric oil.

Fractionation of turmeric oil by vacuum distillation

Turmeric oil (20 g) was subjected to vacuum distillation. The fraction I was collected at 80–110 °C with 10–12% yield. Similarly, the fraction II was collected at 110–120 °C with a 15–17% yield.

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GC Analysis

The GC analysis of turmeric oil and its fractions were carried out using a Shimadzu GC 15A chromatograph equipped with a FID detector and SE-30 column (3.0 m × 0.5 mm i.d.). The oven temperature was programmed as 75 °C for 2 min, increased to 220 °C at the rate of 2 °C/min and at this temperature the column was maintained for 3 min. The injector port and detector temperature were 250 °C. Nitrogen was used as the carrier gas at a flow rate of 30 ml/min. Peak areas were computed by a Shimadzu C-R4A chromatopak data processor.

GC-MS Analysis

Turmeric oil and its fractions were analyzed using a Shimadzu 17A-GC chromatograph equipped with a QP-5000 (quadrapole) mass spectrometer. The turmeric oil, fraction I and fraction II were diluted 25 times with acetone and 1 µl was injected. A fused silica column SPB^{TM-1} (30 m × 0.32 mm I. D., film thickness 0.25 µm) coated with polydimethylsiloxane was used. Helium was the carrier gas at a flow rate of 1 ml/min. The injector port temperature and detector temperature were 250 °C. The oven temperature was programmed to 60 °C for 2 min, increased to 250 °C at a rate of 2 °C/min and at this temperature the column was maintained for 5 min. The split ratio was 1:25 and the ionization voltage 70 eV. Retention indices of all compounds were determined using *n*-alkanes as standards (Jennings and Shibamoto, 1980). The compounds were identified by comparison of Kovats indices with reported values and by matching with NIST-MS library (NIST62-Lib.) and published mass spectra (Adams, 1989; Davis, 1990; Hiserodt *et al.*, 1996).

Antifungal activity

Antifungal activity of turmeric oil and its fractions were tested against *Aspergillus flavus, A. parasiticus, Fusarium moniliforme,* and *Penicillium digitatum* obtained from the Department of Food Microbiology, CFTRI, Mysore, India. The fungal strains were maintained at 4 °C in potato dextrose agar. Strains were grown in potato dextrose agar at 26 °C for 5 days and antifungal activity was evaluated by spore germination method (Paster *et al.*, 1999). Spore suspension (10^3 spores/ml) of each fungus was prepared in sterile water and used for inoculation.

Test material and inoculation

Chloramphenicol rose bengal agar (Hi Media, Mumbai, India) was used for germination of fungal strains. To the flasks containing 20 ml sterile agar and different concentrations of test material diluted in propyleneglycol, 100 µl of spore suspension was inoculated aseptically. In case of control the equivalent amount of propyleneglycol was used. The number of spores germinated was counted at regular intervals and germination of spores was expressed as percentage of control.

Results and Discussion

The spore germination assay of *Aspergillus flavus, A. parasiticus, Fusarium moniliforme* and *Penicillium digitatum* against turmeric oil, fraction I and fraction II showed that fraction II was most effective as it inhibited germination of spores to a greater extent (Fig. 1). In case of *Penicillium digitatum and Aspergillus flavus* 1.5 and 6 mg/ml concentration of fraction II inhibited complete growth for 4 days. Similarly, complete growth inhibition by this fraction was up to 3 days in case of *A. parasiticus* and *Fusarium moniliforme* at 6 mg/ml concentration. Turmeric oil was less effective than fraction II and it was able to suppress complete growth up to 3 days in case of *Penicillium digitatum* and *A. parasiticus* at 3 and 6 mg/ml, respectively, and up to 2 days in case of *Aspergillus flavus* and *Fusarium moniliforme* at 3 mg/ml. Fraction I was least effective and was not able to suppress growth completely even at 6 mg/ml except for *Penicillium digitatum,* whose growth was suppressed completely for 3 days at 6 mg/ml concentration.

