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Antioxidant and cytotoxic effects of essential oil, water and ethanol extracts of major Indian spices

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ABSTRACT

Essential oils, water and ethanol extracts of black pepper, ginger, turmeric, cinnamon, *Garcinia indica*, *G. gummi-gutta*, tamarind and curry leaves were examined for their antioxidant potential and cytotoxicity to cancer cell lines by *in vitro* methods. Essential oils of ginger, turmeric, cinnamon and curry leaf were highly cytotoxic, reducing cell viability to 14 to 30% of untreated control; water and ethanol extracts of *G. indica*, turmeric, cinnamon, tamarind and curry leaf were also cytotoxic, though to a lesser extent (27% to none); ethanol extracts displayed approximately 50% higher cytotoxicity than water extracts. Antioxidant potential of water and ethanol extracts were similar, and decreased after six months of storage in most extracts; most were superior to BHA and BHT. Antioxidant potential of ethanol was also greater than water extracts. Change in essential oil chemoprofile stored at 4^{0}C for a year compared to fresh, most notably *t*-caryophyllene, is also reported here.

Key words: Spice, essential oil, antioxidant potential, cytotoxic effect, storage study.

INTRODUCTION

Spices are treasured for their flavour and aroma, as also for their nutraceutical and therapeutic effects. These properties stem from their unique secondary metabolites-essential oils and oleoresins, and some primary metabolites. The numerous bioactive phytochemicals include flavonoids, terpenoid, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, phthalides. Anticancer effects of medicinal plants are extensively studied *in vitro* based on cytotoxicity screening on human cancer cell lines, to identify potential anticancer plant extracts, mostly banking on traditional knowledge (Nobuji, 9). In this study, an attempt to examine the antioxidant and cytotoxic effects of spice extracts, and look for correlation between the two properties.

MATERIALS AND METHODS

Eight spices used for these studies included dried black pepper (*Piper nigrum* L., variety Thevam) berries, rhizomes of ginger (*Zingiber officinale* Rosc., variety Rejatha) and turmeric (*Curcuma longa* L., variety Alleppey Supreme), cinnamon (*Cinnamomum verum*, variety Nithyashree) bark, rinds of *Garcinia indica* and *G. gummi-gutta*, tamarind (*Tamarindus indica*) pods and curry leaves (*Murraya koenigii*). These were procured from the Experimental Farm of the ICAR-IISR, Peruvannamuzhi, Calicut, Kerala.

Essential oil of black pepper, ginger, turmeric, cinnamon and curry leaves was extracted by steam distillation using Clevenger's distillation apparatus; remaining spices did not yield essential oil. To study antioxidant properties, water and ethanol extracts were used at 10 mg/ml concentrations. Synthetic phenols butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used at 0.1 mg/ml, concentrations normally used in food preservatives.

For cytotoxicity studies on cancer cell lines, essential oils of black pepper, ginger, turmeric, cinnamon and curry leaves were used at 0.01 and 0.02% concentrations in DMSO. The filtered water extracts were lyophilized and ethanol extracts dried at 45-50°C, and used at 25 and 50 µg/ml concentrations in 0.1% DMSO in DMEM (Dulbecco's modified Eagle's medium). In all treatments, DMSO concentration did not exceed 0.1%. Antioxidant potential was assayed by three *in vitro* methods. 1,1-Diphenyl-2-picryl hydrazyl assay (DPPH) was estimated as per the method of Braca *et al*. (4), using 5 to 10 µl of essential oil, 0.5 to 1 ml of water or ethanol extract. DPPH radical scavenging activity was expressed as percentage of blank. Total antioxidant capacity by phosphomolybdenum method was estimated by the method of Prieto *et al*. (10), using 5 to 10 µl of essential oil, 0.5 to 1 ml of water or ethanol extract, and expressed as ascorbic acid equivalents (AAE) (µmol/ ml for essential oils and µmol/ g sample for water and ethanol extracts). Fe (III) to Fe (II) reducing activity was estimated by the method described by Oyaizu (11) in 5 to 10 µl of essential oil, 0.5 to 1 ml of water or ethanol extract, and expressed

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as AAE (µmol/ ml for essential oils and µmol/ g sample for water and ethanol extracts).

