Antibacterial Activity of Turmeric Oil: A Byproduct from Curcumin Manufacture

P. S. Negi, G. K. Jayaprakasha, L. Jagan Mohan Rao, and K. K. Sakariah*

Central Food Technological Research Institute, Mysore 570 013, India

Curcumin, the yellow color pigment of turmeric, is produced industrially from turmeric oleoresin. The mother liquor after isolation of curcumin from oleoresin contains approximately 40% oil. The oil was extracted from the mother liquor using hexane at 60 °C, and the hexane extract was separated into three fractions using silica gel column chromatography. These fractions were tested for antibacterial activity by pour plate method against *Bacillus cereus*, *Bacillus coagulans*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Fraction II eluted with 5% ethyl acetate in hexane was found to be most active fraction. The turmeric oil, fraction I, and fraction II were analyzed by GC and GC−MS. *ar*-Turmerone, turmerone, and curlone were found to be the major compounds present in these fractions along with other oxygenated compounds.

Keywords: *Curcuma longa L.; turmeric oil; ar-turmerone; turmerone; antibacterial activity*

INTRODUCTION

Turmeric (*Curcuma longa* L.) is a tropical herb of the Zingiberaceae family indigenous to southern Asia. Turmeric is used in foods as a condiment. It is also used as an essential ingrediant in medicine as a carminative, anthelmintic, laxative and as a cure for liver ailment (Srimal, 1997). The use of turmeric as an insect repellent (Watt and Bryer-Brandwijk, 1962) and antimicrobial activity of its extracts have long been known (Ammon and Wahl, 1991; Madhyasta and Bhat, 1985; Dahl et al., 1989).

Curcumin, the yellow color pigment of turmeric, is industrially produced using oleoresin of turmeric as the raw material. The mother liquor (approximately 70−80%) after isolation of curcumin from oleoresin has a composition of oil, resin, and unisolable curcumin; this has no commercial value at the present. In this paper, we report the isolation and identifcation of the antibacterial fractions from the leftover turmeric oleoresin after isolation of curcumin. The active fraction was separated, enriched, and tested against a range of pathogenic and spoilage bacteria.

MATERIALS AND METHODS

Materials. All chemicals and solvents used were of AR grade. Mother liquor/curcumin removed turmeric oleoresin (CRTO) was collected from a local oleoresin industry.

Extraction of Turmeric Oil. CRTO (25 g) was extracted three times with hexane (200 mL) for 1 h each time at 60 °C. The extracts were pooled, and the solvent was removed under vacuum (Buchi, Switzerland), which gave 40% (w/w) yield of turmeric oil.

Fractionation of Turmeric Oil. Turmeric oil (5 g) was impregnated with 10 g of silica gel and loaded on to a silica gel column. The column was eluted successively with 1.0 L each of hexane, hexane:ethyl acetate [95:05], and ethyl acetate. The solvents from the eluates were evaporated under vacuum to get three fractions, the yields of which were 18%, 62%, and 19%, respectively.

GC Analysis. The GC analyses of turmeric oil and column fractions were done using a Shimadzu GC 15A chromatography equipped with a FID detector, using SE-30 column (3.0 m × 0.5 mm i.d.). Oven temperature was programmed from 75 °C for 2 min to 220 °C at the rate of 2 °C/min at which temperature the column was maintained for 3 min; injector port temperature was 200 °C; detector temperature was 250 °C; nitrogen as carrier gas was 30 mL/min. Peak areas were computed by a Shimadzu C-R4A chromatopak data processor.

GC−MS Analysis. Turmeric oil and column fractions were analyzed using a Shimadzu 17A-GC chromatograph equipped with a QP-5000 (quadrapole) mass spectrometer. 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 polydimethyl siloxane was used. Helium was the carrier gas at a flow rate of 1 mL/min; injector port temperature was 250 °C; detector temperature was 250 °C; oven temperature was maintained at 60 °C for 2 min and then increased to 250 °C at the rate of 2 °C/min at which temperature the column was maintained for 5 min; splitting ratio was 1:50; ionization voltage was 70 eV. Retention indices of all compounds were determined according to the Kovats method using *n*-alkanes as standards (Jennings and Shibamoto, 1980). The compounds were identified by comparison of Kovats indices and by matching with those of NIST-MS library and published mass spectra (Adams, 1989; Davis, 1990; Hiserodt et al., 1996).

Bacterial Cultures. Bacterial cultures, namely, *Bacillus cereus*, *B. coagulans*, *B. subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were obtained from the Department of Food Microbiology of this Institute and grown in nutrient agar media at 37 °C. Each bacterial strain was transferred from stored slants at 4−5 °C to 10 mL of nutrient broth and cultivated overnight at 37 °C. A preculture was prepared by transferring 1 mL of this culture to 9 mL of nutrient broth and cultivated for 48 h. The cells were harvested by centrifugation (4000 rpm, 5 min), washed, and suspended in saline.

