In vitro antioxidant and anticancer activity of young Zingiber officinale against human breast carcinoma cell lines

Shahedur Rahman1*, Faizus Salehin2 and Asif Iqbal1

Abstract

Background: Ginger is one of the most important spice crops and traditionally has been used as medicinal plant in Bangladesh. The present work is aimed to find out antioxidant and anticancer activities of two Bangladeshi ginger varieties (Fulbaria and Syedpuri) at young age grown under ambient (400 μmol/mol) and elevated (800 μmol/mol) CO2 concentrations against two human breast cancer cell lines (MCF-7 and MDA-MB-231).

Methods: The effects of ginger on MCF-7 and MDA-MB-231 cell lines were determined using TBA (thiobarbituric acid) and MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide] assays. Reversed-phase HPLC was used to assay flavonoids composition among Fulbaria and Syedpuri ginger varieties grown under increasing CO2 concentration from 400 to 800 μmol/mol.

Results: Antioxidant activities in both varieties found increased significantly (P ≤ 0.05) with increasing CO2 concentration from 400 to 800 μmol/mol. High antioxidant activities were observed in the rhizomes of Syedpuri grown under elevated CO2 concentration. The results showed that enriched ginger extract (rhizomes) exhibited the highest anticancer activity on MCF-7 cancer cells with IC50 values of 34.8 and 25.7 μg/ml for Fulbaria and Syedpuri respectively. IC50 values for MDA-MB-231 exhibition were 32.53 and 30.20 μg/ml for rhizomes extract of Fulbaria and Syedpuri accordingly.

Conclusions: Fulbaria and Syedpuri possess antioxidant and anticancer properties especially when grown under elevated CO2 concentration. The use of ginger grown under elevated CO2 concentration may have potential in the treatment and prevention of cancer.

Background

Cancer is a multi-step disease incorporating physical, environmental, metabolic, chemical and genetic factors, which play a direct and/or indirect role in the induction and deterioration of cancers. Diet containing antioxidant rich fruits and vegetables significantly reduces the risk of many cancer diseases suggesting that antioxidants could be effective agents for the inhibition of cancer spread. These agents are present in the diet as a group of compounds with low toxicity, safe and generally accepted [1]. The isolated polyphenols from different plants have been considered as indicator in a number of cancer cell lines at different evolutionary stages of cancer. Anticancer activities of Flavonoids were described in various studies [2]. Some tests showed anti-tumor properties of quercetin including the inhibition of cancer cell proliferation and migration [3]. The isolated polyphenols from strawberry including kaempferol, quercetin, anthocyanins, coumaric acid and ellagic acid were shown to inhibit the growth of human cancer cell lines originated from breast (MCF-7), oral (KB, CAL-27), colon (HT-29, HCT-116), and prostate (LNCaP, DU-145) [4]. Similar results have also been reported in other studies with wine extracts, isolated polyphenols (resveratrol, quercetin, catechin, and epicatechin) and green tea polyphenols (epigallocatechin, epicatechin) [5,6]. Arts et al. reported of catechin’s ability to control postmenopausal cancer in woman [7]. They found that catechin intake may prevent rectal cancer. Epicatechin and gallocatechin-3-gallate induce reduction in...
Epigallocatechin-3-gallate is an effective antiangiogenesis agent, which inhibits tumour cell invasion and proliferation [8]. It also inhibits the growth of the NBT-II bladder tumour cells and breast cancer cell lines [9]. Manthey et al. reported that citrus flavonoids inhibited the growth of HL-60 leukaemia cells [10]. Kaempferol belongs to the flavonoids group. Luo et al. showed kaempferol inhibited the growth of ovarian cancer cell lines (91%) and A2780/CP70 (94%) by concentration of 20 μM and 40 μM respectively [11]. Inhibition of breast cancer cell lines (MCF-7 and MDA-MB-231) by quercetin was reported by Gibellini et al. [12]. In recent years, researches about anticarcinogenic potential of quercetin have exhibited its promise as an anticancer agent. Likewise, in vitro and in vivo studies showed that quercetin was able to inhibit viability of leukemic cells, colon and ovarian carcinoma cells, and especially human breast cancer cells.

