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Biological Studies of Turmeric Oil, Part 1: Selective *in vitro* Anticancer Activity of Turmeric Oil (TO) and TO-Paclitaxel Combination

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The oil from turmeric (*Curcuma longa*) contains several sesquiterpenes with medicinal properties. The oil fractions were purified by repeated high vacuum distillations to constant boiling points and by column chromatography. The major components in the oil fractions were identified as α,β and ar-turmerones. The purified turmeric oil (TO) fractions had growth inhibitory activity against breast (SKBR-3), pancreatic (PANC-1), and prostate (PC-3) cancers, and reduced activity against a non-cancerous cell line, WI-38. A combination of the distillation fraction of turmeric oil and paclitaxel showed substantial increase in growth inhibitory activity against the three cancer cell lines compared with paclitaxel alone, while having reduced activity against the non-cancerous cell line. Percent inhibition may be related to the structural parameters of the turmerones. These results suggest that components in turmeric oil fractions have anti-cancer activity against breast, pancreatic and prostate cancer and a strong positive effect on the activity of paclitaxel.

Keywords: Turmeric oil, Anti-cancer activity, Paclitaxel.

Currently there is a need for novel nontoxic and selective agents to prevent and or treat cancer, particularly breast, pancreatic and prostate malignancies. One method involves selective induction of apoptosis using synergistic molecules blocking multiple pathways. The use of carefully selected components of natural products would be beneficial in this regard. Turmeric (*Curcuma longa* L.), family Zingiberaceae, has been in use for thousands of years in Ayurvedic medicine for cosmetic and medicinal purposes [1]. Turmeric is used as an ingredient in many of the Indian cuisines. India is the largest producer, consumer and exporter of turmeric [1]. One of the main active components, curcumin, has been studied for its anti-oxidant, anti-inflammatory and anticancer activities [2]. The essential oil of turmeric is extracted with either *n*-hexane or by steam distillation and it is reported to have two principal components, α- and β-turmerone [3]. Fractional distillation of turmeric oil under high vacuum is reported to yield two fractions which were used for anti-fungal activity studies [4]. Turmeric oil has been shown to possess anti-microbial, anti-fungal, antiviral, anti-inflammatory and insecticidal activity [4-7]. Early human study of turmeric oil has shown its safety for human use [8]. Turmeric oil has also been studied for anti-cancer activity [9]. The main active constituents responsible for this activity were identified as ar-turmerone and α-turmerone [10,11].

The current study used an oil fraction of narrow boiling range through repeated high vacuum distillations of turmeric oil. The growth inhibitory activity of the fraction was determined using breast, pancreatic and prostate cancer cell lines and a non-cancerous cell line. The oil from the turmeric powder (*C. longa*, Alleppey finger variety) was extracted using filter bags similar to the use of tea bags, thus avoiding the need for filtration. Turmeric oil was purified through repeated distillations under high vacuum to obtain a fraction with steady boiling range, DF-5 (Table 1). The oil was also purified by medium pressure column chromatography to obtain a fraction, CF-3 (Table 2). Components of these fractions were characterized by NMR spectroscopy and GC-MS (Figure 1). The *in vitro* anti-cancer activities of these fractions were studied using breast (SKBR-3), pancreatic (PANC-1) and prostate (PC-3) cancer cell lines and a non-cancerous cell line (WI-38). The effect of turmeric oil (DF-5) on the *in vitro* anti-cancer activity of paclitaxel was also estimated.

Table 1: Distillation of turmeric oil: boiling points and yields.

Distillation Fraction.	Starting qty (g)	Boiling Range (°C)	Yield (g)	Fraction number
1	5.7	115-135	3.54	DF-1
2	3.54	95-112	3.19	DF-2
3	3.10	100-111	2.78	DF-3
4	2.64	100-120	0.57	DF-4
,,	,,	120-123	1.20	DF-5
,,	,,	124	0.26	DF-6

Table 2: Purification by column chromatography.