Growth Inhibition Assay. Turmeric oil and the column fractions were tested for their effect on the growth of different bacteria by the method of Chen et al. (1998) with slight modification. To flasks containing 20 mL of melted nutrient agar different concentrations of test material in propylene glycol were added. In the case of the control, an equivalent amount of propylene glycol was added. One hundred microliters (about 10^3 cfu/mL) of each bacterium to be tested was inoculated into the flasks under aseptic conditions. The media

10.1021/jf990308d CCC: $18.00
Published on Web 09/24/1999

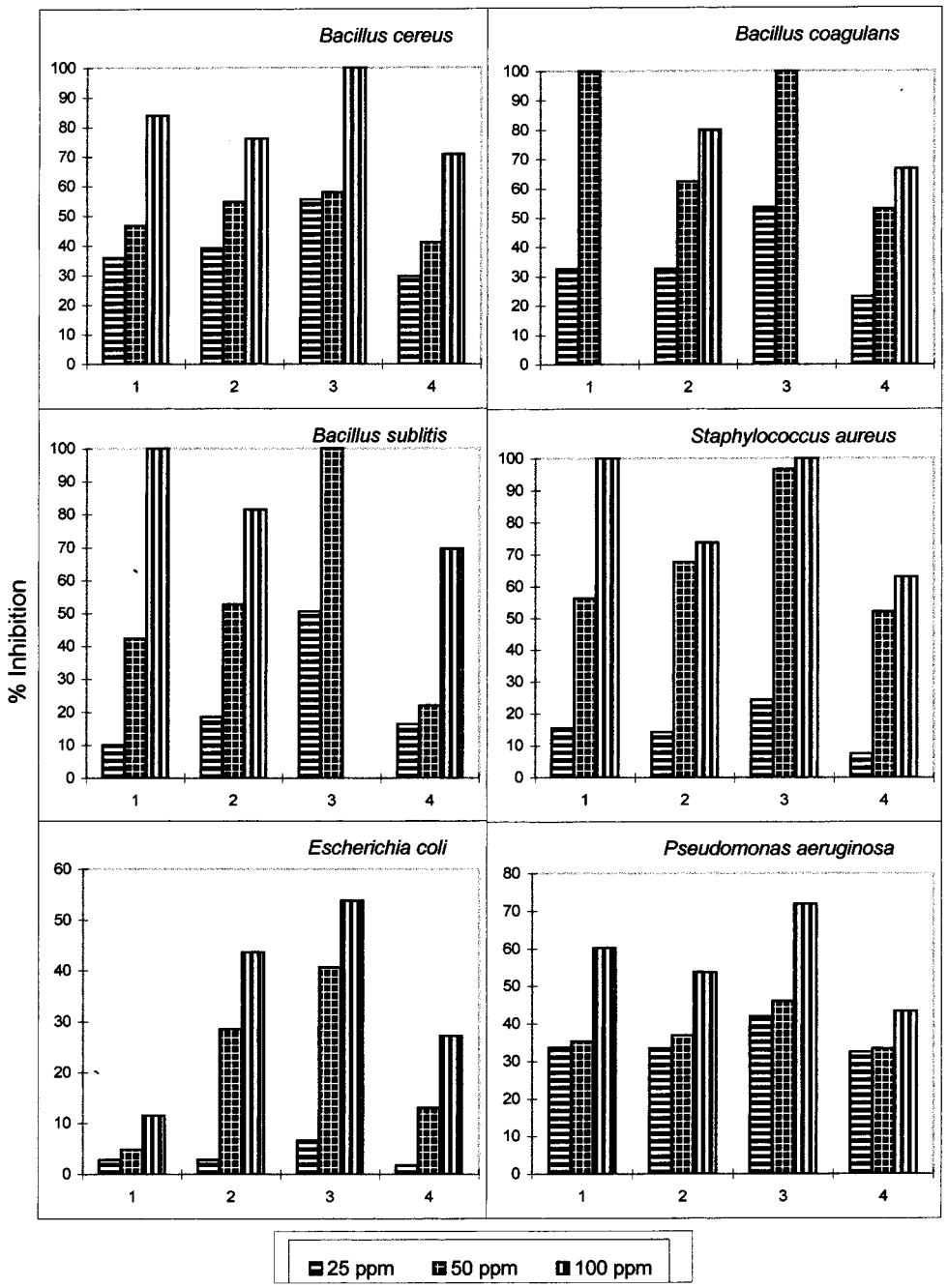


Figure 1. Inhibition of bacterial growth by turmeric oil and its fractions: 1, turmeric oil; 2, fraction I; 3, fraction II; 4, fraction III.

was then poured into sterilized Petri plates in quadruplet and incubated at 37 °C for 20−24 h. The colonies developed after incubation were counted, and the inhibitory effect was calculated according to Rico-Munoz and Davidson (1983) using the following formula:

$$\% \text{ inhibition} = (1 - T/C) \times 100$$

where T is cfu/mL of test sample and C is cfu/mL of control.