Total phenols content was estimated in 5 to 10 µl of essential oil, 0.5 to 1 ml of water or ethanol extract by method of Singleton *et al*. (13), and expressed as gallic acid equivalents (GAE)/ ml for essential oils and GAE/ g sample for water and ethanol extracts. Each value is a mean of three replicates. ANOVA was done using the MStatC package. DPPH values were subjected to arcsine transformation for ANOVA. Assays of cytotoxicity activity *in vitro* was performed on the following human cancer cell lines, cervical cancer cell line (HeLa), breast cancer cells (MDA-MB-231), liver cancer cells (HepG2) and skin cancer cells (A375), obtained from National Centre for Cell Science, Pune. These were maintained in DMEM containing 10% fetal bovine serum and antibiotic-antimycotic. Cells were seeded at a density of 5 × 10^3 cells/ well in 96-well plates and treated with spice essential oils (0.01 and 0.02%) or water or ethanol extracts (25 and 50 µg/ml). After incubating at 37°C in 5% CO_2 for 48 h, cytotoxic effect of the extracts was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium) assay (Anto *et al*., 3). Relative cell viability (%) was calculated by comparing the viability of treated cells with that of DMEM control.

RESULTS AND DISCUSSION

The study on antioxidant activity of essential oils of black pepper, ginger, turmeric, cinnamon and curry leaves (Table 1) revealed that cinnamon essential

Table 1. Antioxidant activity of essential oil of spices at different months of storage.

Spice	Months after extraction				
	0	3	6	9	12
DPPH radical scavenging activity (% of control)*					
Black pepper	40.00 (41.32)	41.88 (44.59)	35.97 (34.49)	35.83 (34.35)	24.64 (17.45)
Ginger	68.54^{b} (86.60)	60.28^{d} (75.41)	53.93 (65.34)	60.80^{d} (76.19)	39.94 (41.23)
Turmeric	63.75^{c} (80.44)	48.92 (56.81)	47.40 (54.20)	52.03 (62.15)	36.31 (35.06)
Cinnamon	71.76^{a} (90.20)	70.93^{ab} (89.31)	73.01^{a} (91.40)	73.04^{a} (91.43)	60.06^{d} (75.09)
Curry leaf	49.59 (57.97)	34.12 (31.48)	31.66 (27.59)	30.74 (26.37)	14.22 (6.04)
$CD_{0.05}$ = 4.07					
Fe(III) to Fe(II) reducing activity (µmol ascorbic acid equivalent/ ml)					
Black pepper	21.22	23.23	57.95^{a}	49.52	42.19
Ginger	15.86	32.21	40.54	25.90	24.45
Turmeric	14.84	26.50	35.26	19.22	20.85
Cinnamon	50.25^{bc}	53.72^{a}	56.03^{a}	51.65^{bc}	52.05^{bc}
Curry leaf	15.19	20.38	46.88	50.25^{bc}	27.27
$CD_{0.05}$ = 4.92					
Total antioxidant potential by phosphomolybdenum method (µmol ascorbic acid equivalent/ ml)					
Black pepper	294.99	373.80	431.28	525.11^{c}	724.16^{b}
Ginger	549.58^{c}	854.24^{a}	464.86	481.02	496.32
Turmeric	897.50^{a}	874.27^{a}	534.76^{c}	386.02	334.70
Cinnamon	107.95	113.47	113.92	117.07	211.71
Curry leaf	495.00	451.70	464.86	381.02	357.47
$CD_{0.05}$ = 120.9					
Total phenols (mg gallic acid equivalent/ ml)					
Black pepper	1.041	1.270	1.027	0.943	0.012
Ginger	2.886^{bc}	3.166^{b}	2.836^{bc}	1.336	0.019
Turmeric	1.527	1.563	0.540	0.558	0.007
Cinnamon	4.865^{a}	5.359^{a}	2.515^{bc}	2.304	0.021
Curry leaf	1.470	2.735^{bc}	1.327	2.035	0.019
$CD_{0.05}$ = 0.50					

*Arc Sine transformed values; values in parentheses are original means

oil had the most DPPH radical scavenging activity (71.76%), followed by ginger (68.54%) and turmeric (63.75%); activity of curry leaves and black pepper were much lower. The activity generally decreased with storage time; but in cinnamon it remained high even till nine months after extraction. Essential oil of cinnamon (50.25 µmol/ ml) followed by black pepper (21.22 µmol /ml) had the most Fe(III) to Fe(II) reducing activity (FRA), even up to 12 months after extraction. Highest FRA was at 6th month after extraction in most oils: Black pepper (57.95 µmol/ ml) and cinnamon (~55 µmol/ ml) had greater FRA than other oils.