Eluting solvent	Volume	Fractions	Quantity
n-Hexane	1L	CF-1	0.14g
		CF-2	0.04g
0.5% Ethyl acetate, *n*-Hexane	1L	--	--
1% Ethyl acetate, *n*-Hexane	1L	CF-3	2.44g
		CF-4	1.86g
2% Ethyl acetate, *n*-Hexane	2L	CF-5	0.82g
		CF-6	0.22g
		CF-7	0.04g
5% Ethyl acetate, *n*-Hexane	1L	CF-8	0.10g
		CF-9	0.06g
Methanol	250 mL	CF-10	1.32g
Total obtained from column			7.04g

Figure 1: Turmeric oil components

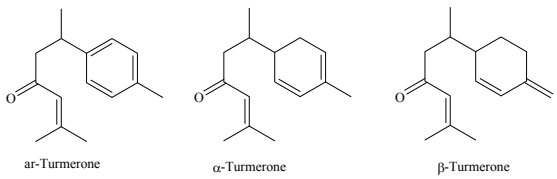

Figure 2: Turmeric oil DF-5 (µg/100 µL), percent inhibition 48 h

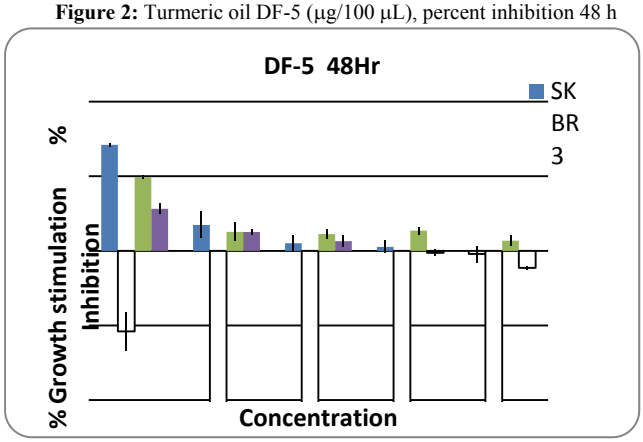

Figure 3: Turmeric oil CF-3 (µg/100 µL), percent inhibition 48 h

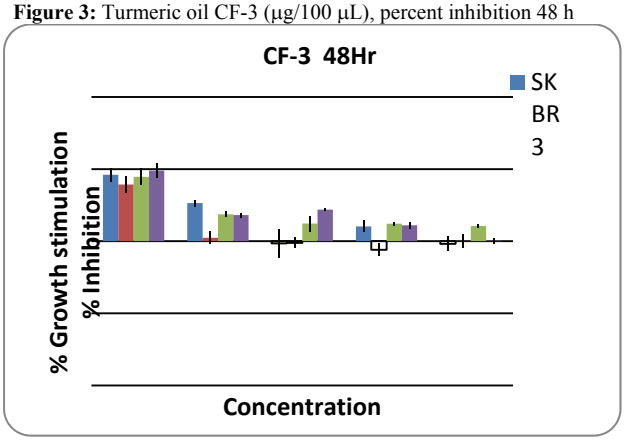

Figure 4: Paclitaxel, percent inhibition 48 h

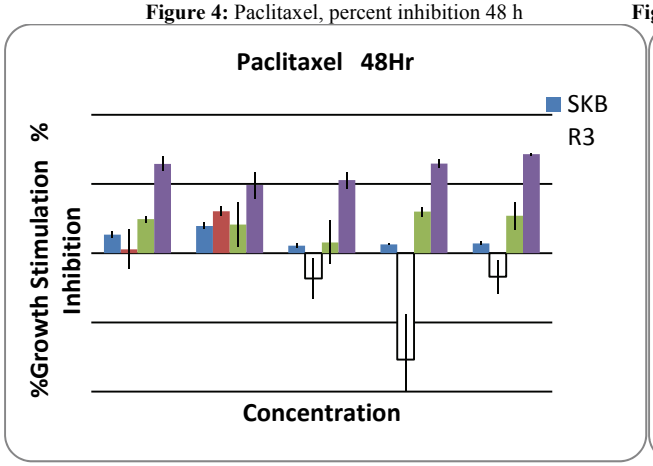

Figure 5: Combination of paclitaxel and turmeric oil, percent inhibition 48 h

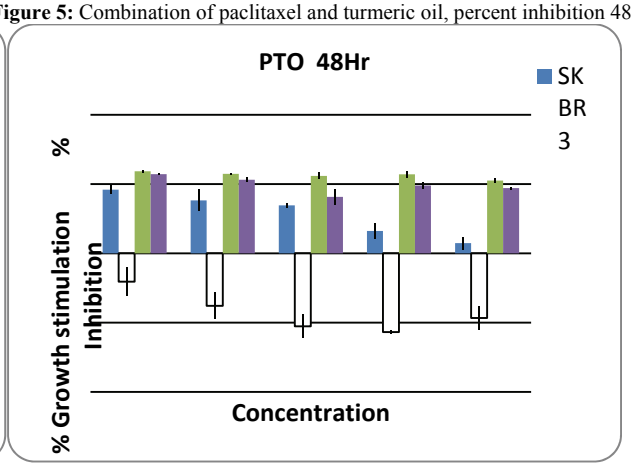

Table 3: Summary of IC_{50} (M) values for DF-5 and CF-3

Drug	Cell Line	24 hours		48 hours	
