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CYTOTOXIC ACTIVITY OF SOME EGYPTIAN CEREAL AND LEGUMES

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ABSTRACT

The analysis of common Egyptian cereal and legume varieties (low tannin sorghum var. Dorado, high tannin sorghum var. Asyout 14, lentil var. Giza 4) and whole grain barley flour (Telbina) revealed that high tannin sorghum had the highest total phenols content (69.2 mg/100 g) followed by cooked lentil (57.4 mg/100 g). Ferulic and syrnigic acids were the major phenolic compounds in the both samples. The results showed also that high tannin sorghum and cooked lentil had high vitamin B-3 and B-6 contents. Cooked lentil showed high amount of folic acid (200 μg/100 g) compared with (70 μg/100 g) in high tannin sorghum. Samples were also evaluated for any cytotoxic activity against the human tumor cell lines (breast carcinoma cell line (MCF7), liver carcinoma cell line (Hepg2), brain tumor cell line (U251) and cervix carcinoma cell line (Hela). The results showed that high tannin sorghum had good cytotoxic activity against all cell lines tested, which cooked lentil was found to have only cytotoxic activity against brain tumor (U251) and cervix cell line (Hela). Telbina was proven to have some effects against breast and brain tumor, while low tannin sorghum had some effects against Hela and liver tumor.

Key words: Egyptian cereal - Legume varieties - Cytotoxic activity - Barley flour – Sorghum - Lentil - Human tumor cell lines.

INTRODUCTION

Cancer risk is not simply dependent on the presence of carcinogens but on interactions between carcinogens and anti-carcinogens in foods and the body's defense systems. Little is known about the amount of a natural toxin and natural cancer-preventing nutrients in different foods or in the overall diet (WCRF, 1997).

Polyphenolic compounds are widely distributed in the vegetable kingdom and are therefore consumed regularly in the human diet.

Epidemiological studies suggest that foods rich in polyphenolic compounds contribute to reducing cancer (Josefa *et al.*, 2004).

Plant flavonoids, polyphenols and related compounds are diverse group of phytochemicals that widely occur in variety of plants and have gained attention due to their antioxidant capacity that benefits human health. Some of these compounds are associated to cereal and legumes and effected as phytochemicals against cancer growth (Gutierrez Uribe *et al.*, 2005).

Cereal phenolic is primarily located in the grain outer layers. Phenolic acids in sorghum include benzoic and cinnamic acid derivatives. Recent evidence indicates that consumption foods rich in phenolics may help reduce the risk of strokes, coronary heart disease, certain cancers and liver disorders through their antioxidant activity (Cotelle *et al.*, 1996).

Phenolic acids are another class of complicated phytochemicals. Syringaldehyde, O-coumaric acid, and gentistic acid are the three major ones in soybeans. Many *in vitro* and *in vivo* studies have demonstrated anticarcenogenic properties of phenolic acids, including ferulic acid, chlorogenic acid, caffic acid, and ellagic acid (Wang and Wixon, 1999).

Plant phenols may interfere with all of the cancer process, potentially resulting in a reduction of cancer risk. If DNA damage has been caused and the very active DNA repair mechanisms of the body fail, an initiated cell might turn into tumor cell. However, such initiated cells may be killed by apoptosis and phenols have been reported to stimulate apoptosis (Chandra *et al.*, 2000).

Phenolic compounds have antioxidant, antiinflammatory, antitumoral and oestrogenic activates, which might suggest their potential in the prevention of coronary heart disease and cancer (Francisco and Juan, 2001).

Andlauer and Furst (1999) reported that compounds in vegetables possessing similar or antagenistic properties to those in physiological estrogens are called phytoestrogens. Lignans and isoflavonoides are two classes of phytoestrogens. Lignans in soybeans and lentil have been found to be slightly estrogenic, because of their structural similarity to estrogen and it has been suggested that they may protect against estrogen dependent cancer. The highest levels of lignin's in the diet are found in those from countries or regions with a low incidence of cancer.