Total antioxidant potential was highest and at par in turmeric (874-898 µmol/ ml) and ginger (550-854 µmol/ ml) up to three months of extraction, followed by black pepper (295-724 µmol/ml) and curry leaf (357-495 µmol/ml), and least in cinnamon (107-212 µmol/ ml). No specific pattern in activity was discernible with respect to time of storage. Cinnamon essential oil had the maximum phenols content (5.36 mg GAE/ ml), followed by ginger (3.17 mg GAE/ml) and curry leaf (2.74 mg GAE/ ml); black pepper (1.27 mg GAE/ ml) and turmeric (1.56 mg GAE /ml) were on par. Phenol content was high till the 3rd month after extraction, after which there was a gradual decline.

The major volatiles in spice essential oils were reported earlier (Shamina *et al*., 12; Anon, 2), and could be responsible for the observed nutraceutical properties. In black pepper, the major volatiles are D-limonene (20.7%), caryophyllene (18%), sabinene (12%), δ-3-carene (11%), α- pinene (6.5%) and β-pinene (10%). Ginger major volatiles include zingiberene (23.5%), farnesene (13.8%), citral (8.4%), β-sesquiphellandrene (9.2%), camphene (6.8%), z-citral (5.5%), α-curcumene (5.4%), β-phellandrene (3.7%), 1,8-cineole (3.2%). Turmeric volatiles include turmerone (38%), curlone (22%), ar-turmerone (17.8%), l-phellandrene (6%), 1,8-cineole (3.4%), zingiberene (2.1%); and in cinnamon, cinnamaldehyde (66.7%), *t*-caryophyllene (9%), benzyl benzoate (4.5%), linalool (3.3%), cinnamyl acetate (2.2%). Curry leaves contain *t*-caryophyllene (34%); b-phellandrene (10%) and a-selinene (10%), and the minor components a-pinene, a-humulene, a-guaiene and epiglobulol. It was also reported that in essential oil of black pepper, *t*-caryophyllene decreased from 18 to 11%, while *t*-caryophyllene oxide increased from 0.24 to 9.6%, from immediately after extraction to 12 months after storage at 4^{0}C. Similarly in curry leaves, *t*-caryophyllene, the major component present up to ~26% till the 3rd month after extraction, was reduced to 0.5% by 9th month, almost all of which was oxidized to *t*-caryophyllene oxide (Anon, 1). The change in chemoprofile of essential oils is reflected in change in antioxidant character on storage.

Antioxidant activity of water and ethanol extracts is summarised in Tables 2 and 3. As in essential oil, cinnamon ethanol extract had the most DPPH radical scavenging activity (75.67%), followed by turmeric (69.67%) and curry leaves (65.85%) all at 6th month after extraction; ginger, *G. gummi-gutta* and *G. indica* were at par. BHA had higher activities compared to black pepper or tamarind. BHT had the least activity (Table 3). In water extracts, curry leaves had the most activity (55-64%), followed by cinnamon (48-68%) and black pepper (52-61%) (at par), *G. gummi-gutta* and *G. indica*; ginger had the least activity. The activity decreased significantly with storage in water extracts; but, in curry leaves water and ethanol extracts and most ethanol extracts the activity peaked around 6 months of storage. Activity of BHT and tamarind was the least.

Curry leaves water extracts had highest FRA (132-305 µmol/ ml), followed by cinnamon (114-198 µmol/ml) and *G. indica* (91-137 µmol/ml) and least in ginger (19-35 µmol/ ml). In ethanol extracts, turmeric had higher activities (152-379 µmol/ ml), followed by curry leaves (151-299 µmol/ ml); cinnamon (131-216 µmol/ ml) and *G. indica* (95-252 µmol/ ml), all at par; BHA and BHT had least activity. Ethanol extracts had almost 50% higher activities than water extracts. In both extracts, activity increased with storage time.