		IC_{50}	Std. dev.	IC_{50}	Std. dev.
DF-5	SKBR-3	5.77E-03	2.88E-04	2.33E-04	1.86E-05
DF-5	PANC-1	2.95E-03	1.80E-04	3.22E-04	2.57E-05
DF-5	PC-3	1.36E-03	1.21E-04	4.98E-04	7.96E-05
DF-5	WI 38	1.40E+05	4.21E+01	2.98E-03	2.27E-04
CF-3	SKBR-3	3.43E-04	3.29E-05	3.18E-04	4.77E-05
CF-3	PANC-1	3.95E-04	1.86E-05	4.02E-04	3.22E-05
CF-3	PC-3	3.27E-04	3.92E-05	3.34E-04	4.01E-05
CF-3	WI 38	3.24E-04	2.56E-05	3.28E-04	3.60E-05

Table 4: Summary of IC_{50} (M) values for taxol and PTO (combination)

Drug	Cell Line	24 hours		48 hours	
		IC_{50}	Std. dev.	IC_{50}	Std. dev.
Taxol	SKBR-3	6.94 E-05	5.85 E-06	3.23 E-06	3.97 E-07
Taxol	PANC-1	1.53 E-07	4.22 E-08	1.08 E-07	2.98 E-08
Taxol	PC-3	5.81 E-08	2.66 E-09	8.03 E-08	5.20 E-09
Taxol	WI 38	1.91 E-05	1.14 E-06	1.91 E-05	1.14 E-06
PTO	SKBR-3	5.54 E-07	3.38 E-08	3.09 E-07	2.16 E-08
PTO	PANC-1	9.90 E-09	1.09 E-08	4.60 E-09	3.68 E-10
PTO	PC-3	6.48 E-08	3.24 E-09	1.76 E-08	1.11 E-09
PTO	WI 38	4.13 E-07	1.66 E-08	8.00 E-04	4.22 E-05

