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## ***In Vitro* Anti Proliferative Activity, Antioxidant Potential and Total Phenolic Compounds of Black Tea Extract**

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### **ABSTRACT**

Natural products, mainly isolated from medicinal plants, have considered as valuable sources for herbal anticancer drugs. The present study aimed to determine total phenolic and flavonoid contents, antioxidant activity, and anti proliferative activity of black tea (*Camellia sinensis* Kuntze) extract *in vitro*. Crude ethyl alcohol extract of black tea was prepared. To determine antioxidant activity, total phenol, and flavonoids content of the extract, the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay, Folin-Ciocalteu method and aluminum chloride colorimetric method were used, respectively. The anti proliferative activity in cancerous (AGS) and normal (HDFs) cell lines was tested by MTT [3-(4, 5-dimethylthiazol-2-yl) 2, 5 di phenyl tetrazolium bromide] assay. The IC<sub>50</sub> of DPPH radical assay was 8±1.41 µg/ml, compared with butylated hydroxytoluene (BHT) with IC<sub>50</sub> 25.41±1.89 µg/ml. The total phenol and flavonoid contents were 341.8±4.41 mg GAE/g and 21.1±2.11 mg RUT /g, respectively. The extract showed higher anti proliferative activity against the cancer cell line than normal cell line. 48 hours after treatment, crude ethyl alcohol extract inhibited the proliferation of AGS and HDFs cells with IC<sub>50</sub> values of 264.3 µg/ml and 689.5 µg/ml, respectively. This study revealed that the crude ethyl alcohol extract of black tea suppresses the proliferation of gastric carcinoma cells. It is a rich resource of natural antioxidants and can prevent a lot of diseases such as cancer.

**Key Words:** Black tea, Gastric cancer, Free radical scavengers, Cytotoxicity.

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### **INTRODUCTION**

Gastric cancer is one of the most notable reason of cancer-related deaths worldwide and a main cause of cancer death in Asia [1]. Treatment option for these patients includes chemotherapy and surgery. But, these treatments have many side effects and are inefficient [2]. Many studies on protective and therapeutic effects of drugs on gastric cancer has been reported so far [3]. Various flavonoids have been found to inhibit the development of cancer. Epidemiological studies have shown the relationship between consumption of flavonoids in fruits and

vegetables with reduce the incidence of cancer [4]. Medicinal plants have been used to the treatment of human diseases for many years [5, 6]. A number of medicinal plants have been hopeful results as therapeutic agents [7-15]. Plants have the ability to synthesize a wide row of compounds which have long been used as remedies. Some of them are now being collected and examined to identify possible sources of drugs [13, 16-29].

Teas from *Camellia sinensis* are widely consumed in more than fifty centuries in the world [30]. Different teas from *C. sinensis* are obtained on the basis auto-



oxidation process catalyzed by polyphenol oxidase and peroxidase enzymes. Thus, the teas are classified as oxidized (black tea), semioxidized (oolong tea), non-oxidized (green and white tea), and post-fermented (pu-erh tea). The most consumed teas are the green and black teas [31]. Several classes of compounds have been identified in different types of *C. sinensis* teas such as phenolic compounds, terpenes, alkaloids. Also vitamins (such as A, K, B, and C), tocopherols, carotenoids, and minerals such as manganese, phosphorus, potassium, fluorine, magnesium, and iron have been identified in the *C. sinensis* [32-34]. Moreover, the primary metabolism products such as polysaccharides, proteins and lipids have been identified in this plant [34, 35]. The compounds found in teas presented different biological properties, as established by the number of *in vivo* and *in vitro* studies [36], the main ones being antioxidant activity [37], anti-inflammatory [38], antisepticemic [35], anticarcinogenic [39-41], hypocholesterolemic [42], antibacterial effects [43], preventive effects on postoperative intra-abdominal adhesions [44], protective effects for the renal injury induced by contrast media [45]. Based on our information has been no reported about antioxidant activities and anti-proliferative effect of black tea in gastric cancer cell line (AGS) so far. Therefore, this research was purposed to evaluate the antioxidant and anti proliferative activity of *C. sinensis* Kuntze *in vitro*.

## MATERIALS AND METHODS

### Preparation of hydroalcoholic extract

Black tea was purchased from a local market. The leaves powder (100 g) was dissolved in 70% ethyl alcohol (400ml) and kept at room temperature (RT) for 96 h. Subsequently, the mixture was filtered and concentrated under nearly vacuum pressure and at 40°C using rotary evaporator. The extracts were kept in sterile bottles under refrigerated conditions until further use. The extracts were suspended at 37°C in dimethyl sulphoxide (DMSO) to give a stock solution of 25 mg/mL, dissolved in culture medium, filtered (Millipore® 0.22 µm) and stored (4°C) until use. The remaining DMSO in the wells (maximal 0.2%) did not significant effect on the experiment results [46].