The chemical constituents of turmeric oil, fraction I and fraction II were determined by GC and identified by GC-MS. Table I shows chemical constituents of turmeric oil, fraction I and fraction II, and their retention time and Kovats indices. The data indicated the presence of twenty-five compounds in turmeric oil and fraction II. Aromatic turmerone (21.4%), α-zingiberene (15%), β-(*Z*)-farnesene (13.96%), aromatic curcumene (10.3%), turmerone (6.2%) and curlone (5.1%) were found to be the major compounds in turmeric oil. Frac-

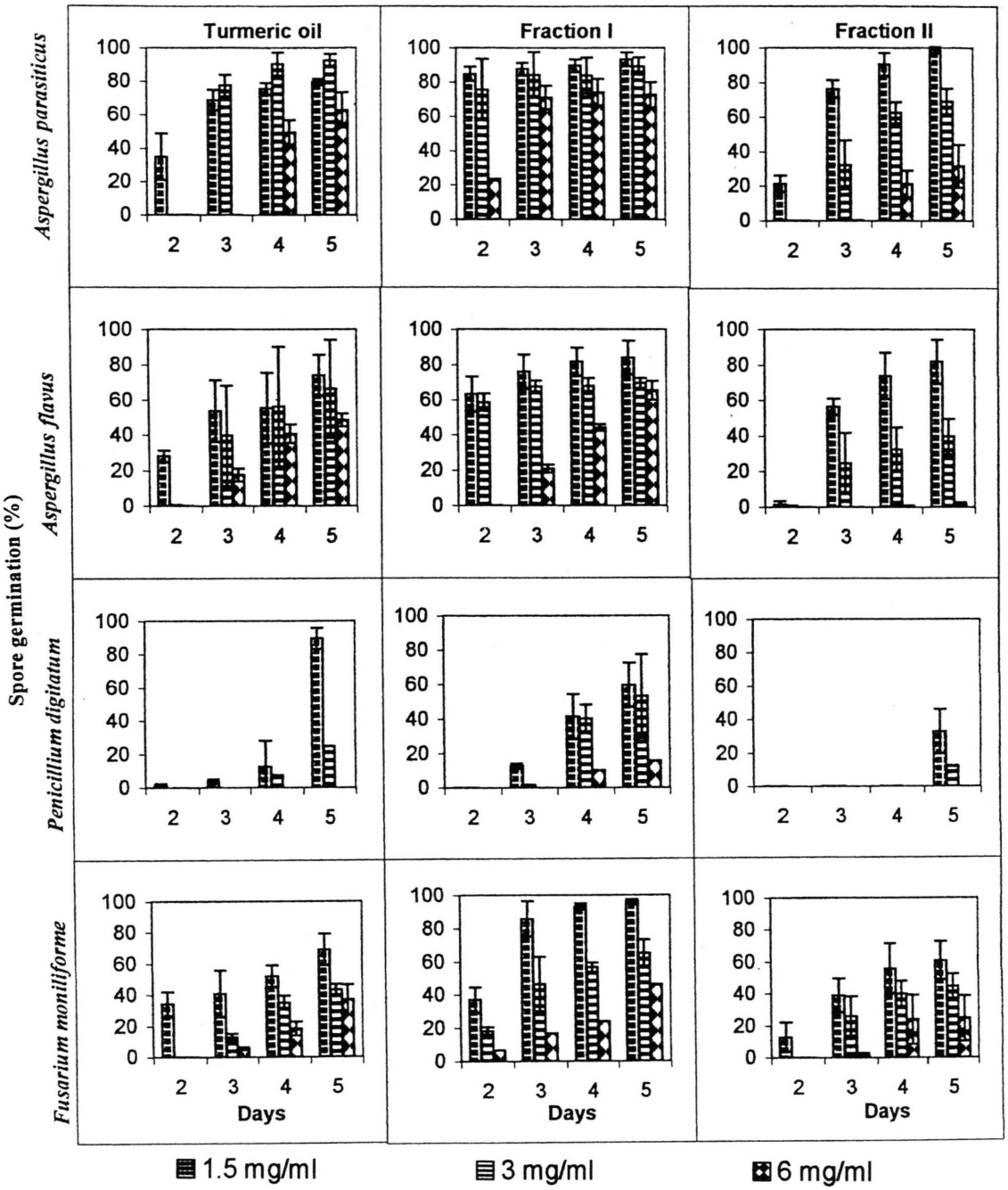

Fig. 1. Effect of turmeric oil, fraction I and fraction II on spore germination of different fungi.

tion I contained α-zingiberene (28%), β-(*Z*)-farnesene (21%), aromatic curcumene (18%) whereas fraction II contained aromatic turmerone (52.6%), turmerone (11.5%) and curlone (8.5%) as the major components. Further, oxygenated compounds (6–9) were enriched in fraction II (Fig. 2).

The comparison of chemical composition showed that the fraction II has high concentration

Table I. Chemical composition of turmeric oil, fraction I and fraction II.