Turmeric powder contains two main sets of active ingredients, curcuminoids as the yellow solid component and sesquiterpenoids as the oil component. Extraction of turmeric oil in this study yielded a crude fraction devoid of curcuminoid compounds. The fraction DF-5 (Table 1), obtained after repeated distillations, has a narrow boiling range of 120-123°C under high vacuum (~0.5 mm Hg). GC-MS and NMR analysis showed that this fraction contained ar-turmerone (53%) and α- and β-turmerones/curlone (47%) as major components. The fraction, CF-3 (Table 2), obtained through column chromatographic purification, has minimal ar-turmerone (1.8%) and the rest is α- and β-turmerones (98.2%). The IC_{50} values on the three cancer cell lines and for the non-cancerous cell line for 24 hours and 48 hours are given in Table 3. At 24 hours, CF-3 has higher anticancer activity compared with DF-5 in all the three cancer cell lines (IC_{50} values, 10^{-4}M vs. 10^{-3}M). The activity of DF-5 on WI 38 is extremely low at 24 hours (1.40E+05) compared with its effect on the cancer cells. At 48 hours the IC_{50} values were comparable for both fractions; however, DF-5 still had relatively low activity on the non-cancerous cells, WI 38. The percentage inhibition of various cell lines after 48 hours is shown in Figures 2 and 3. At 60 µg/mL, DF-5 had stronger anti-cancer activity (71%) than CF-3 (46%) on the SKBR-3 cell line. The inhibitory activity was similar for both fractions for the PANC-1 cell line. However, for the PC-3 cell line, CF-3 had higher activity (49%) compared with DF-5 (28%). These results may be attributed to the composition of the turmerones in the fractions (ar-turmerone and α- and β-turmerones). Thus, optimum activity may depend on a combination of the different sesquiterpene compounds in the mixture. Since the structural difference between ar-turmerone and α- and β- turmerones is the presence of an aromatic ring system for ar-turmerone, the modulating effect may be attributed to this ring system.

At the 48 hour time point bar graphs (Figures 2 and 3), turmeric oil fraction, DF-5, at the higher dose inhibited the cancer cell lines, but none of the doses affected the normal tissue WI-38 cell line. Thus, this fraction is specific and lacks a general toxicity to cells or tissue. CF-3 behaved in a similar way to DF-5 except that it inhibited WI-38 growth.

Anti-cancer activity of turmeric oil distillation fraction and paclitaxel: Paclitaxel is used as a broad spectrum chemotherapeutic agent for ovarian, breast and non-small cell lung cancers, and a variety of solid tumors [14]. However, significant toxicities, such as myelosuppression and peripheral neuropathy, limit the effectiveness of this promising cancer drug [15]. Synergism of anti-cancer activity of paclitaxel and curcumin was reported [16]. We checked the effect of this drug in combination with turmeric oil towards cytotoxicity against the four cell lines in the present study. Paclitaxel-turmeric oil (PTO) combination [paclitaxel (1 mg) and turmeric oil, DF-5 (10 mg)] were used for *in vitro* studies on the four cell lines. Paclitaxel at the 48 hour treatment point was effective in inhibiting prostate (PC-3) cancer cells at all concentrations (~60% inhibition), and at the 300 and 150 nM concentrations inhibited the other cancer cell lines more modestly. On the other hand, at the same doses, PTO was more effective as it inhibited PANC-1 and PC-3 by approximately ~60% at all concentrations when compared with untreated cells. SKBR3 cell growth proliferation was also inhibited with PTO, albeit more modestly up to 45%. In addition to the better inhibition of these cancer cells, PTO did not affect non-cancerous (WI-38) cell growth. Paclitaxel, however, did have some effect on this non-cancerous cell growth. The results of the experiments are shown in Figures 4 and 5. IC_{50} values are summarized in Table 4. Paclitaxel does not show much inhibitory activity on breast cancer cells at the concentrations used (Figure 4). The paclitaxel-TO combination (PTO) at the same concentrations of paclitaxel showed considerably increased activity towards the breast cancer cell line (Figure 5), while demonstrating no inhibitory activity on the normal cell line. The IC_{50} values (Table 4) indicated increase in activity for the combination compared with taxol for the cancer cell lines, but decrease in activity towards the non-cancerous cell line for the combination compared with taxol. Further work needs to be done to determine synergy and establish the effect of the turmerones using pure components of the TO fraction.