The minimum inhibitory concentration (MIC) was reported as the lowest concentration of the compound capable of inhibiting the complete growth of the bacterium being tested (Naganawa et al., 1996).

RESULTS AND DISCUSSION

The number of colonies developed after incubation was taken as the index of growth inhibition. The results show that the turmeric oil and its column fractions exhibited an antibacterial effect against all the bacteria tested (Figure 1). Fraction II eluted by 5% ethyl acetate in hexane was found to be the most effective. In the case of Gram-positive bacteria, fraction II brought about complete inhibition of growth at the 50 ppm level except for *B. cereus*, while for Gram-negative bacteria a higher concentration (200 ppm) was required to inhibit complete growth. Fraction I was more effective against *B. cereus* (54.7% inhibition), *B. subtilis* (52.7% inhibition), *B. coagulens* (62.4% inhibition), and *S. aureus* (67.5% inhibition) whereas against Gram-negative bacteria it was less effective (*E. coli*, 28.5% inhibition; *P. aeruginosa*, 36.8% inhibition) at 50 ppm. Fraction III was least effective. Turmeric oil was found to be more effective than fraction III against Gram-positive bacteria, but it

Table 1. Composition of Turmeric Oil, Fractions I and II[a]

RT (min)	compound	% peak area content: turmeric oil (%)	fraction I (%)	fraction II (%)	KI^{cal}	identification
38.4	*ar*-curcumene	3.49	8.66	0.11	1449	MS, RI
43.7	α-zingiberene	2.48	6.48	0.10	1465	MS, RI
44.4	*β*-bisabolene	2.10	5.07	tr	1478	MS, RI
45.6	*β*-*trans*-farnesen	6.57	16.45	0.23	1488	MS, RI
50.0	*ar*-turmerol	0.87	1.00	0.71	1541	MS, RI
52.3	**3**[b]	1.90	1.80	1.90	1580	MS, RI
52.4	caryophyllen oxide	1.03	2.05	1.00	1588	MS, RI
56.8	*ar*-turmerone	62.00	41.36	77.85	1611	MS, RI
58.4	turmerone	5.09	4.50	5.17	1622	MS, RI
62.2	curlone	3.88	2.55	5.30	1650	MS, RI
65.1	**4**[b]	0.43	tr	1.11	1685	MS, RI
67.6	**5**[b]	tr	tr	1.00	1702	MS, RI
70.4	**6**[b]	tr	tr	1.84	1717	MS, RI

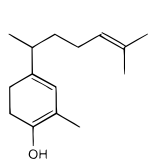
[a] MS, mass spectra. RI, retention index. KI, Kovats indices. tr, less than 0.09%. [b] MS was compared with that of Hiserodt et al. (1996).

Table 2. Minimum Inhibitory Concentration (MIC) of Turmeric Oil and Fraction II

bacteria	MIC (ppm): turmeric oil	fraction II
Gram Positive		
B. cereus	200	100
B. coagulans	50	50
B. subtilis	100	50
S. aureus	100	50
Gram Negative		
E. coli	>200	200
P. aeruginosa	>200	200

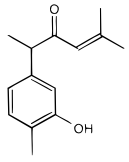
exhibited around 5% inhibition in *E. coli* and 35% inhibition in *P. aeruginosa* at 50 ppm concentration.

The chief constituents of turmeric oil, fractions I and II, were analyzed by GC and GC−MS. GC−MS data showing retention time (RT), Kovats indices (KI), and chemical constituents of turmeric oil, fraction I, and fraction II are presented in Table 1. Thirteen components were identified in turmeric oil, fraction I, and fraction II. *ar*-Turmerone (62.0%), *trans*-*β*-farnesene (6.6%), turmerone (5.1%), and curlone (3.9%) were found to be the major compounds in turmeric oil whereas fraction II contained *ar*-turmerone (77.9%), curlone (5.3%), and turmerone (5.2%) as the major components. Further oxygenated compounds (**4**−**6**) were enriched in fraction II. *ar*-Turmerone and turmerone are found to be the two major constituents of the *Curcuma longa*, which confirms the earlier reports (Govindarajan, 1980).