Dry beans are stable food for many Latin American and African countries and largely recognized as a beneficial source of proteins. There are several publications linking bean consumption to reduce risk of diabetes, obesity, cancer and cardiovascular disease. The potential health benefits of beans have been attributed to the presence of micronutrients, such as phenolic compounds, that possess antioxidant properties. Ferulic acid, P-coumaric acid and sinapic acid were three main phenolic acids identified in

all cultivars, however, quantifiable amounts of caffic acid were isolated from only two cultivars (Luthria and Pastor, 2005).

Noting the recent accumulation of evidence supporting the amazing health benefits of legumes (beans, peas and lentils), one researcher concludes: "they have acquired the status stable food for anyone who wants to eat a healthy diet" (Leterma, 2002).

There was one plant super food that was evidently so powerful at guarding against breast cancer, though, that one could find a protective effect eating as two measly servings a week, legumes. Young women eating just 2 to 4 servings a week of beans or lentils seemed to cut their risk of developing breast cancer by about a quarter, compared to those that ate servings less than once a month (Adebamova *et al.*, 2005).

Studies have shown that ample amounts of fruits and vegetables may be a key factor in preventing cancer. This may be due to the presence of vitamin B_6. Other foods that deliver B_6 include poultry, fish, meat and legumes (Thompson, 2005)

The objective of the research was to evaluate some common Egyptian cereals and legumes for any cytotoxic against human tumor cell lines and identification of their phenolic acids and soluble vitamin.

MATERIALS AND METHODS

Materials:

Low tannin sorghum (*Sorghum bicolor* var. Dorado), high tannin sorghum (Asyout 14) and lentil (*Lens culinaris* var. Giza 4) seeds were obtained from Field Crops Research Institute, Agricultural Research Center, Giza, Egypt, whole grain barley flour (Telbina) was obtained from the local market.

Preparation of samples:

Dehulled lentil seeds was cleaned, washed and cooked until become soft, then dried in laboratory oven at 60 °C overnight. Sorghum grains were cleaned and milled.

Analytical methods:

Total and free phenols were determined according to the methods described by Hahn and Rooney (1984). Conjugated phenols were calculated by difference.

The methanolic extract of the materials under study was subjected to HPLC (Model hp1050) equipped with UV detector for the identification and determination of the phenolic compounds according to the method described by Gertz (1990).

Folic acid was determined according to the method mentioned by Cavalli-Sforza (1980).

B vitamins (B_1, B_2, B_3, B_6, B_{12}) and vitamin C were identified and determined according to the method described by Gertz (1990).

Measurement of potential cytotoxic by SRB assay

The four samples under studying (high tannin sorghum, low tannin sorghum, Telbina and cooked lentil) were tested for any cytotoxic activity against human tumor cell lines, i.e. breast carcinoma cell line (MCF7), liver carcinoma cell line, brain tumor cell line (U251) and cervix carcinoma cell line (Hela). The tests were carried out at National Cancer Institute, Cairo, Egypt.

Potential cytotoxic of samples were tested using the method of Skehan *et al.* (1990). Cells were plated in 96-multiwell plate (104 cells/well) for 24 hrs. before treatment with the sample to allow attachment of cell to the wall of the plate. Different concentrations of the sample under study (0, 1, 2.5, 5 and 10 µg/ml water extract) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the sample for 48 hrs at 37°C and in atmosphere of 5% CO_2. After 48 hrs cells were fixed, washed and stained with Sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and sample concentration is plotted to get the survival curve of each tumor cell line after the specified compound (sample).

The 50% growth inhibition (CI_{50}) and the 10% growth inhibition (CI_{10}) were the parameters used for evaluation.

RESULTS AND DISCUSSION

From Table (1) it was observed that, high tannin sorghum (Asyout 14) had the highest total phenols content (69.2 mg/100g), followed by cooked lentil (57.40 mg/100 g). Low tannin sorghum (Doradow) had half the amount present in the high tannin sorghum (35.59 mg/100 g). On the other hand, Telbina showed total phenol amounted in 42.80 mg/100 g. Most of phenols content of tested samples present in a free state except lentil, which had an equal amount of free and conjugated phenols (28.7 mg/100 g).