### Determination of the free-radical scavenging activity

The free-radical scavenging activity was measured by the 2,2 diphenyl-1-picrylhydrazyl (DPPH) method described by Moon and Terao, with some modification [47]. Different amounts of the extract and methanol were added to a solution of 0.3 mg/mL methanolic solution of DPPH to make up a total volume of 3.0 mL. After standing for 15 min at room temperature, the absorbance was measured at 517 nm using UV-Vis spectrophotometer (UNICO 2100: USA). Butylated hydroxytoluene (BHT) was used as positive control. Inhibition of free radical by DPPH was calculated as follows:

$$\text{Antiradical activity (\%)} = \frac{(A \text{ control} - A \text{ sample})}{A \text{ control}} \times 100.$$

### Determination of total phenolic content

The total phenolic content of the black tea extract was determined using Folin-Ciocalteu method [48], with some modification. Briefly, 0.1 ml of the diluted sample was added to 0.5 ml of 10% (v/v) Folin-Ciocalteu reagent and kept at RT for 3-8 min. Subsequently, 0.4 ml of 7.5% (w/v) sodium carbonate solution was added to the mixture. After being kept in total darkness for 30 min., the absorbance of the reaction mixture was measured at 765 nm using a UV-Vis spectrophotometer (UNICO 2100: USA). Amounts of total phenolic were calculated using a gallic acid calibration curve.

### Determination of total flavonoid content

The total flavonoid content of the extract was measured as previously reported method [49]. Briefly, 0.5 ml of diluted plant material was independently mixed with 1.5 ml of methanol, 0.1 ml of 10% (w/v) aluminum chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water. Following incubation at RT for 40 min, the absorbance of the reaction mixture was read at 415 nm using a UV-Vis spectrophotometer (UNICO 2100: USA). The results were expressed in mg of rutin equivalents of dry plant matter by comparison with the standard curve, which was made in the same condition.

### Cells and cell culture

AGS (human gastric carcinoma) cell line was purchased from Pasteure Institute of Iran and Human dermal fibroblasts (HDFs) cell line was kindly provided by the Cellular and Molecular Research Center of Shahrekord University of Medical Science, Iran. All the cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen-Gibco, Carlsbad, California,) supplemented with 10% of fetal bovine serum (FBS; Gibco), 100 µg/mL of streptomycin (Sigma-Aldrich Chemicals, St. Louis, MO, USA), 100 UI/mL of penicillin (Sigma) and 0.25 µg·mL<sup>-1</sup> amphotericin B (Gibco), at 37 °C in a humidified air atmosphere containing 5% (V/V) CO<sub>2</sub>.

### MTT assay

The cells were seeded into 96-well plates (SPL Life Sciences, Korea) at a density of 5000 cells per well in a final volume of 100 µL per well. After incubation at 37 °C with 5% CO<sub>2</sub> for 24 h, overlay medium was aspirated to allow the cells attach to the bottom of each well. Subsequently, the cells were treated with various concentrations of the extract and incubated at 37 °C with 5% CO<sub>2</sub>. The number of living cells was determined by the ability to cleave the tetrazolium salt MTT [3-(4, 5-dimethylthiazol-2-ol) 2, 5 diphenyl tetrazolium bromide] by the mitochondrial enzyme succinate dehydrogenase which develops a formazan blue color product. The procedure was as described previously [50].

Each experiment was carried out in triplicate and the percentage survival of the treated cancer and normal cultured cells was calculated according to the formula as follows:

Percentage of survival (%) = (Absorbance of treated cells/ Absorbance of control) × 100

The 50% inhibitory concentration (IC<sub>50</sub>) was calculated by regression analysis and related models with probit regression model procedure, using the SPSS software (version 16.0).

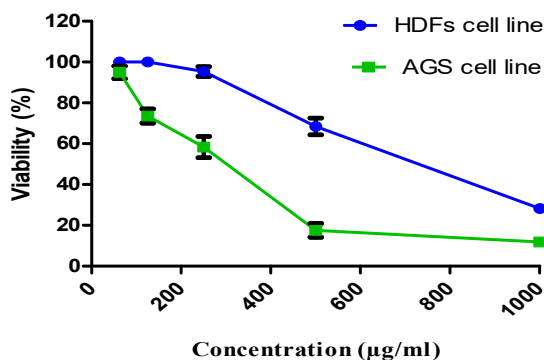
## RESULTS

### Antioxidant capacity, phenolic compounds, and flavonoids

Total phenolic and flavonoid amounts of black tea extract were 341.8±4.41 mg GAE/g and 21.1±2.11 mg RUT/g, respectively. The antioxidant activity of crude extract and BHT was 8±1.41 µg/ml and 25.41±1.89 µg/ml, respectively (table1).