In conclusion, repeated fractional distillation of turmeric oil yields a narrow boiling range containing α– and β–turmerones with ar-turmerone as the major component. The turmeric oil obtained by selective distillation has anti-cancer properties against three cancer cell lines, SKBR-3, PC-3 and PANC-1, and considerably less activity against the non-cancerous cell line, WI-38. TO fraction enhanced the anti-cancer activity of paclitaxel and the fraction containing ar-turmerone has growth stimulating activity on the non-cancerous cell line with reduced toxicity for the paclitaxel formulation. Further studies are needed to establish the structure activity relationship of the turmerones for the selectivity. Unlike other cancer drugs used in chemotherapy turmeric components are not toxic and are good candidates for further investigation for cancer treatment and prevention as well as for development of new formulations for cancer drugs.

Experimental

General: NMR, Bruker (400 MHz); Mass spectra supplemental data); HPLC: (Hewlett Packard HP 1090 Series II liquid chromatography), C-18 column, (X-terra MS C-18, 5 μm, 4.6 x 150 mm), UV detector (254 nm); mobile phase, acetonitrile/water 85-15, TLC plates were obtained from Sigma- Aldrich, silica gel (thickness 200 μm, polyester support).

Turmeric oil extraction and distillation: Turmeric powder obtained from fresh turmeric roots (95 g) was placed in Whatman filter paper pouches, stirred with 1.2 L of *n*-hexane for 24 h and the solvent concentrated to obtain a red oil (5.2 g). The residue in the pouch was re-extracted by stirring overnight with 500 mL of *n*-hexane, and concentrated to obtain an additional 0.5 g of material; both extracts were combined (5.7 g). The turmeric oil obtained by solvent extraction was further purified by repeated high vacuum distillations (<1 torr) to obtain a clear light yellow fraction (1.20 g), DF-5, boiling at 120-123°C (Table 1). HPLC showed peaks at (RT, min) 2.85, 3.59, and 4.23. Based on GC-MS and NMR spectroscopy, the major components in DF-5 were identified as ar-turmerone (53.2%), α-turmerone (38.3%), and β-turmerone (8.7%) [3,9].

Purification by column chromatography: Distilled turmeric oil fractions from different batches were collected (8.00 g) and purified on a silica gel column eluted with *n*-hexane and *n*-hexane-ethyl acetate mixtures (from 0.5% to 5% ethyl acetate) to obtain column fraction CF-3 (2.44 g) (Table 2). HPLC showed peaks at (RT, min) 2.89, 2.95, 3.64, and 4.24. TLC showed one spot since the components were not separated (ethyl acetate/*n*-hexane; 15/85) Rf = 0.42, Elemental analysis gave C: 73.86%; H 8.79%. Based on GC-MS and NMR spectroscopy, the major components in CF-3 were identified as α-turmerone (77.8%), β-turmerone (20.4%) and ar-turmerone (1.8%) [3,9].

In vitro studies: Cell lines obtained from American Type Tissue Culture Collection (Manassas, VA) were maintained at 37ºC in a humidified atmosphere containing 5% CO_2. Cells were cultured in RPMI-1640 (Invitrogen) supplemented with 10% FBS (CellGen). Anti-cancer cell culture assays were performed with 3 cancer cell culture lines namely: (1) breast, SKBR-3 (HTB-30), (2) pancreatic, PANC-1 (CRL-1469) and (3) prostate, PC-3 (CRL-1435) and one benign non-cancerous cell line, WI-38 (CCL-75). The compounds were tested for their growth inhibition characteristics with 5 serial dilutions in the culture medium per compound. Cell proliferation was determined using Resazurin (Alamar Blue, Invitrogen) [12,13]. Percent growth inhibition was determined by measurement of fluorescence at 24 and 48 h after addition of compounds, relative to the cell population without any treatment.

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