



ANTI-TUMOR ACTIVITIES AND OIL CONSTITUENTS OF *ZINGIBER OFFICINALE* (ZINGIBERACEAE)

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ABSTRACT

The phytochemical studies on *Zingiber officinale* rhizomes revealed that it contains traces of flavonoid, carbohydrates, tannins, sterols or terpenoids and it is free from alkaloids. Assay of essential oil of *Zingiber officinale* rhizome was also carried out. The alcoholic extract of the plant rhizome, was examined against five tumor cell lines, BHK-2, HEPG-2, MCF-7, HCT-2 and HEP-2, using SRB assay. All cell lines were growth inhibited in a dose dependent manner after exposure to the plant extract. The antitumor activity of the plant extract using E.A.C. method, showed a high activity against mice tumor.

INTRODUCTION

Zingiberaceae, is a family of flowering plants consisting of aromatic perennial herbs with creeping rhizomal or tuberous rhizomes, comprising 52 genera and more than 1300 species, distributed throughout tropical Africa, Asia, and America. Many species are important ornamental plants as Zingiber (Kress, 2005).

There are various reports on the family Zingiberaceae in different parts of the world as it is used in folk medicine and yield medicinal substances (Janssen *et al* 1996; Mackeen *et al* 1997; Mohamed and Lajis, 2004; Kress *et al* 2005; Aguilardo, 2007; Dan *et al* 2007; Ibrahim *et al* 2007; Kaewsri and Paisooksantivatana, 2007 and Sharma *et al* 2007). So far, few works have been done on Zingiberaceae with regard to their essential oil composition (Zoghbi *et al* 1996; Vanida *et al* 2005; Zoghbi and Maria, 2005; Dan *et al* 2007 and Natta *et al* 2008). Cancer, after cardiovascular disease, is the second leading cause of death.

(Odunsanja, 2001; Celinger *et al* 2002 and Turgay and Turrstan, 2005). It is a complex disease, normally associated with a wide range of escalating effect both at the molecular and cellular levels. It therefore seems unlikely that chemoprevention follows simplistic rules and formulation success in primary prevention is reflected in the decreasing incidence of lung cancer particularly in men which parallel on earlier decrease in the consumption of tobacco (Bertram and Vine, 2005).

There are various reports on the effect of plant extract of several medicinal plants on different cancer cell lines (Li, 1996; Darzynkiewicz and Traganos, 2000; Dennis *et al* 2000; Li *et al* 2006 and Khosite *et al* 2008). Many workers, studied the antitumor activity of Zingiber extract (Matthes *et al* 1980; Havrilesky *et al* 1995; Bordia *et al* 1997; Vimala *et al* 1999; Habsah *et al* 2000 and Murrakumi *et al* 2004).

The objectives of this work are to evaluate the chemical composition of the essential oil of *Zingiber officinale* and assess its economic and medical values importance in production of bioactive materials affect on cancer cells.

MATERIALS AND METHODS

The Plant material of *Zingiber officinale* rhizome was obtained from Agricultural Research Institute, Egypt, 2007. The samples were air dried at room temperature, ground to coarse powder and stored in plastic bags in a dry dark place until used.

A- The plant Rhizome Extraction

One hundred grams of the dry plant rhizomes samples were extracted, using soxhlet apparatus by 70% ethyl alcohol. The obtained residue was dried and weighed.

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B- Preliminary Phytochemical Screening

The ethyl alcohol 70% extract was used for detecting the presence of flavonoids, carbohydrates, tannins, sterols, terpenoids and alkaloids.

C- Extraction of the essential oil

One hundred of dry rhizomes powder was subjected to steam distillation for 6 hours using a Dean – Stark apparatus (distillator). The essential oil was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure at room temperature and subjected to GC-Mass.

Gas liquid Chromatography and Gas liquid Chromatography Flame Ionization Detector Analysis (GC-MS and GC-FID analyses)

Constituents of the essential oil were analyzed by GC-MS and the major component found was further characterized by GC. Quantitative determination was carried out using linear calibration graphs, obtained from standard solutions of authentic compounds diluted with hexane in the concentration range of 50-150 ppm. Each dilution was carried out in triplicate and the mean value was used.