The Zingiberaceae family is well-known in Southeast Asia and many of its species are being used as traditional medicine, which is found to be effective in the treatment of several diseases. Zingiber officinale is generally used as a culinary spice in Bangladesh and as well as for the treatment of oral diseases, leucorrhoea, stomach pain, stomach discomfort, diuretic, inflammation and dysentery. Shukla et al. reported cancer preventive properties of ginger and showed that this ability is related to flavonoid and polyphenolic components of fresh ginger extract especially quercetin [13]. Kuokkanen et al. showed that the concentration of total phenolics was significantly increased in the birch leaves produced in the CO2-enriched air, as has also been observed in the experiments of Ibrahim et al. [14,15]. Emerging management strategies are using eco-physiological factors to elevate phytochemical concentrations in food crops. Some eco-physiological conditions that are thought to have significant impact on the enhancement of health-promoting phytochemicals in a number of plants include environmental conditions, cultural and management practices [16]. In addition, there is an increasing interest in using appropriate strategies of management practices to improve the quality of food crops by enhancing their nutritive and health-promoting properties. The results of previous studies indicated that the synthesis of phenolics and flavonoids in ginger can be increased and affected by using CO2 enrichment and following that, the antioxidant activity in young ginger extracts could also be improved [17]. Information about anticancer and antioxidant activities of enriched ginger by elevated CO2 concentration is scarce. On the other hand, the impacts of cultural conditions and CO2 concentration on biopharmaceutical production in herbs have not been widely investigated and it is needed to be understood, especially when the objective is the optimization of the herb chemistry. In this study, we aimed to explore antioxidant potential and anticancer activities of two Bangladeshi ginger varieties (Zingiber officinale) at young age and grown under different CO2 concentration.

**Methods**

**Plant material**

Two varieties of Zingiber officinale Roscoe (Fulbaria and Syedpuri) rhizomes were germinated for two and half weeks and then transferred to polyethylene bags which were filled with soilless mixture of burnt rice husk and coco peat in a ratio 1:1. After two and half weeks, those plants were transferred to CO2 growth chamber with two different CO2 concentrations (400 μmol/mol, ambient; 800 μmol/mol, elevated CO2 concentration). Pure carbon dioxide (99.6% purity) was supplied from high pressure carbon dioxide cylinder and injected through a pressure regulator into the growth chamber. Irradiance, relative humidity and air temperature of chamber were controlled using integrated control, monitoring and data management system software. Plants were harvested at 15 weeks and aerial parts and rhizomes separated and freeze dried and kept in -90°C for future analysis.

**Extract preparation**

Aerial parts and rhizomes were dried (freeze dry) to constant weights. Aerial parts and rhizomes (1 g) were powdered and extracted using methanol (50 ml), with continuous swirl for 1 h at room temperature using an orbital shaker. Extracts were filtered under suction, evaporated and crude extract stored at -25°C. These crude extracts were used in this study [18].

**Determination of antioxidant activity**

**TBA assay**

The method of Ottolenghi (1959) was used to determine the TBA (thiobarbituric acid) values of the samples [19]. The formation of malonaldehyde is the basis for the well-known TBA method used for evaluating the extent of lipid peroxidation. At low pH and high temperature (100°C), malonaldehyde binds TBA to form a red complex that can be measured at 532 nm. The increase amount of the red pigment formed correlates with the oxidative rancidity of the lipid. 2 ml of 20% trichloroacetic acid (CCl3COOH) and 2 ml TBA aqueous solution were added to 1 ml of sample solution and incubated. The mixture was then placed in a boiling water bath for 10 min. After cooling, it was centrifuged at 3,000 rpm for 20 min and the absorbance of the supernatant was measured at 532 nm. Antioxidant activity was determined based on the absorbance.
Cell culture and treatment
Human breast cancer cell lines (MCF-7 and MDA-MB-231) were obtained from the American Tissue Culture Collection (ATCC) (Rockville, MD) and were cultured in 100 µl of Roswell Park Memorial Institute medium (RPMI) 1640 media supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin. MCF-7 and MDA-MB-231 cells were incubated overnight at 37°C in 5% CO2 for cells attachment.