Awika *et al.* (2001) reported that phenols (mg/100 mg) were highest in the brown sorghum (1.1-2.45 mg/100 mg) and lowest in white sorghum (0.07). Especially sorghum high in phenols could be a good source of antioxidants in foods. Beta *et al.* (1999) reported that barley contains 0.2 to 0.4% phenolic by weight of grain.

Because of its high content of total, free and conjugated phenols, high sorghum tannin and cooked lentil were selected for fractionation of phenolic compounds and the data were recorded in Table (2). The obtained results showed that ferulic acid (24.14%) and syringic acid (32.14%) were major

phenols in sorghum, which constituted 56.28% followed by benzoic acid (14.26%), protocatechuic acid (5.10%) and vanillin (5.56%). Other components constituted < 10%.

Table (1): Total, free and conjugated phenols in sorghum, lentil and Telbina (mg/100 g).

Sample	Phenol content (mg/100 g)		
	Total	Free	Conjugated
High tannin sorghum	69.20	49.30	19.90
Lentil	57.40	28.70	28.70
Low tannin sorghum	35.59	32.39	3.20
Telbina	42.80	36.70	6.10

Table (2): Relative concentration percentage of phenolic compounds of high tannin sorghum and cooked lentils.

Phenolic compound	High tannin sorghum	Cooked lentils
Ferulic acid	24.14	40.23
Syringic acid	32.14	22.87
Cinnamic acid	--	5.06
Protocattechuic acid	5.10	6.91
Vanilline	5.56	10.60
Caffic acid	2.62	1.23
P-coumaric acid	0.61	0.67
Resorcinol	1.35	1.75
Salicylic acid	1.26	--
Benzoic acid	14.26	5.44
Apigenin	3.23	5.19

The same Table revealed that ferulic acid (40.23%) constituted the major component in lentil followed by syrngic acid (22.87%) which constituted 63.10% followed by vanillin (10.60%) and protocatechine acid (6.91%), benzoic acid (5.44%), apigenin (5.19%) and cinnamic acid (5.06%). Other components constituted < 5%. Andlauer and Furst (1999) reported that major functional antioxidant in cereal grains are ferulic acid, vanillic acid and P-cumaric acid. It is conceivable that the profound antioxidative action of wheat bran extract is owing to the synergy of the various phenolic acids, as well as due to presence of other biologically active constituents. In addition, combinations of phenolic acids are claimed to have anticarcinogenic activities. NGSP (2003) reported that, antioxidant rich sorghum varieties currently being studied offer high levels of phenols and tannins, which are two components that have been linked to cancer

prevention and improved cardiovascular health. Early results from studies suggest that certain grain sorghum varieties may be a powerhouse of cancer-fighting, heart-healthy compounds on par with blue berries and cranberry.

Whether a compound initiates or inhibits the cancer process may depend on how much are exposed to some chemicals, such as caffic acid found naturally in many fruits and vegetables, are carcinogenic at high doses and protective at low doses. At a concentration of 2% caffic acid, rats develop stomach tumors. At a concentration 0.0005% caffic acid inhibits cancer from developing (WCRF, 1997).

In parallel with that high tannin contents, high tannin sorghum and cooked lentil were taken into consideration for vitamin B determination to get the relationship between B vitamins and phenolic compounds. Table (3) showed soluble vitamin contents (mg/100 g) in high tannin sorghum and cooked lentil. It observed that sorghum has high content of vit. B_3 (2.53 mg) followed by vit. B_6 (1.75 mg), while it has 0.65 mg of vit. B_1 and 0.19 B_2. It has low vit B_{12} content (0.199 mg). Concerning to folic acid content, sorghum has 70 µg. It has little amount of vit. C (1.95 mg).

Table (3): Soluble vitamin content (mg/100 g) in high tannin sorghum and cooked lentil.