Ethanol extracts had ~4 times as much total antioxidant potential as water extracts. Tamarind had higher activities (880 µmol/ ml, 3rd month after extraction) and 1895 µmol/ ml (9th month) in water and ethanol extracts respectively, followed by *G. gummi-gutta* and *G. indica*. The activity in both extracts remained high even after a year of extraction. Water extracts of turmeric and ginger at 12 months after extraction had least activity. All spice extracts were superior to BHA and BHT. Ethanol extracted ~50% greater phenol than water. In both solvents, phenol content decreased with storage. Turmeric ethanol extract had the most phenol content (74 mg GAE/ mg), followed by curry leaves water extract (53 mg GAE/ mg), *G. indica* (23 mg GAE/ mg)*;* tamarind (10 mg GAE/ mg) had the least.

Natural sources of polyphenols and alkaloids, abundant in secondary metabolites of spices, are promising alternatives, as results of this study suggests that most spice extracts are better antioxidants. The antioxidant activity of plant extracts containing polyphenols is due to their hydrogen atom or electron donor ability and thus to capture free radicals. While, Fe (III) reduction is an indicator of electron donor activity; in DPPH radical scavenging test hydrogen atoms are also involved. The essential

Table 2. Antioxidant activity of water extracts of spices at different months of storage.

Spice	Months after extraction				
	0	3	6	9	12
DPPH radical scavenging activity (% of control)*					
Black pepper	60.64 (75.85)	57.61 (71.31)	55.03 (67.16)	54.74 (66.67)	52.03 (62.04)
Ginger	33.92 (31.16)	39.09 (39.75)	33.13 (29.88)	28.03 (22.09)	25.87 (19.03)
Turmeric	47.71 (54.71)	48.06 (55.33)	41.93 (44.65)	31.38 (27.12)	27.63 (21.51)
Cinnamon	68.15^{cd} (86.13)	58.29 (72.34)	56.48 (69.49)	54.03 (65.48)	48.17 (55.52)
Garcinia gummi-gutta	53.65 (64.86)	38.47 (38.73)	39.38 (40.26)	34.75 (32.50)	32.03 (28.13)
G. indica	46.46 (52.54)	42.76 (46.11)	46.82 (53.18)	49.51 (57.86)	46.39 (52.43)
Tamarind	47.50 (54.35)	37.39 (36.89)	36.50 (35.39)	34.76 (32.50)	27.36 (21.14)
Curry leaf	54.54 (66.31)	63.53 (80.13)	63.65 (80.30)	62.21 (78.28)	55.50 (67.93)
$CD_{0.05}$ = 2.14					
Fe(III) to Fe(II) reducing activity (µmol ascorbic acid equivalent/ g dw)					
Black pepper	60.98	56.06	88.96	101.90	137.13
Ginger	19.00	25.45	35.49	19.45	22.28
Turmeric	39.49	52.52	62.08	65.88	81.65
Cinnamon	153.00	133.33	114.38	197.90	183.68
G. gummi-gutta	81.71	64.53	82.25	91.68	79.35
G. indica	106.57	90.51	121.41	136.98	137.40
Tamarind	33.08	36.38	52.82	49.95	48.38
Curry leaf	132.11	235.49^{c}	250.15^{b}	252.63^{b}	304.85^{a}
$CD_{0.05}$ = 13.91					
Total antioxidant potential by phosphomolybdenum method (µmol ascorbic acid equivalent/ g dw)					
Black pepper	91.17	74.70	70.90	57.02	55.44
Ginger	80.62	58.10	58.00	41.69	42.80
Turmeric	79.75	85.50	74.60	54.15	38.04
Cinnamon	74.00	78.00	67.75	66.14	66.61
G. gummi-gutta	286.45	251.00	247.97	267.93	272.75
G. indica	142.02	133.45	210.77	229.30	183.86
Tamarind	587.00^{b}	632.00^{b}	635.77^{b}	880.45^{a}	469.66^{c}
Curry leaf	122.06	182.00	124.06	100.76	68.55
$CD_{0.05}$ = 53.13					
Total phenols (mg gallic acid equivalent/ mg dw)					
Black pepper	13.761	12.350	6.951	9.495	0.111
Ginger	10.949	10.827	4.192	4.216	0.053
Turmeric	11.954	11.936	6.182	7.747	0.084
Cinnamon	19.242	14.272	7.037	6.916	0.190
G. gummi-gutta	13.318	10.061	8.721	5.318	0.099
G. indica	19.414	13.777	13.439	8.098	0.146
Tamarind	10.359	9.846	7.021	3.637	0.071
Curry leaf	55.250^{a}	53.181^{b}	22.714	31.930	0.376
$CD_{0.05}$ = 1.076					