Both non invasive MCF-7 and highly invasive MDA-MB231 cancer cells were used in this study to verify the effectiveness of ginger extract against them.

Determination of anticancer activity

M TT assay
The assay detects the reduction of MTT [3-(4, 5-dimethylthiazolyl)-2, 5-diphenyl-tetrazolium bromide] by mitochondrial dehydrogenase to blue formazan product, which reflects the normal functioning of mitochondria and hence the cell viability. The experiment was conducted as described by Mosmann (1983) [21]. Briefly, the cancer cells were seeded in 96-well plates at a density of 1 × 10^4 cells/well in 100 µl RPMI. After twenty-four hours of seeding, the medium was removed and then the cells were incubated for 3 days with RPMI with the absence and/or the presence of various concentration of ginger extracts. Ginger extract was added at various concentrations ranging from 4.6, 9.3, 18.7, 37.5, 75, 150 and 300 µg/ml. After incubation, 20 µl of MTT reagent was added into each well. These plates were incubated again for 4 h in CO2 incubator at 37°C. The resulting MTT-products were taken both before and after hydrolysis, were filtered through a 0.45 µm filter [23].

Viability % = (optical density of sample/optical density of control) × 100

IC50 values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell line.

Same batch of ginger extracts were used for both TBA and MTT assay.

High performance liquid chromatography (HPLC)

Flavonoid extract preparation
Aliquots of aerial parts and rhizomes (0.25 g) were extracted with 60% aqueous methanol (20 ml). 6 M HCl (5 ml) was added to each extract to give a 25 ml solution of 1.2 M HCl in 50% aqueous methanol. Extracts were refluxed at 90°C for 2 h. Extract aliquots of 500 µl, taken both before and after hydrolysis, were filtered through a 0.45 µm filter [23].

Analysis of flavonoids composition
Reversed-phase HPLC was used to assay flavonoid compositions. The Agilent HPLC system used consisted of a model 1100 pump equipped with a multi-solvent delivery system and an L-7400 ultraviolet (UV) detector. The column was an Agilent C18 (5 µm, 4.0 mm internal diameter 250 mm). The mobile phase composed of: (A) 2% acetic acid (CH3COOH) and (B) 0.5% acetic acid-acetonitrile (CH3CN), (50:50 v/v), and gradient elution was performed as follows: 0 min, 95:5; 10 min, 90:10; 40 min, 60:40; 55 min, 45:55; 60 min, 20:80 and 65 min, 0:100. The mobile phase was filtered under vacuum through a 0.45 µm membrane filter before use. The flow rate was 1 ml/min and UV absorbance was measured at 280-365 nm. The operating temperature was maintained at room temperature [24]. Identification of the flavonoids was achieved by comparison with retention times of standards, UV spectra and calculation of UV absorbance ratios after coinjection of samples and standards [25].

Statistical analysis
The experimental results were expressed as mean ± standard deviation of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA) and the differences among samples were determined by Duncan’s Multiple Range Test using the SPSS v14 and MSTATC programs. P-value of ≤ 0.05 was regarded as significant.

Results and discussion

Antioxidant activity
The results obtained from the preliminary analysis of antioxidant activity are shown in Table 1. According to the data obtained significant differences were observed among treatments for antioxidant activities. From the

| CO2 (µmol/mol) | Varieties | Parts   | TBA          |
|---------------|-----------|---------|--------------|
|               | Fulbaria  | Aerial parts | 69.29 ± 1.22  |
|               | Fulbaria  | Rhizomes  | 67.93 ± 1.81  |
| 400           | Syedpuri  | Aerial parts | 70.59 ± 1.52  |
|               | Syedpuri  | Rhizomes  | 67.79 ± 1.04  |
|               | Fulbaria  | Aerial parts | 71.01 ± 1.71  |
|               | Fulbaria  | Rhizomes  | 75.05 ± 1.63  |
| 800           | Syedpuri  | Aerial parts | 73.78 ± 1.21  |
|               | Syedpuri  | Rhizomes  | 77.98 ± 1.20  |

All analyses are the mean of triplicate measurements ± standard deviation. Means not sharing a common letter were significantly different at P < 0.05. Results expressed in percent.