Vitamin (mg/100 g)	Sorghum	Lentil
B_1	0.650	0.330
B_2	0.19	0.07
B_3	2.530	1.066
B_6	1.750	1.100
B_{12}	0.199	traces
Folic acid (µg)	70.0	200.0
C	1.950	3.800

Regarding to lentil, it has high amounts of B-vitamins (1.1 mg B_6, 1.06 mg B_3, 0.33 mg B_1 and 0.07 mg B_2, respectively). In addition, it has high folic acid concentration (200 µg). This means that lentil is an excellent source of folic acid. In contrast, lentil has low vitamin C content (3.3 mg), These soluble vitamins play an important role in the etiology cardiovascular disease and cancer (Zhang *et al.*, 2003).

Folate is an essential component in the human diet. It is involved as a cofactor in many metabolic reactions, including the biosynthesis of the building blocks of DNA and RNA, the ribonucleotides. Recently, claims that high-folate diets protect against cardiovascular disease and cancer (Sybesma *et al.*, 2003).

Vitamin B_6 helps the brain function (it plays a role in the development of neurotranumitters), helps prevent heart disease and light color cancer. The

recommended daily allowance for vitamin B6 is 2 mg for men and 1.6 mg for women. Researchers found a clear association between high B_6 known as P5P (pyridoxal-5-phosphate) concentration and reduced risk of colorectal cancer (Thompson, 2005).

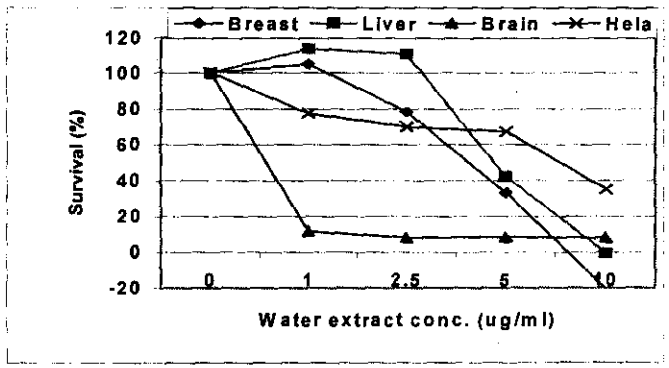
It could be seen from Table (4) and Fig. (1) that high tannin sorghum was proven to have good cytotoxic activity against the three human tumor cell lines (breast, liver and brain). It reduced the survival of human breast carcinoma cell line to 50% and 10% at concentration of 4.0 and 7.0 μg/ml respectively. While, it reduced the survival of liver carcinoma cell line to 50% and 10% at concentration 4.8 and 8.5 μg/ml respectively. It reduced the survival of brain carcinoma cell line to 50% and 10% at concentration 0.6 and 1 μg/ml, respectively. While, it was proven to have some cytotoxic activity against cervex carcinoma cell line (Hela). It reduced the survival cells to 50% at concentration of 7.5 μg/ml.

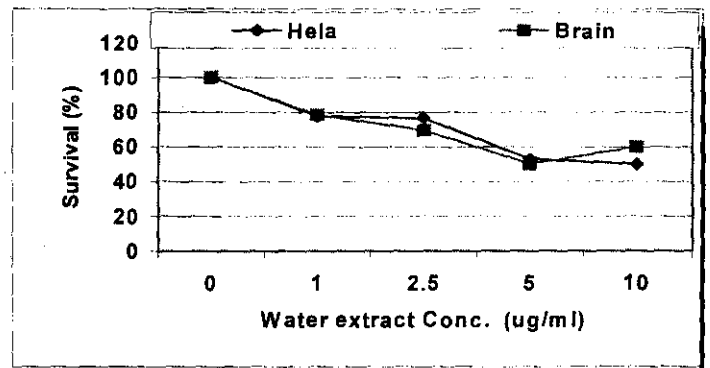
Table (4): The cytotoxic activity of high and low tannin sorghum, Telbina and cooked lentil against human carcinoma cell lines.