Compound 4 (MW=220)

Compound 5 (MW=216)



Compound 6 (MW=218)

The MIC levels of turmeric oil and fraction II are presented in Table 2. MIC levels of fraction II were less than or equal to 100 ppm for Gram-positive bacteria, whereas in case of Gram-negative bacteria 200 ppm of the same fraction was required to bring about complete inhibition of growth. MIC levels of fraction II were lower due to enrichment of *ar*-turmerone, turmerone, curlone, **4**, **5**, and **6** in this fraction, and probably the additive/synergistic effect of these compounds may be responsible for high antibacterial activity. Similarly, growth of *E. coli* and *P. aeruginosa* was inhibited completely at 200 ppm of fraction II, whereas the percentages of inhibition were 35% and 67%, respectively, at the same concentration of the turmeric oil.

Results of the present study are consistent with the antibacterial activity of spices and essential oils reported by earlier workers (Sivropoulou et al., 1996; Naganawa et al., 1996). In addition to the nature of growth medium, physical and chemical properties of antimicrobial compounds may play a role in determining the inhibitory effect, which depends on their ability to diffuse in the medium. Comparison of data from different studies is limited due to differences in test methodologies (Carson et al., 1995; Remmal et al., 1993).

CONCLUSION

Turmeric oil was found to possess antibacterial activity toward a number of bacteria. Major constituents possessing antibacterial properties can be isolated by eluation with 5% ethyl acetate in hexane using silica gel column chromatography. The results of the present study are indicative of the utilization of turmeric oil having no commercial application as a preservative agent.

ACKNOWLEDGMENT

We thank Central Instrumentation Facilities for assistance in obtaining the instrumental analysis.

LITERATURE CITED

Adams, R. P. *Identification of Essential Oils by Ion Trap Mass Spectrometry*; Academic Press: New York, 1989; p 29.

Ammon, H. P. T.; Wahl, M. A. Pharmacology of *Curcuma longa*. *Planta Med*. **1991**, *57*, 1−7.

Carson, C. F.; Hammer, K. A.; Riley, T. V. Broth microdilution methods for determining the succeptibility of *Escherichia coli and Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Microbios* **1995**, *82*, 181−184.

Chen, C.-S.; Liau, W.-Y.; Tsai, G.-J. Antibacterial effects of N-sulfonated and N-sulfobenzoyl chitosan and application to oyster preservation. *J. Food Protect.* **1998**, *64*, 1124−1128.

Dahl, T. A.; McGowan, W. M.; Shand, M. A.; Srinivasan, V. A. Phytokilling of bacteria by the natural dye curcumin. *Arch. Microbiol*. **1989**, *151*, 183−185.

Davis, W. Gas Chromatographic retention indices of Monoterpenes and Sesquiterpenes on methyl Silicone and Carbowax 20M phases. *J. Chromatogr*. **1990**, *50*, 1−24.

Govindarajan, V. S. Turmeric−Chemistry, Technology and Quality. *CRC Crit. Rev. Food Sci. Nutr*. **1980**, *12*, 199−301.

Hiserodt, R.; Hartman, T. G.; Ho, C.-T.; Rosen, R. T. Characterization of powdered turmeric by liquid chromatography−mass spectrometry and gas chromatography−mass spectrometry, *J. Chromatogr.* **1996**, *740*, 51−63.

Jennings, W.; Shibamoto, T. *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*; Academic Press: New York, 1980; p 29.

Madhyastha, M. S.; Bhat, R. V. Evaluation of substrate potentiality and inhibitory effects to identify high risk spices for aflatoxin contamination. *J. Food Sci.* **1985**, *50*, 376−378.

Naganawa, R.; Iwata, N.; Ishikawa, K.; Fukuda, H.; Fujino, T.; Sujuki, A. Inhibition of microbial growth by Ajoene, a sulfur-containing compound derived from garlic. *Appl. Environ. Microbiol.* **1996**, *62*, 4238−4242.

Remmal, A.; Bouchikhi, T.; Rhayour, K.; Ettayebi, M. Improved methods for the determination of antimicrobial activity of essential oils in agar medium. *J. Essential Oil Res.* **1993**, *5*, 179−184.

Rico-Munoz, E.; Davidson, P. M. Effect of corn oil and casein on the antimicrobial activity of phenolic antioxidants. *J. Food Sci.* **1983**, *48*, 1284−1288.

Sivropoulou, A.; Papanikolaou, E.; Nikolaou, C.; Kokkini, S.; Lanaras, T.; Arsenakis, M. Antimicrobial and cytotoxic activities of Origanum essential oil. *J. Agric. Food Chem.* **1996**, *44*, 1202−1205.

Srimal, R. C. Turmeric: a brief review of medicinal properties. *Fitoterapia* **1997**, *LXVIII*, 483−493.

Watt, J. M.; Breyer-Brandwijk, M. G. *The Medicinal and Poisonous Plants of Southern and Eastern Africa*; E & S Livingstone, Ltd.: Edinburgh, Scotland, 1962; p 1062.

Received for review April 1, 1999. Revised manuscript received July 23, 1999. Accepted July 28, 1999.

JF990308D