Table 1 Antioxidant activity of Zingiber officinale extracts grown under different CO2 concentrations (measured by the TBA method)
result, the antioxidant activity of aerial parts was higher than rhizomes extracts in both varieties that were grown under ambient CO2 concentration. The results also had indicated that antioxidant activities increased significantly by elevated CO2 concentration. Antioxidant activity was enhanced in rhizomes by elevated CO2 concentration more than in aerial parts with highest value of TBA (77.98%) were obtained from Syedpuri rhizomes. The aerial parts extract of Fulbaria and Syedpuri in ambient and elevated CO2 condition exhibited strong potential of free radical scavenging activity. According to the results, TBA content of the Syedpuri aerial parts grown in ambient CO2 concentration reached to 70.59%, while at the same extract concentration, that of the rhizomes was 67.79%. In ambient CO2 concentration, differences between aerial parts and rhizomes in both varieties for TBA activity was not significant, while in elevated CO2 concentration significant differences was observed between different parts of each variety. Many researchers had shown that high total flavonoids content increases antioxidant activity and there was a linear correlation between flavonoids content and antioxidant activity [18,26].

**Anticancer activity**

As shown in Table 2, parts (aerial parts and rhizomes) of two ginger varieties were found to express MCF-7 and MDA-MB-231 cancer cell inhibitory activity when tested at concentrations of 4.6-300 μg/ml. At a concentration of 37.5 μg/ml, though, most of the extracts exhibited strong anticancer activity towards MCF-7 and MDA-MB-231 cells, at this concentration, extract of Syedpuri rhizomes grown under elevated CO2 concentration exhibit lowest MCF-7 and MDA-MB-231 cell viability at 39.01% and 40.16% respectively. Moreover, MCF-7 and MDA-MB-231 treated with tamoxifen (positive control) showed 24.9% and 26.7% viability in same concentration (37.5 μg/μl). In contrast, for MCF-7 cell, the anticancer activity of aerial parts extract in ambient and elevated CO2 concentration was significantly stronger than that of the rhizomes extract especially in Syedpuri variety. In addition, for MDA-MB-231 cell, the anticancer activity of aerial parts extract in ambient CO2 concentration was significantly stronger than that of the rhizomes extracts, but, with increasing of CO2 concentration anticancer power increased significantly in rhizomes of both varieties. However, of all extracts investigated, Syedpuri rhizomes that were obtained from plants grown under elevated CO2 concentration exhibited the strongest anticancer activities towards cancer cells. The IC50 values for MCF-7 and MDA-MB-231 cells were 25.7 and 30.2 μg/ml respectively (Table 3). While IC50 value of rhizomes extract of Syedpuri grown in ambient CO2 for MCF-7 and MDA-MB-231 cells were 47 and 38.8 μg/ml accordingly. However, with the increase of CO2 concentration, IC50 value decreased significantly in both varieties. Furthermore, IC50 values of tamoxifen as a positive control for MCF-7 and MDA-MB-231 cells were 19.7 and 22.89 μg/ml respectively.

**HPLC analysis of flavonoids**

The results obtained from the preliminary analysis of flavonoids are shown in Table 4. Increasing the CO2 concentration from 400 to 800 μmol/mol resulted in enhanced quercetin, catechin, kaempferol and fisetin levels in the aerial parts and rhizomes of both varieties. On the other hand, the contents of epicatechin and morin decreased in ginger parts with rising of CO2 concentration from ambient to 800 μmol/mol. Some study results indicated that increasing the CO2 concentration from 400 to 800 μmol/mol resulted in enhanced quercetin, catechin, kaempferol and fisetin levels in the aerial parts.