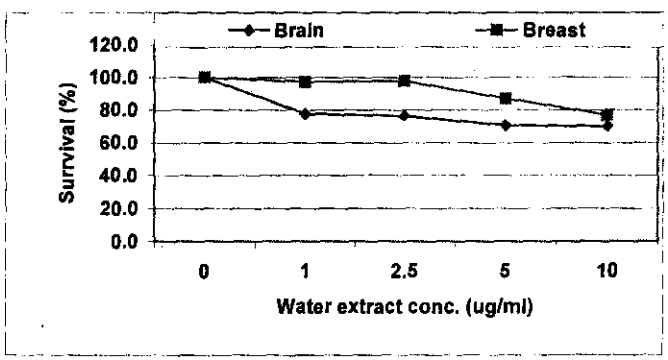
Sample (μg/ml)	Human carcinoma cell lines							
	Breast (MCF7)		Liver (Hepg 2)		Brain (U251)		Cervex (Hela)	
	IC_{50}	IC_{10}	IC_{50}	IC_{10}	IC_{50}	IC_{10}	IC_{50}	IC_{10}
High tannin sorghum	4	7	4.8	8.5	0.6	1.0	7.6	--
Low tannin sorghum	--	--	--	--	--	--	--	--
Telbina	--	--	--	--	--	--	--	--
Cooked lentil	--	--	--	--	5.0	--	10.0	--

IC_{50}: Dose of the extract, which reduces survival to 50%.
IC_{10}: Dose of the extract, which reduces survival to 10%.
-- : No activity

Cooked lentil was proven to have some activity against human breast carcinoma cell lines (inhibited cell growth by 20%), but showed good cytotoxic activity against the human brain and cervex carcinoma cell lines. It reduced the survival brain and cervex carcinoma cell lines to 50% at concentrations of 5 and 10 μg/ml, respectively. on the other hand, cooked lentil was proven to have no cytotoxic activity against liver carcinoma cell line. Telbina proven to have some activity against breast and brain carcinoma cell lines, it inhibition about 30% of the cells activity at concentration of 10 and 5 μg/ml, respectively. While, low tannin sorghum showed some activity against human liver and Hela carcinoma cell lines. It inhibited about 30% of cell activity at concentration of 10 μg/ml.







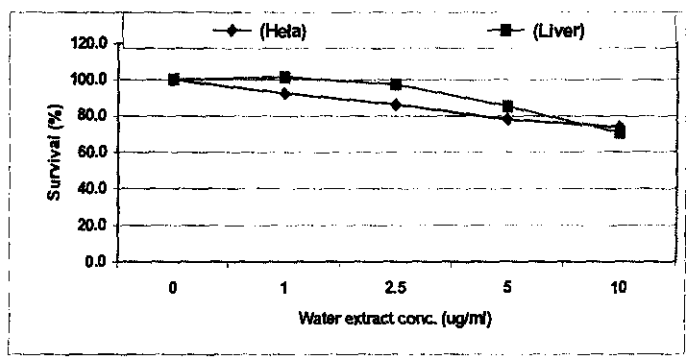


Fig. (1): The cytotoxic activity of a: High tannin sorghum against breast (MCF7), liver (Hepg2), brain (U251) and Hela cell lines b: cooked lentil against Hela and brain (U251) cell line, c: Telbina against brain (U251) and breast (MCF7) cell lines d: Low tannin sorghum against Hela and liver (Hepg2) cell lines

Telbina and low tannin sorghum failed to reduce survival to 50% or 10% of the cell lines under study. A major phenolic compound in cooked lentil was ferulic acid (40.23%) which may attributed the high activity against tumor cells (Borislav, 2004 and Lee, 2005). Lentil activity against brain and cervix cell lines may refereed to high folic acid (B9) content (200 μg/100 g).

Ferulic acid is a highly abundant phenolic phytochemical which is present in plant cell walls. It may have significant health benefits through its antioxidant and anti-cancer activity. Animal studies and *in vitro* studies suggest that ferulic acid may have direct antitumor activity against breast cancer and liver cancer (Borislav 2004 and Lee, 2005). The effect of ferulic acid as anti-cancer appear to be due to its ability to prevent the conversion of the nitrites used in foods into cancer causing chemicals.

The safety of ferulic acid is well established both by animal studies and by its consumption as part of people's daily diet for thousands of years, it is a significant component of grains, seeds, leafy vegetables and other food plants.

Washington State University researchers suggest that consuming more vitamin B_6 can ward off cancer.