*Arc Sine transformed values; values in parentheses are original means

Table 3. Antioxidant activity of ethanol extracts of spices at different months of storage.

Spice	Months after extraction				
	0	3	6	9	12
DPPH radical scavenging activity (% of control)*					
Black pepper	68.53 (86.60)	60.60 (75.90)	50.64 (59.75)	42.17 (45.08)	36.18 (34.84)
Ginger	65.66 (82.97)	68.88^{bc} (86.31)	63.53 (80.09)	55.19 (67.42)	50.83 (60.11)
Turmeric	56.07 (68.85)	65.18 (82.38)	69.67^{bc} (87.93)	64.13 (80.96)	57.80 (71.60)
Cinnamon	66.57^{de} (84.17)	70.01^{bc} (88.32)	75.67^{a} (93.86)	70.63^{b} (88.99)	60.07 (75.10)
Garcinia gummi-gutta	58.82 (73.19)	59.73 (74.59)	65.37 (82.63)	65.91 (83.34)	52.24 (62.50)
G. indica	59.78 (74.64)	62.08 (78.08)	65.37 (82.63)	60.40 (75.60)	54.72 (66.64)
Tamarind	51.92 (61.96)	50.84 (60.12)	39.57 (40.57)	39.13 (39.83)	26.08 (19.34)
Curry leaf	56.98 (70.29)	63.24 (79.72)	65.85 (83.27)	63.69 (80.36)	57.15 (70.59)
BHA	(77.00)	69.67	64.96	66.85	69.49
BHT	71.96	15.16	4.03	4.64	1.79
$CD_{0.05}$ = 2.14					
Fe(III) to Fe(II) reducing activity (µmol ascorbic acid equivalent/ g dw)					
Black pepper	53.78	61.87	80.35	108.03	105.28
Ginger	95.88	104.78	140.78	169.13	156.88
Turmeric	174.89	151.74	233.06	375.25^{a}	378.88^{a}
Cinnamon	141.75	159.10	131.01	208.05	216.08
G. gummi-gutta	85.04	77.72	110.48	130.95	139.58
G. indica	101.82	95.46	147.01	217.13	252.25^{c}
Tamarind	44.33	51.83	75.90	62.13	66.53
Curry leaf	167.55	150.57	153.90	298.53^{b}	288.10^{b}
BHA	65.18	42.68	35.69	16.25	6.48
BHT	75.62	43.49	32.05	19.30	13.83
$CD_{0.05}$ = 20.93					
Total antioxidant potential by phosphomolybdenum method (µmol ascorbic acid equivalent/ g dw)					
Black pepper	46.07	109.50	148.00	85.96	74.41
Ginger	188.27	175.50	224.60	305.55	333.61
Turmeric	371.20	313.50	293.35	502.40	492.94
Cinnamon	247.80	231.50	211.49	157.24	160.51
G. gummi-gutta	311.10	572.00	534.95	720.73^{cd}	736.37^{cd}
G. indica	163.10	526.00	478.14	776.48^{c}	728.32^{c}
Tamarind	1307.75^{b}	1894.55^{a}	774.30^{c}	646.06	606.55
Curry leaf	354.70	252.65	317.09	476.48	579.00
BHA	76.36	62.50	43.52	8.13	3.38
BHT	2.03	0.95	0.82	0.36	0.11
$CD_{0.05}$ = 121.80					
Total phenols (mg gallic acid eq./ mg dw)					
Black pepper	13.157	16.417	7.764	9.653	0.094
Ginger	16.445	20.610	10.435	13.134	0.144

Contd...