### Table 2: Anticancer activities of Zingiber officinale extracts against MCF-7 and MDA-MB-231 cell lines (determined by the MTT assay at concentration 37.5 μg/ml)

| CO2 (μmol/mol) | Varieties | Parts | MCF-7 | MDA-MB-231 |
|----------------|-----------|-------|-------|------------|
| 400            | Fulbaria  | Aerial parts | 59.65 ± 2.55b | 63.31 ± 1.85a |
|                |           | Rhizomes    | 57.56 ± 1.68b | 69.41 ± 2.30c |
| 800            | Fulbaria  | Aerial parts | 50.65 ± 0.56a | 58.12 ± 1.09a |
|                |           | Rhizomes    | 56.98 ± 1.74b | 66.61 ± 2.31c |
|                | Syedpuri  | Aerial parts | 40.37 ± 1.46c | 48.16 ± 1.03c |
|                |           | Rhizomes    | 48.97 ± 1.04d | 44.35 ± 1.86d |

All analyses are the mean of triplicate measurements ± standard deviation. Results expressed in percent of cell viability.

### Table 3: IC50 values of Zingiber officinale extracts against MCF-7 and MDA-MB-231 cancer cell lines (expressed in μg/ml)

| CO2 (μmol/mol) | Varieties | Parts | MCF-7 | MDA-MB-231 |
|----------------|-----------|-------|-------|------------|
| 400            | Fulbaria  | Aerial parts | 51.39 ± 1.32b | 56.12 ± 2.15a |
|                |           | Rhizomes    | 52.01 ± 2.11b | 62.81 ± 1.60a |
| 800            | Fulbaria  | Aerial parts | 36.80 ± 1.32a | 46.87 ± 0.45a |
|                |           | Rhizomes    | 47.00 ± 1.16b | 38.80 ± 1.81b |

All analyses are the mean of triplicate measurements ± standard deviation. Results expressed in percent of cell viability.
Table 4 The concentrations of some flavonoids compounds in two varieties of *Zingiber officinale*, Fulbaria and Syedpuri grown under various CO₂ concentrations

| Flavonoid compounds | Fulbaria | Syedpuri |
|---------------------|----------|----------|
|                     | 400      | 800      | 400    | 800    |
|                     | Aerial parts | Rhizomes | Aerial parts | Rhizomes | Aerial parts | Rhizomes | Aerial parts | Rhizomes |
| Quercetin           | 0.961 ± 0.013<sup>a</sup> | 0.894 ± 0.039<sup>a</sup> | 1.22 ± 0.06<sup>a</sup> | 1.19 ± 0.022<sup>bc</sup> | 0.985 ± 0.032<sup>a</sup> | 1.33 ± 0.124<sup>b</sup> | 1.26 ± 0.01<sup>b</sup> |
| Epicatechin         | 0.128 ± 0.028<sup>b</sup> | 0.085 ± 0.007<sup>de</sup> | 0.073 ± 0.009<sup>c</sup> | 0.049 ± 0.018<sup>c</sup> | 0.12 ± 0.004<sup>b</sup> | 0.103 ± 0.0034<sup>de</sup> | 0.096 ± 0.021<sup>bc</sup> | 0.038 ± 0.009<sup>c</sup> |
| Catechin            | 0.416 ± 0.024<sup>c</sup> | 0.492 ± 0.020<sup>bc</sup> | 0.673 ± 0.044<sup>bc</sup> | 0.637 ± 0.044<sup>c</sup> | 0.668 ± 0.079<sup>a,d</sup> | 0.533 ± 0.034<sup>bc</sup> | 0.734 ± 0.014<sup>b</sup> | 0.684 ± 0.05<sup>c</sup> |
| Kaempferol          | 0.041 ± 0.006<sup>d</sup> | 0.052 ± 0.003<sup>c,d</sup> | 0.117 ± 0.014<sup>a</sup> | 0.147 ± 0.023<sup>a</sup> | 0.051 ± 0.002<sup>c</sup> | 0.067 ± 0.005<sup>c</sup> | 0.162 ± 0.011<sup>bc</sup> | 0.184 ± 0.019<sup>b</sup> |
| Fisetin             | 0.982 ± 0.022<sup>d</sup> | 0.633 ± 0.033<sup>F</sup> | 2.051 ± 0.27<sup>a</sup> | 2.88 ± 0.19<sup>b</sup> | 1.53 ± 0.121<sup>F</sup> | 1.32 ± 0.13<sup>c</sup> | 2.37 ± 0.397<sup>bc</sup> | 3.12 ± 0.18<sup>b</sup> |
| Morin               | 0.532 ± 0.057<sup>d</sup> | 0.464 ± 0.014<sup>abc</sup> | 0.491 ± 0.052<sup>d</sup> | 0.876 ± 0.046<sup>c</sup> | 0.765 ± 0.024<sup>c</sup> | 0.607 ± 0.006<sup>c</sup> | 0.662 ± 0.029<sup>a</sup> | 0.517 ± 0.025<sup>d</sup> |