GC-MS analysis was performed on a HP 5890 GC-HP 5972 mass selective detector. The GC was fitted with HP-INNOWAX column. The inlet temperature was set at 250°C and the oven temperature was programmed from 70°C to 220°C (15 minutes) at 10°C/minute. The mass spectrometer was run in electron ionization mode, scanning at 45-550 amu, with a solvent delay time of 3.0 minutes and a transfer line temperature of 300°C. The relative proportion of each individual component of the oil was expressed as a percentage, relative to the total peak area.

Quantitative analysis was performed by GC on a Hewlett Packard HP-6890 series analyzer fitted with a flame ionization detector (FID) using a Shimadzu CBP 5: capillary column (25.0 m x 220 µm x 0.25 µm) with 5% methyl siloxane as the stationary phase. The carrier gas was helium at a flow rate of 3.5 mL/min. Injector and detector temperature were 250 and 300°C, respectively, and the oven temperature was programmed from 70°C to 220°C (15 minutes) at 10°C/minute with a running time of 30 min and an injection volume of 2 µL.

D- Measurement of Potential Cytotoxicity by SRB assay

The measurement of potential cytotoxicity of isolated drug from *Zingiber officinale* was carried out in National Cancer Institute, Cancer Biology Department, Pharmacology Unit, Cairo University. Cells were plated in 96-multiwell plate (10⁴ cells/well) for 24hrs before treatment with the compound (s) to allow attachment of cell to the wall of the plate. Different concentrations of the test extract (0, 1, 2.5, 5 and 10 µg/ml), added to the cell monolayer triplicate wells, were prepared for each individual dose. Monolayer cells were incubated with the compound (s) for 48hrs at 37°C and in atmosphere of 5% CO₂. After 48hrs, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with the EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration, is plotted to get the survival curve of each tumor cell line after the specified compound.

Anti -tumor activity of the (E.A.C)

Procedure

One ml of tumor cells which is drawn from mice being (E.A.C). A set of sterile test tubes used, where 2.5x10⁵ tumour cells per ml were suspended in phosphate buffer saline. Make 3 tubes with 3 different concentrations for the extract (25, 50, 100 µg/ml). Added 2.5x10⁵ tumour cells for each tube. Kept at 37°C for 2 hours. Take sample cells volume by volume with trypan blue on slide and cover it. Examine under microscope. Dead cells stained blue and live cell not stained. Then carried out to calculate the percentage of non viable cells (McIlmans et al 1957).

RESULTS AND DISCUSSION

The preliminary phytochemical screening of *Zingiber officinale* rhizome revealed that it contains flavonoids, tannins, carbohydrates, sterols and free from alkaloids. The essential oil from *Zingiber officinale* was obtained as a pale yellow oil in 4.0/100g dry wt. According to GC-MS analysis under the conditions described above, trans anethole (40 mg%) was detected as the main component of the essential oil (Table, 1).

Tabel 1. Oil Constituents of *Zingiber Officinale* Using GC-Mass

No	Oil Constituents	R.T	% mg dry wt.	M wt.	M. Formula
1	1,5-Heptadien-3-yne	4.8	10.32	92	C ₇ H ₈
2	Beta-Pinene	7.3	15.68	136	C ₁₀ H ₁₆
3	Estragole (Cumicaldehyde)	8.8	5.9	148	C ₁₀ H ₁₂ O
4	Trans-anethole	9.1	40	148	C ₁₀ H ₁₂ O
5	1,8 - Cineole	9.4	9.1	154	C ₁₀ H ₁₈ O
6	1,3 - isobenzo-furandione 4, 7-dimethyle	9.8	21	176	C ₁₀ H ₈ O ₃
7	Cyclo pentene -1-un-decanoic acid	11.5	3.2	252	C ₁₆ H ₂₈ O

Trans-anethole occurs naturally in the volatile oil of more than 20 species of plants (Newbrene *et al* 1999). It has a sweet and aromatic odor and has been used as a fragrance or flavoring in a variety of foods, alcoholic beverages and cosmetic products. Trans-anethole has been reported to increase salivary secretion (Fox, 1987) and has carminative and expectorant activity, it is widely used in therapeutics (Gracza, 1981). Thus the consumption of using *Z. officinale* rhizomes as herbal medicine is partially explained. Vieira *et al* (2001) reported that volatile and essential oils of some plants were used as phago-inhibitors and growth inhibitor, where the oxygenated compounds have anti-cancer effect. Li, (1996) reported that elemene and pinene were used for cure of bladder tumor.