Table 3 Contd...

Spice	Months after extraction				
	0	3	6	9	12
Turmeric	74.007[a]	64.556[b]	34.453[d]	20.750	0.377
Cinnamon	25.105	20.237	11.136	6.880	0.231
G. gummi-gutta	19.649	15.892	13.166	7.367	0.162
G. indica	20.997	22.678	21.364	9.711	0.249
Tamarind	9.958	9.550	7.038	3.132	0.067
Curry leaf	39.131[c]	33.426[d]	27.645	15.523	0.293
$CD_{0.05}$ = 3.352					

*Arc Sine transformed values; values in parentheses are original means

oil antioxidant activity is attributed to the major and minor constituents, and to synergy among them, cinnamaldehyde in cinnamon (Sivakumar *et al*., 14), and piperine, a nonvolatile in black pepper (Mittal and Gupta, 8) are known antioxidants. The -OH (in *para* position) and phenol groups, and β-diketone moiety (H atom donor) are attributed antioxidant activity of curcuminoids in turmeric (Masuda *et al*., 6).

Spice essential oils markedly decreased cell viability of cancer cells (Table 4). Ginger, turmeric, cinnamon and curry leaves were significantly superior to black pepper (Fig. 1). Turmeric, cinnamon and curry leaf reduced cell viability of HeLa (cervical cancer) cells by 14-18%, irrespective of concentration, indicating that the IC50 of these extracts is below 0.01%. Cell viability was reduced by half when concentration of ginger essential oil was doubled from 0.01 to 0.02%. The decrease in viability of breast cancer cells, MDA-MB-231, was on par (24% to 30%) in all spices tested, at both concentrations, except black pepper, which was only 1/3rd as effective. Cell viability of liver cancer cells, HepG2, was reduced the most by cinnamon and ginger (at 0.02%), the rest were at par, black pepper was least effective. In A375 (skin cancer) cells too, black pepper was only ½ as effective as the rest. Essential oils have been used for antimicrobial, antiparasitical, insecticidal, medicinal and cosmetic applications since the Middle Ages; these findings corroborate these applications.

Results of water and ethanol extracts were quite different from essential oil (Table 5). In HeLa cells, the most effective treatments were ethanol extracts of turmeric (27% decrease in cell viability) and *G. indica* (37%) at 50 µg/ml. Other promising treatments were turmeric water extract (62%), and ethanol extracts of curry leaves (75%, at 50 µg/ml) and *G. indica* (77%, at 25 µg/ml). Cell viability of MDA-MB-231 cells was reduced the most by *G. indica* (51-72%), followed by

Table 4. Effect of spice essential oils on cell viability in cancer cell lines.

Spice	Conc. (%)	Percentage viability of cancer cells compared to untreated control			
		HeLa	MDA-MB-231	HepG2	A375
Black pepper	0.01	[d]84.85	[b]64.01	[d]72.86	[c]50.70
	0.02	[c]43.91	[b]53.77	[e]82.47	[c]52.29
Ginger	0.01	[b]23.53	[a]27.00	[c]37.32	[ab]24.10
	0.02	[a]14.03	[a]24.21	[a]19.12	[b]28.49
Turmeric	0.01	[b]17.66	[a]30.50	[ab]24.79	[ab]25.10
	0.02	[b]17.74	[a]25.79	[ab]20.44	[ab]23.99
Cinnamon	0.01	[b]16.14	[a]26.12	[a]17.34	[a]21.66
	0.02	[b]15.24	[a]25.46	[a]16.63	[a]20.50
Curry leaf	0.01	[b]15.65	[a]29.66	[ab]25.35	[ab]24.67
	0.02	[b]15.34	[a]27.56	[ab]20.66	[ab]23.19
DMSO	0.1	93.45	60.89	98.25	66.93
$CD_{0.05}$		10.09	14.71	9.29	6.30