All analyses are the mean of triplicate measurements ± standard deviation. Means not sharing a common letter were significantly different at P ≤ 0.05. Results expressed in mg/g of dry plant material.
parts and rhizomes of *Zingiber officinale* varieties and following that, the antioxidant activity in young ginger extracts could also be improved [25]. Findings of this current study supported previous researcher’s findings and showed that anticancer effect of ginger extracts increase with increasing CO₂ concentration.

Flavonoids are among the best candidates for mediating the protective effect of diets which are found in fruits and vegetables with respect to colorectal cancer. Study shows relative activity being as quercetin > apigenin > fisetin > kaempferol. Quercetin belongs to the flavonoids group due to its powerful antioxidant activity. Previous studies showed that quercetin may help to prevent cancer, especially prostate cancer [27]. Scambia *et al.* reported quercetin inhibited human breast cancer cells (MCF-7 and MDA-MB231) significantly [28]. Dutt *et al.* explained mechanism of breast cancer inhibition by quercetin [29]. In ginger quercetin is abundant flavonoid compound [25,26,30]. Antioxidant activity of quercetin was believed to have cytoprotective role against oxidative stress. It seemed that quercetin not only protects cells from free radical damage through antioxidant effect, but also motivates apoptotic cell death via pro-oxidant activity and inhibits tumourigenesis. Hence, anticancer power maybe related to quercetin content in those varieties. In addition, flavonoid compounds could probably be responsible for the anticancer activity of *Zingiber officinale*. Further research is required to untangle the specific bioactive compounds responsible for the anticancer properties of the extracts of *Zingiber officinale* varieties.

Conclusions

Currently, about 50% of drugs used in clinical trials for anticancer activity were isolated from natural sources such as herbs and spices or related to them [31]. A number of active compounds such as flavonoids, diterpenoids, triterpenoids and alkaloids have been shown to possess anticancer activity. According to the report of the American National Cancer Institute (NCI), the criterion of anticancer activity for the crude extracts of herbs is an IC₅₀<30 μg/ml [32]. Thus, according to the results from current study seems that enriched ginger varieties developed by elevated CO₂ concentration could be employed in ethno-medicine in the treatment of cancerous diseases.

There are some limitations of this study. Relationship between flavonoids concentration and antioxidant activity were not determined. Moreover, only cytotoxicity was determined but apoptosis and cell cycle analysis were not performed.

Our results in this study indicate that some compounds in Bangladeshi ginger varieties at young age possess anticancer activities and may contribute in the therapeutic effect of this medicinal herb. However, there is a need of detailed scientific study on traditional medical practices to ensure that valuable therapeutic knowledge of some plants is preserved and also to provide scientific evidence for their efficacies.

Acknowledgements

We thank staffs and kind support of the Department of Biotechnology and Genetic Engineering, Islamic University.

Author details

1. Department of Biotechnology and Genetic Engineering, Islamic University, Kushabia-7003, Bangladesh. 2. Department of Biotechnology and Genetic Engineering, University of Development Alternative, Dhaka, Bangladesh.