**Table 1.** DPPH radical-scavenging activity of the crude ethyl alcohol of black tea

Sample	Concentration (µg/ml)	Scavenging of DPPH radical activity inhibition (%)	DPPH-radical scavenging activity IC <sub>50</sub> (µg/ml)
Black tea extract	30	99±2.2	8±1.41
	20	98±1.7	
	10	70±1.5	
	5	23.15±2.6	
	2.5	21.7±0.9	
BHT	50	90.8±1.5	25.41±1.89
	40	78.3±1.2	
	30	55.5±0.7	
	20	40.09±1.7	
	10	22±1.06	



**Figure 1.** Anti proliferative activity of crude ethyl alcohol extract of black tea on AGS (cancerous) and HDFs (normal) cell lines. AGS (cancerous) and HDFs (normal) cell lines were treated with different concentrations of the extract for 48 h and cell viability was determined using MTT assay. AGS: Human Gastric Carcinoma; HDFs: Human Dermal Fibroblast.

### Antiproliferative activity

The results showed that cell viability of AGS (cancerous) and HDFs (normal) cell lines was significantly reduced in a dose-dependent manner following treatment with the crude ethyl alcohol extract (Figure 1). Based on Probit regression model, antiproliferative activity of the crude ethyl alcohol extract on the two cell lines studied was significantly different ( $P < 0.001$ ). The IC<sub>50</sub> value of the extract for AGS and HDFs was 264.3 µg/mL (CI 95%: 227.3-307.4) and 689.5 µg/mL (CI95%: 650.6-730.7), respectively.

## DISCUSSION

In recent years, considerable efforts have been done to identify naturally occurring compounds and related synthetic agents that can prevent the development and recurrence of cancer. Several medicinal plants have been found to have cancer cell proliferation effects [51-55]. They can induce apoptosis in various tumor cells via their antioxidant and anti-inflammatory effects [56]. The beneficial effects of plant extracts are derived from their constituent phytochemicals that include polyphenols, alkaloids, and nitrogen and sulfur containing compounds [57]. Phytochemicals extracted from plants are excellent chemotherapeutic agents, which are easily available, inexpensive, nontoxic, and well tolerated [58]. Actually natural antioxidants are rich resources for prevention and treatment of the diseases [59-66]. Our results showed that cell viability of AGS was significantly reduced in a dose-dependent manner, following treatment with crude ethyl alcohol extract of black tea. Also the extract has the phenolic and flavonoid compounds that caused to show the antioxidant activity more than BHT.

Some plant antioxidants have been suggested to contribute to their anti-carcinogenic effects and their flavanols have been reported to inhibit cancer cell proliferation *in vitro* [67]. Tea is one of the most popular beverages consumed around the world, second only to water. Polyphenols are the naturally occurring compounds in fresh tea leaves and account for its pungency and unique flavor. The four primary polyphenols in tea leaves are epigallocatechin, epicatechin, epicatechin gallate, and epigallocatechin gallate [68]. There has been extensive *in vitro* research regarding the possible cancer prevention mechanisms by green and black tea extracts and their polyphenols using human breast cancer cell lines. These studies suggested that multiple mechanisms are involved, including induction of apoptosis [69], cell cycle arrest [70], down-regulation of telomerase [71], inhibition of vascular endothelial growth factor [72] and suppression of aromatase activity [73]. Both green and black tea extracts also have demonstrated cancer preventive properties in carcinogen-induced or transplanted mammary tumors in experimental animal studies. Green tea extracts or catechins fed to rodents after administration of chemical carcinogens decreased the size and multiplicity of mammary tumors [74, 75].

Although less extensively studied, black tea extracts, given before carcinogen challenge, have been shown to reduce the tumor number, size and multiplicity in carcinogen-treated rats on a high fat diet [76, 77]. However, the relationship between tea consumption and human cancer incidence is an important concern. Whereas some studies have shown a protective effect of tea consumption against certain types of cancers, many laboratory studies have demonstrated inhibitory effects of tea preparations and tea polyphenols against tumor formation and growth. This

inhibitory activity is believed to be mainly due to the antioxidant and possible anti proliferative effects of polyphenolic compounds in green and black tea. These polyphenolics may also inhibit carcinogenesis by blocking the endogenous formation of N-nitroso compounds, suppressing the activation of carcinogens, and trapping of genotoxic agents [78]. Therefore, in consistent with the above mentioned reports, our results may indicate that polyphenolics can be responsible for anti-proliferative activity of crude ethyl alcohol extract of black tea. Actually use of medicinal plants has caused a decrease in incidence of different diseases due to their effects in protecting against oxidative damage and decreasing inflammation (23, 79-82).

## CONCLUSION

Based on our findings of the present study, probably due to the polyphenolic compounds of crude ethyl alcohol extract of black tea and its relatively high antioxidant activity, it can suppress the proliferation of gastric carcinoma cells.

## CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

## ACKNOWLEDGMENT

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