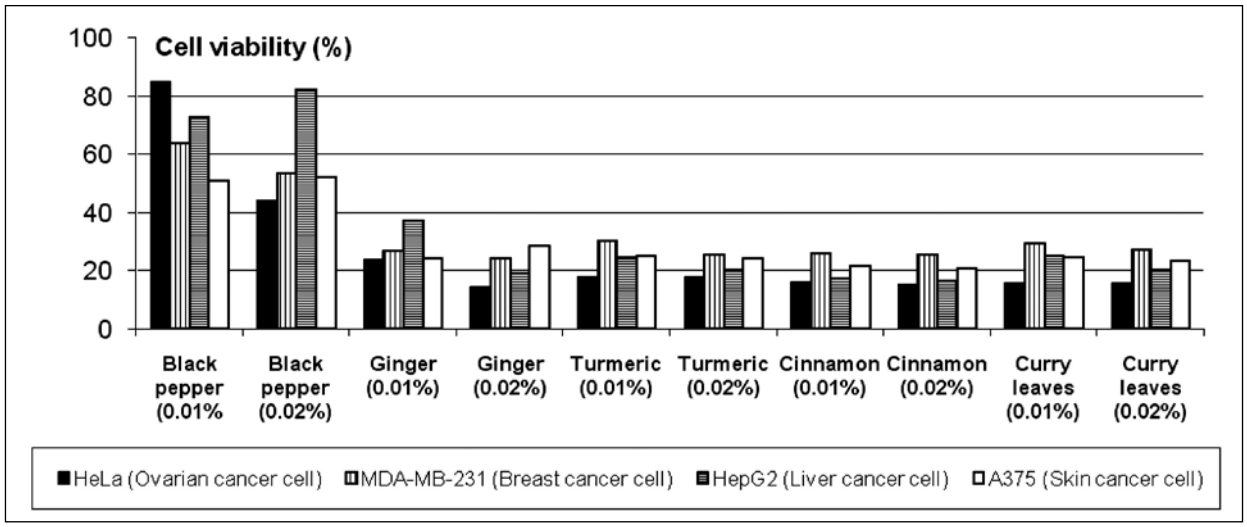

Fig. 1. Cell viability of four cancer cells treated with essential oil of spices.

Table 5. Effect of spice water and ethanol extracts of on cell viability (%) in cancer cell lines.

Spice	Conc. (µg/ ml)	HeLa	MDA-MB-231	HepG2	A375
Water extract					
Black pepper	25	108.76	104.32	106.29	123.43
	50	101.28	105.44	122.20	144.39
Ginger	25	107.16	103.62	96.95	131.16
	50	95.23	94.02	109.52	134.48
Turmeric	25	102.08	91.65	90.96	139.05
	50	61.64^{b}	82.56	63.85^{b}	129.42
Cinnamon	25	98.08	70.77^{b}	84.26	117.84
	50	87.91	63.55^{ab}	74.40^{c}	111.26
G. indica	25	99.68	71.99^{b}	76.27^{c}	106.94
	50	96.82	51.46^{a}	76.20^{c}	126.85
G. gummi-gutta	25	108.71	70.18^{b}	118.51	121.74
	50	112.20	50.28^{a}	94.39	145.88
Tamarind	25	108.63	79.21	109.88	156.03
	50	112.31	77.36	109.64	145.08
Curry leaf	25	108.92	77.10	82.47	118.50
	50	107.56	61.07^{ab}	106.10	150.57
DMSO	0.1%	108.89	78.58	76.06^{c}	94.62
Ethanol extract					
Black pepper	25	115.38	89.56	109.12	142.29
	50	94.35	115.21	112.20	120.60
Ginger	25	117.59	90.08	87.16	143.97
	50	99.12	83.39	94.31	131.99
Turmeric	25	96.44	71.30^{b}	90.05	121.22
	50	27.27^{a}	52.52^{a}	85.93	49.11^{ab}
Cinnamon	25	114.41	84.07	106.45	124.37

Contd...

Table 5 Contd...