Sl. No.	RT [min]	Compound	Peak areas (%)			KI	Identification method
			Turmeric oil	Fra.I	Fra. II		
1	5.8	α-phellandrene	0.42	0.92	Tr.	998	MS, KI
2	6.3	*p*-cymene	0.35	0.40	Tr.	1008	MS, KI
3	6.5	1,8-cineole	1.24	1.30	Tr.	1024	MS, KI
4	8.1	*trans*-ocimene	0.36	0.40	Tr.	1044	MS, KI
5	12.2	α-terpineol	0.22	0.30	Tr.	1149	MS, KI
6	22.2	α-*cis*-bergamotene	2.27	3.10	Tr.	1376	MS, KI
7	23.9	caryophyllene	Tr.	0.25	Tr.	1377	MS, KI
8	25.2	aromatic curcumene	10.30	18.0	3.02	1447	MS, KI
9	26.2	α-zingiberene	15.03	28.0	4.12	1467	MS, KI
10	26.4	β-bisabolene	3.55	5.50	1.22	1476	MS, KI
11	27.2	β-(*Z*)-farnesene	13.96	21.00	4.71	1493	MS, KI
12	27.7	bisabolene *cis*-γ	0.99	2.40	0.34	1500	MS, KI
13	27.8	caryophyllene oxide	1.07	1.50	Tr.	1555	MS, KI
14	29.1	aromatic turmerol	0.52	1.10	0.89	1570	MS, KI
15	29.7	dehydrocurcumene	2.31	3.00	1.00	1576	MS
16	30.9	compound (3)*	0.91	0.60	1.90	1589	MS
17	33.3	aromatic turmerone	21.40	3.50	52.60	1628	MS, KI
18	33.5	turmerone	6.20	1.00	11.50	1632	MS, KI
19	34.6	curlone	5.10	1.10	8.50	1655	MS, KI
20	37.3	compound (**6**)*	0.42	–	3.80	1710	MS
21	38.3	compound (**7**)*	Tr.	–	0.82	1733	MS
22	42.4	compound (**8**)*	Tr.	–	0.74	1862	MS
23	42.8	compound (**9**)*	Tr.	–	0.66	1867	MS
24	48.3	hexadecanoic acid	Tr.	–	0.73	1946	MS
25	56.6	heptadeconic acid	0.26	–	0.79	2124	MS

MS: Identification based on mass spectral data.
KI: Kovats indices on SPB column.
Tr.: less than 0.09%.
RT: Retention time
* : MS was compared with that of Hiserodt *et al.* (1996).

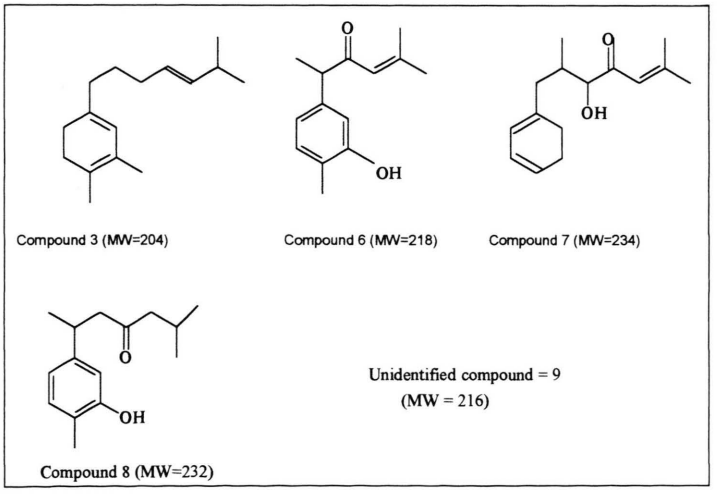

Fig. 2. Structures of compounds identified in turmeric oil.

of aromatic turmerone, turmerone, curlone and compounds (6–9) as compared to turmeric oil. High antifungal activity of fraction II may be attributed to the enrichment of these compounds. Aromatic turmerone has been implicated in many biological activities (Baik *et al.,* 1993; Ferreira *et al.,* 1992; Whalon *et al.,* 1998; Helen *et al.,* 1982; Roth *et al.,* 1998). Probably aromatic turmerone alone or in synergy with turmerone, curlone and compounds (6–9) is responsible for the higher antifungal activity of this fraction. Turmeric oil is less effective antifungal agent as compared to fraction II. Fraction I have the least concentration of aromatic turmerone and least antifungal activity. A positive correlation was observed between concentrations of *Aromatic* turmerone, turmerone, curlone and compounds (6–9) and antifungal activity.

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