Pullman (2003) reported that vitamin B6 converts the vitamin folate to a form that the body can use to produce thymine (B_1), a component of DNA. If the body doesn't have enough vitamin B_6, it doesn't make enough thymine and it tries to make do by substituting uracil. Uracil is not a normal component of DNA, and its presence stresses normal DNA repair mechanisms in the cell. This inefficiency in the normal repair mechanisms leads to breaks in DNA strands and instability of chromosomes. A possible first step in the development of cancerous cells.

From the above mentioned data, it could be concluded that the presence of phenolic compounds and vitamin B complex in diet may be prevent or protect cancer. It could be recommended that high tannin sorghum meal can be used as seasoning spices agents. On the other hand, one cup of lentil soap is very important daily to prevent or protect tumor.

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النشاط المضاد للسرطان فى بعض الحبوب والبقول المصرية

منى عبد المنعم محمد على
معهد بحوث تكنولوجيا الأغذية-مركز البحوث الزراعية-الجيزة-مصر

تمت الدراسة على كل من للذرة الرفيعة عالية التانينات (صنف اسيوط١٤) والمنخفضة التانينات (صنف دورادو) ومطحون حبوب الشعير الكاملة (التلبينة) والعدس (صنف جيزة ٤) وتبين من النتائج ارتفاع محتوى الفينولات الكلية فى الذرة الرفيعة (صنف أسيوط ١٤) مقارنة بالأنواع الأخرى تحت الدراسة حيث بلغت نسبة الفينولات الكلية ٦٩,٢ مللجم/١٠٠ جم يليها العدس المطبوخ ٥٧,٤ مللجم/١٠٠ جم.

تم التعرف على مكونات الذرة الرفيعة (أسيوط ١٤) والعدس المطبوخ من المركبات الفينولية باستخدام جهاز الفصل الكروماتوجرافى HPLC وقد تم التعرف على إحدى عشر مركب فينولى وتبين ان حمض الفيروليك والسيرنجيك هما المركبان الأساسيان حيث يمثلان ٥٦,٢٨%، ٦٣,١% من محتوى المركبات الفينولية فى كل من الذرة الرفيعة (أسيوط ١٤) والعدس المطبوخ على التوالى.

كما تم تفريد وتقدير الفيتامينات القابلة للذوبان فى الماء (فيتامين ب١، ب٢، ب٣، ب٦، ب١٢ وحمض الفوليك باستخدام جهاز الفصل الكروماتوجرافى HPLC وتبين من نتائج التحليل احتواء كل من الذرة الرفيعة (صنف أسيوط ١٤) والعدس المطبوخ على نسبة عالية من فيتامين ب٣، ب٦ وكذلك ارتفاع محتوى حمض الفوليك فى العدس المطبوخ (٢٠٠ ميكروجرام).

تم تقييم الحبوب والبقول موضع الدراسة كمواد مثبطة للخلايا السرطانية لاربع أنواع من الأورام هم سرطان الثدى وسرطان الكبد وسرطان المخ وسرطان عنق الرحم وقد أظهرت الدراسة ان الذرة الرفيعة عالية التانينات (صنف أسيوط ١٤) كان لها نشاط مثبط جيد فى جميع أنواع السرطانات المختبرة حيث انخفضت الخلايا المتبقية الى ٥٠%، ١٠% عند التركيزات المقترحة. بينما كان العدس المطبوخ ذو تأثير فى تثبيط ٥٠% من الخلايا السرطانية فى حالة سرطان المخ وسرطان عنق الرحم.

واظهرت النتائج أيضا أن للذرة الرفيعة منخفضة التانينات (صنف دورادو) بعض التأثير المثبط على ورم سرطان عنق الرحم والكبد بينما فى حالة مطحون الشعير الكاملة (التلبينة) أظهرت النتائج وجود بعض التأثير المثبط على ورم الثدى والمخ ولكن لم يحدث انخفاض للخلايا المتبقية الى ٥٠% أو ١٠% فى جميع أنواع السرطانات المختبرة عند التركيزات المقترحة فى كلا النوعين.

قام بتحكيم هذا البحث: أ.د./ كمال رشدى و أ.د./ أحمد بسيونى