Spice	Conc. (µg/ ml)	HeLa	MDA-MB-231	HepG2	A375
	50	104.24	89.12	108.10	125.08
G. indica	25	76.53^{b}	51.21^{a}	61.94^{b}	86.61
	50	37.05^{a}	52.52^{a}	47.05^{a}	35.58^{a}
G. gummi-gutta	25	104.32	100.45	114.33	136.73
	50	108.65	106.77	100.19	130.43
Tamarind	25	122.53	70.45^{b}	109.06	140.81
	50	113.58	66.47^{ab}	104.08	153.28
Curry leaf	25	103.60	74.93^{bc}	78.77^{c}	106.32
	50	74.52b	60.60^{ab}	37.97^{a}	91.10
DMSO	0.1%	111.47	95.47	92.73	79.75
$CD_{0.05}$		15.32	15.33	14.50	18.15

curry leaves and tamarind (60-79%), turmeric and cinnamon (52-92%); the water and ethanol extracts were at par. Cell viability of MDA-MB-231 cells was reduced by about half by *G. gummi-gutta*, *G. indica* and turmeric. Tamarind, curry leaves and cinnamon were also effective at both concentrations, indicating that 25 µg/ml was enough to compromise cell's viability. Cell viability of HepG2 cells decreased the most due to ethanol extracts of curry leaves (38%) and *G. indica* (47%). Other effective treatments included water extracts of turmeric (64%), cinnamon (74%) and *G. indica* (76%). A375 was most affected by ethanol extracts of *G. indica* (36%) and turmeric (49%) (*at par*). The rest did not affect cell viability of A375 cells significantly. Almost 2/3rd of HeLa and A375 cells and half the MDA-MB-231 and HepG2 cells subjected to 50 µg/ ml of *G. indica* were killed. Flavonoids in alcoholic extracts prevent or inhibit cancer development by affecting metabolic pathways such as activation of glycolytic enzymes or protein synthesis, DNA scission by inducing topoisomerase I- and II-mediated DNA cleavage complex (López-Lázaro *et al*., 5).

Spice volatile essential oils are a mixture of terpenes and terpenoids, phenol-derived aromatic components and aliphatic components. Except black pepper, essential oils were more cytotoxic than water and ethanol extracts, against the four cancer cells studied. Of water and ethanol extracts, *G. indica* and turmeric, and in some cells cinnamon, tamarind and curry leaves were most effective. In general, ethanol extracts were superior to water extracts, and higher concentration correlated with lower cell viability. Antioxidant potential of water and ethanol extracts also had a similar trend, where cinnamon, turmeric, curry leaves and *Garcinia* spp. were superior. DPPH radical scavenging activity peaked around six months of storage in most ethanol extracts and around nine months in essential oil. FRA was most at six months in essential oils and increased with storage till a year in water and ethanol extracts. Total antioxidant potential peaked around three months in essential oils. In tamarind water and ethanol extracts the activity remained high even after a year of extraction.

Inhibition of human cancer cells by essential oils of several plants (eugenol, geraniol, farnesyl and geranyl-geranyl) has been reported. Essential oils are cytotoxic to eukaryotic cells as they are prooxidants, affecting inner cell membranes and mitochondria, but are usually non-genotoxic, long-term. This can be taken advantage for formulating antiseptic, antimicrobial and insecticidal agents. Prooxidant activity of essential oils and polyphenols can reduce tumor volume or cell proliferation by apoptotic and/or necrotic effects (Yoo *et al*., 15; Mazie `res *et al*., 7). Prooxidant radical production of essential oils can be controlled, dosages determined and targeted without being toxic or mutagenic to healthy tissues, thus they could make their foray into modern medicine.

To conclude, since this study reveals that essential oils are significantly cytotoxic to these four cancer cell lines. Studies on combinations of these oils, in various dosages for drug and their delivery to specific tumours, would be an exciting field of study. This study has also shortlisted the potential anticancer drug candidates in ethanol and water extracts of *G. indica*, turmeric, cinnamon, tamarind and curry leaf. The antioxidant potential and cytotoxicity of cancer cell lines are in broad conformity, and prove the superiority of some spices over others. Identification of bioactive phytochemicals in these spice extracts is another area for study. Though our previous studies have identified the major compounds, the complete

chemoprofiling is far from over. Thus, exploration and validation of nutraceutical properties of spices is a potential area for research and application.

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