Authors’ contributions

SR and FS participated in the design, coordinating and carried out the study and also drafted the manuscript. AI performed the statistical analysis. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 7 August 2011 Accepted: 20 September 2011

Published: 20 September 2011

References

1. Fresco P, Borges F, Diniz C, Marques MP: New insights on the anticancer properties of dietary polyphenols. *Med Res Rev* 2006, 26:747-766.
2. Mavundza EJ, Tshikalange TE, Lall N, Hussein AA, Mudau FN, Meyer JJM: Antioxidant activity and cytotoxicity effect of flavonoids isolated from *Atherixia phyllicoides*. *J Med Plant Res* 2010, 4:2584-2587.
3. Rim JH, Park JW, Min DS, Chang JS, Lee YH, Park YB, Choi KS, Kwon TK: NAG-1 up-regulation mediated by EGR-1 and p53 is critical for quercetin-induced apoptosis in HCT116 colon carcinoma cells. *Apostosis* 2006, 12:411-421.
4. Zhang J, Li Q, Di X, Liu ZH, Xu G: Layer-by-layer assembly of multicoloured semiconductor quantum dots towards efficient blue, green, red and full color optical films. *Nanotechnology* 2008, 19:435-606.
5. Kampa M, Hatzioglou A, Notas G, Damanaki A, Bakogiorgou E, Gerneti C, Kouromalis E, Martin PM, Castanias E: Wine antioxidant polyphenols inhibit the proliferation of human prostate cancer cell lines. *Nutr Cancer* 2003, 47:225-233.
6. Weisburg JH, Weissman DB, Sedaghat T, Babich H: In vitro anti-cancer of epigallocatechin gallate and tea extracts to cancerous and normal cells from the human oral cavity. *Basic Clin Pharmacol Toxicol* 2004, 95:191-200.
7. Arts IC, Jacobs DRJ, Gross M, Harnack LJ, Folsom AR: Dietary catechins and cancer incidence among postmenopausal women: the Iowa Women’s Health Study (United States). *Cancer Causes Control* 2002, 13:373-382.
8. Fang T, Chang E, Shih C: Green tea catechin inhibits ephrin-A1-mediated cell migration and angiogenesis of human umbilical vein endothelial cells. *J Nucl Biochem* 2007, 18:391-399.
9. Chen JJ, Ye ZQ, Koo MML: Growth inhibition and cell cycle arrest effects of epigallocatechin gallate in the NBT-II bladder tumour cell line. *BJU Int* 2004, 93:1082-1086.
10. Manthey JA, Grohmann K, Guthrie N: Biological properties of citrus flavonoids pertaining to cancer and inflammation. *Curr Med Chem* 2001, 8:135-153.
11. Luo H, Rankin GO, Liu L, Daddysman MK, Jiang BH, Chen YC: Kaempferol inhibits angiogenesis and VEGF expression through both HIF dependent and independent pathways in human ovarian cancer cells. *Nutr Cancer* 2009, 61:554-563.
12. Gabelini L, Pinti M, Nasi M, De Bisio S, Roat E, Bertoncelli L, Cossarizza A: Interfering with ROS Metabolism in Cancer Cells: The Potential Role of Quercetin. *Cancers* 2010, 2:1288-1311.
13. Shukla Y, Prasad S, Tripathi C, Singh M, George J, Kalra N: In vitro and in vivo modulation of testosterone mediated alterations in apoptosis related proteins by [6]-gingerol. *Mol Nutr Food Res* 2007, 51:1492-1502.
14. Kuokkanen K, Julkunen-Titto R, Keinanen M, Niemela P, Tahvanainen J: The effect of elevated CO₂ and temperature on the secondary chemistry of Betula pendula seedlings. Trees 2001, 15:378-384.
15. Itharat A, Houghton PJ, Eno-Amooquaye E, Burke PJ, Sampson JH, Raman A: The Relationship between Phenolics and Flavanon Production with Total Non Structural Carbohydrate and Photosynthetic Rate in Lysisia purulia Benth. under High CO₂ and Nitrogen Fertilization. Molecules 2011, 16:162-174.