Cytotoxicity effect

Cytotoxicity effect of *Z. officinale* extract was examined against five cell lines liver, cervical, colon, larynx and breast cell lines, HEPG2, HELA, HCT2, HEP2 and MCF7. The plant extract dilutions were 1, 2.5, 5 and 10 µg/ml for each cell line under examination. When the concentration of the plant extract increase the number of cell survival are decreased. For HEPG2 cell lines the survival cells ranged from 0.6 to 0.2, while for HELA cell line the survival cells ranged from 0.9 to 0.2. On the other hand for HCT2, the number of survival cells ranged between 0.8 to 0.4. For HEP2 the survival cells ranged from 1.02 to 0.5 and for MCF7 survival cells ranged between 0.9 to 0.43. IC50 was recorded in different values for each cell

line, it recorded 4.22µg/ml for HEPG2, 4.78 µg/ml for HELA, 3.16 µg/ml for HCT2; and 3.32 µg/ml for MCF7. (Table 2).

The effect of *Zingiber officinale* extract on the proliferation of five cancer cell lines were determined using SRB assay. All cell lines were growth inhibited in a dose dependent manner after exposure to the plant extract. When the activity of *Z. officinale* extract on five cancer cell lines showed a significantly different ratio against HEPG2, HELA, HCT2, HEP2 and MCF7, the result showed that the plant extract had selectively toxic against the five cancer lines (Fig. 1 and Table 2). Our results agree with assumption of Vieira *et al* (2001) as essential oils of some plants used as growth inhibitors. The results agree with Khosite *et al* (2008) as they examined plant extract against some cell lines and finally agree with Darzynkiewicz *et al* (2000) results, on prostate cancer.

Antitumor activity of *Z. officinale* extract against mice tumour gave good results, as the plant extract concentrations were 1, 2.5, 10 µg/ml, exhibited a good cytotoxicity ranging between 1.04 to 0.15 which gave an assumption that *Z. officinale* can be used as antitumour therapy.

The mechanism of action is unclear and possibly multiple compounds in the plant extract are involved. Plant derived sesquiterpenes which were reported to be the main constituents in *Zingiber officinale*. This results agree with the assumptions of Matthes *et al* (1980) and Murrakumi *et al* (2004) who reported that cytotoxic components of *Zingiber zotmbet* were terpenes, which agree with our results.