16. Schreiner M: Vegetable crop management strategies to increase the quantity of phytochemicals. Eur J Nutr 2005, 44:85-94.
17. Malikov VM, Yuledashiev MP: Phenolic compounds of plants of the Scutellaria L. genus: distribution, structure, and properties. Chem Nat Compd 2002, 38:358-406.
18. Ghasemzadeh A, Jaafar HZE, Rahmat A: Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (Zingiber officinalis Roscoe). Molecules 2010, 15:4324-4333.
19. Oitojenghi A: Interaction of ascorbic acid and mitochondria lipids. Arch Biochem Biophys 1959, 79:355.
20. Jin S, Zhang YF, Kang XM, Wang JX, Zhao WH: Daidzein induces MCF-7 breast cancer cell apoptosis via the mitochondrial pathway. Ann Oncol 2010, 21:263-268.
21. Mosmann T: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983, 65:55-63.
22. Lau CS, Ho CY, Kim CF, Leung KN, Fung KP, Tse TF, Chan HL, Chow MS: Cytotoxic activities of Coriolus versicolor (Yunzhi) extract on human leukemia and lymphoma cells by induction of apoptosis. Life Sci 2004, 75:797-808.
23. Crozier A, Jensen E, Lean MEJ, McDonald MS: Quantitative analysis of flavonoids by reversed-phase high performance liquid chromatography. J Chromatogr 1997, 761:315-321.
24. Wang TC, Chuang YC, Yu YH: Quantification of bioactive compounds in citrus fruits cultivated in Taiwan. Food Chem 2007, 102:1163-1171.
25. Ghasemzadeh A, Jaafar HZE, Rahmat A: Elevated carbon dioxide increases contents of flavonoids and phenolic compounds, and antioxidant activities in Malaysian young ginger (Zingiber officinalis Roscoe.) varieties. Molecules 2010, 15:7907-7922.
26. Ghasemzadeh A, Jaafar HZE, Rahmat A: Identification and concentration of some flavonoid components in Malaysian young ginger (Zingiber officinalis Roscoe) varieties by a high performance liquid chromatography method. Molecules 2010, 15:6231-6243.
27. Rietjens IM, Boersma MG, van der Woude H, Jeurissen SM, Schutte ME, Alink GM: Flavonoids and alkylbenzenes: mechanisms of mutagenic action and carcinogenic risk. Mutat Res 2005, 574:124-138.
28. Scambia G, Ranelletti FO, Panici PB: Quercetin potentiates the effect of adriamycin in a multidrug-resistant MCF-7 human breast cancer cell line: P-glycoprotein as a possible target. Cancer Chemother Pharmacol 1994, 34:459-464.
29. Du G, Lin H, Wang M, Zhang S, Wu X, Lu L, Ji L, Yu L: Quercetin greatly improved therapeutic index of doxorubicin against 4T1 breast cancer by its opposing effects on HIF-1α in tumor and normal cells. Cancer Chemother Pharmacol 2010, 65:277-287.
30. Khaki AA, Khaki A, Ahmadi-Ashtiani HR, Rasegar H, Rezaazadeh Sh, Babazadeh D, Zahedi A, Ghanbari Z: Treatment Effects of Ginger Rhizome & Extract of Carrot seed on Diabetic Nephropathy in Rat. J Med Plant 2010, 9:75-80.
31. Newman DJ, Cogg GM: Natural products as sources of new drugs over the last 25 years. J Nat Prod 2007, 70:461-477.
32. Itharat A, Houghton PJ, Eno-Amooquaye E, Burke PJ, Sampson JH, Raman A: In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. J Ethnopharmacol 2004, 90:53-58.

Pre-publication history
The pre-publication history for this paper can be accessed here: http://www.biomedcentral.com/1472-6882/11/76/prepub

doi:10.1186/1472-6882-11-76
Cite this article as: Rahman et al.: In vitro antioxidant and anticancer activity of young Zingiber officinale against human breast carcinoma cell lines. BMC Complementary and Alternative Medicine 2011 11:76.