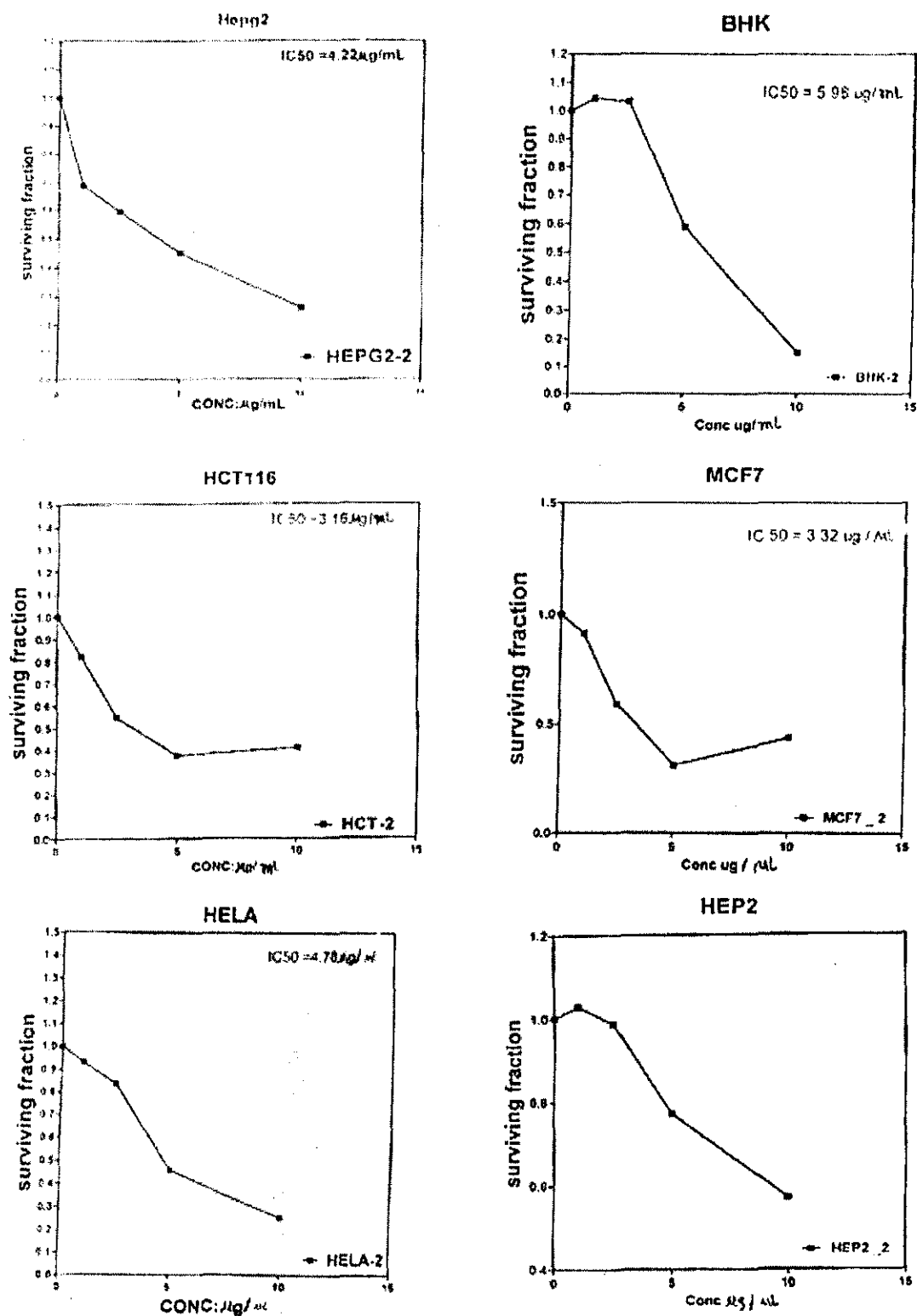
Fig. 1. The effect of *Zingiber officinale* alcohol extract on the different cancer cell line

Table 2. The effect of *Zingiber officinale* alcohol extract on the different cancer cell line

Conc. µg/ml.	HEPG2	HELA2	HCT2	HEP2	MCF7	BHK2
0.0	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
1.0	0.682887	0.930727	0.822504	1.028848	0.915621	1.045400
2.5	0.591361	0.836923	0.545791	0.987588	0.591297	1.045400
5.0	0.448573	0.456677	0.374606	0.772182	0.313673	
10.0	0.259726	0.253126	0.408537	0.571433	0.437872	

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النشاط المضاد للخلايا السرطانية والمحتوى الزيتي لنبات الزنجبيل
Zingiber officinale

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الموجز

BHK-2, HEPG-2, MCF-7, HCT-2 and HEP-2،
وهي سرطان الكبد والمثانة والقولون والثدي والمرئ
باستخدام طريقة SAB، انه ذات فاعلية وإيجابي
التأثير على هذه الأنواع من الخلايا.
كما انه تم دراسة النشاط المستخلص الكحولي ضد
خلايا التدرن في الفئران باستخدام طريقة E.A.C.
وقد أعطت نتائج جيدة .

اثبتت الدراسة الفيتو كيميائية على ريزومات نبات
الزنجبيل، انه يحتوى على الفلافونويدات والسكريات
والتانينات والتربينات. كما انه لا يحتوى على
القلويدات. وأثبتت النتائج ان المحتوى الزيتي للنبات
به العديد من المركبات التربينية بنسب مختلفة.
وقد دلت دراسة النشاط لمستخلص النبات الكحولي
ضد خمس انواع من الخلايا المسرطنة: