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ANTICANCER ACTIVITIES OF SOME CHEMICAL CONSTITUENTS FROM STRAWBERRY (*Fragaria x ananassa*) FRUITS.

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ABSTRACT

The present study was carried out to evaluate the anticancer activities of strawberry (*Fragaria x ananassa*) fruits extracts. The methanolic (80%) extract was evaporated to dryness, the residue (crude extract) (CE) was fractionated by using different solvents producing, ethyl acetate fraction (EAF) and butane fraction (BUF). Completely maturated fruits moisture, sugars, fiber, pH value, vitamin C and minerals including K , P, Ca, Mg and Na were measured and the values established as , 88.9% , 6.2% , 2.2 % , 4.2, 60 (mg/100g), 160 (mg/100g), 23 (mg/100g), 22 (mg/100g), 12(mg/ 100g), and 3 (mg/100g) respectively. EAF extract contained 43.13%, 43.15% and 2.33% from phenols, flavonoids and anthocyanins respectively, while, BUF extract which contained 37.34% ., 44.64% and 2.21% respectively, and CE extract contained 26.19%, 21.92% and 0.13% respectively . The results revealed that all extracts of strawberry fruits were the most effective as anticancer agent since then activity ranged from 70.1 to 81.7 % at concentration of 10 µg/ml .

INTRODUCTION

In recent years, increasing attention has been paid by consumers to the health and nutritional aspects (vitamins content, mineral elements, antioxidants, etc.) of horticultural products (Scalzo *et al*., 2005). Fruits and vegetables are known lately to strongly contribute in reducing risks of diseases of various etiology as cancer and heart stroke. This fact is attributed to the large amounts of antioxidants they contain (Kris-Etherton *et al*. 2002 and Ha¨kkinen *et al*., 1999). Phenolic compounds are a group of biologically active molecules present as metabolites in plants. The interest for these natural compounds has increased in the last years due to their antioxidative, and anticarcinogenic activity and relationship to human health. It has been demonstrated that some health benefits of food depend on the presence of these antioxidants, which occur in fruits and vegetables in general. In this study, strawberries were used as sample due to the fact that a number of phenolic compounds have been detected in berries conferring them antioxidative and anticarcinogenic properties (reviewed in Hannum, 2004). Antioxidant compounds are produced by the plant to protect the cell against the attack from other cell chemical species as free radicals and reactive oxygen species. Free radicals are constantly produced by the cell metabolism (Benavente-Garcı´a *et al*., 1997). Phenolic compounds contain aromatic ring(s) bearing hydroxyl group(s) and can range from simple molecules to very large oligomers. They frequently occur naturally in glycosylated forms, which make them more water-soluble although the higher molecular weight oligomers are more insoluble (Bravo, 1998). Phenolic compounds are

abundant in highly colored fruits, and due to their popularity and high consumption, these fruits serve as one of our most important dietary sources of phenolics (Williner, *et al*., 2003). Strawberries contain high levels of antioxidant compounds such as anthocyanins, flavonoids, and phenolic acids, which provide protection against harmful free radicals. Antioxidants have also been associated with lower occurrences and mortality rates due to cancer and heart disease as well as offering a number of other health benefits (Wang and Zheng, 2001). In fact, polyphenolic compounds of plants are well known as exhibit contrasting pharmacological actions, such as prooxidant activity and the induction of cell death (Lapidot, *et al*., 2002). In this regard, cell culture is a powerful technique for studying physiological, biochemical and toxicological processes modulated by pure phytochemicals in vitro (Glei *et al*. 2003). HepG2 cell line has been widely used in biochemical and nutritional studies because it is considered one of the experimental models that more closely resembles the human hepatocyte in culture (Ramos, *et al*. 2005). In addition, steady-state functioning of the antioxidant defenses in HepG2 is relatively higher than in hepatocytes and other non-transformed cells. Therefore, variations in the responses to different conditions are more easily detected (Alia, *et al*., 2005).

The present works takes into consideration the use of strawberry extracts as the source of phenolic compounds especially flavonoids to evaluate their activities as anticancer agents. Also, separation and identification on some chemical components of different fractions (ethyl acetate fraction and butane fraction) from each methanolic extracts and its effects as anticancer were studied.

MATERIALS AND METHODS

Materials:

Fresh strawberry (*Fragaria x ananassa*) fruit was obtained from the Strawberry and Now Traditional Crops Improvement Center. Faculty of Agriculture. Ain Shams University.

Extractions:

- Methanolic extract (crude extract CE): Fresh strawberry fruits were washed and freeze dried immediately. Then the dry materials were ground, The powdered strawberry were macerated in methanol 80% (1:3 w/v) for 24h . The methanolic extracts were filtered and evaporated to dryness under vacuum, the residue was named crude extract (CE).
- The crude extracts (CE) were dissolved in distilled water and them partitioned with ethyl acetate (6 times x 200). The ethyl acetate layers dehydrated with Na2SO4 and evaporated to dryness. The residue were named ethyl acetate fraction (EAF). The remaining water layer then were partitioned with n – butanol (6 times x 200ml). The butanol layers dehydrated with Na2 SO4 were evaporated to dryness . The residues were named butanol fraction (BUF).

Chemical analysis:

– Moisture, sugars, minerals, vitamin C and fiber contents were determined using the methods described by AOAC. (2005).

– pH values were measured according to Ling (1963) using digital pH meter,
- Total phenol content was determind by colorimetric method of Shahidi and Naezk (1995).
- Total flavonoids contents were determined by the aluminum chloride colorimetric assay according to Marinova *et al*. (2005).
- Proanthocyanidins content was determined according to the methods of Bahorun *et al*. (1994).

Anticancer activity (sytotoxicity activity) against tumor cell lines (HEPG2):

Cytotoxicity was determined in National Cancer Institute. Cairo Univ, according to the method described by Skehan and Streng (1990).

HPLC analysis:

The separation of Phenolic compounds from fractions were performed in National Research Center by HPLC (HP) equipped with a Hewlett- Packard 1050 photodiode array detector (Agilent Technologies Palo Alto. Calif. U.S.A.) with Hewlett – packared. HPLC, Chem. Station software and auto sampler. Using a PDS- column C 18 – 5 micron (150mm x4-6mm, operated at 4Sº C . The solvent system used was gradient of A (acetic 2.5%) B (acetic 8%) and C (acetonitrile). The Solvent flow rate was 1 ml/min. Ingection volume 50 µl. Phenolic compounds were assayed by external standard calibration at 280 nm.

RESULTS AND DISCUSSION

The chemical composition of strawberry (*Fragaria X ananassa*) fruits are given in Table (1). The results of the analyses were established to give nutrient values per 100 g. Moisture, sugars, fiber, pH value and vitamin C were found 88.9%, 6.2%, 2.2%, 4.2 and 60 (mg/100g), respectively. Also, mineral contents are presented in the same table . According to results, potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg) and sodium (Na) contents were 160, 23, 22, 12 and 3 (mg/100g), respectively. These results are in agreement with those obtained by Demir *et al.* (2002) and Ozcan, & Hacıseferogulları (2007).

Table (1): Chemical properties of strawberry fruits.

Properties	Values	Minerals	Values (mg/100g)
Moisture (%)	**88.9**	**Potassium k**	**160**
Sugars (%)	**6.2**	**Phosphorus P**	**23**
Fiber (%)	**2.2**	**Calcium Ca**	**22**
pH	**4.2**	**Magnesium Mg**	**12**
Vitamin C(mg)	**60.0**	**Sodium Na**	**3**

Targeted analyses of strawberry fruits methanol extract (CF), ethyl acetate fraction (EAF) and butanol fraction (BUF) detected total phenols, flavonoids and anthocyanins content (Table2). The observed data indicted that EAF extract contained the higher content of total phenols (43.13%) followed by BUF extract (37.34%), and CF extract (26.19%).

Regarding total flavonoids, BUF extracts contained higher content (44.64) followed by EAF, extracts (43.15%), then Cu extracts (21.92%). Also,

the results showed that EAF contained relatively higher total anthocyanins content (2.33%) followed by BUF extracts (2.21%) whereas, Cu contained the lowest total anthocyanins content (0.13%).

Table (2): Total phenolics, Flavonols and anthocyanins contents in strawberry fruits.

Compounds %	Extracts		
	Cu	EAF	BUF
Total Phenols	26.19	43.13	37.34
Total Flavonoids	21.92	43.15	44.64
Total Anthocyanins	0.13	2.33	2.21

Data in Table (3) indicated that ethyl acetate fraction (EAF) of strawberry fruits extract contained 18 components which could be identified as catechin (13.1%) quercetin (12.2%) and rutin (12.2%) as the major components. In addition to moderate amounts of gallic acid (5.2%), coumaric acid (4.3%), epicatechin (3.8%), quercetin (3.5%), apigenin (3.4%) Chlorogenic (3.3%), quercetin 3- glucosode (3.1%) and ellagic acid (1.9%) respectively. On the other hand, EAF extract contained low contents from luteolin, rosmarinic, ferulic, Kaemeferol, cinnamic and naringenin. Regarding butanol fraction (BUF) extract, data in table (3), also showed that the presence of high contents of coumaric (8.9%), catechin (7.3%), epicatechin (7.1%), rutin (6.2%), quercetin 3- glucoside (5.1%), and hyperoside (5.1%). Also, BUF extract contained moderate amounts of caffeic (4.2), ellagic acid (2.2%), and gallic acid (2.2%) respectively. The lowest contents were butealin, quercitrin, kampferol, quercetin, cinnamic, and naringenin, respectively .

Table (3): HPLC analysis for chemical constituents (%) of ethyl acetate fractions (EAF) and butanol fractions (BUF) from strawberry fruits.

Chemical constituents	extracts	
	EAF	BUF
Gallic acid	5.2	2.2
Catechin	13.1	7.3
Chlorogenic	3.3	--
Caffic	--	4.2
Epicatechin	3.8	7.1
Coumaric	4.3	8.9
Rutin	12.2	6.2
Ellagic acid	1.9	2.2
Hyperoside	--	5.1
Ferulic	0.8	--
Queitrin	3.5	1.5
Luteolin	1.3	1.5
Apigenin	3.4	--
Naringenin	0.2	0.3
Cinnamic	0.2	0.5
Kaompferol	0.4	1.2
Rosmarinic	1.3	--
Quercetin-3-glucoside	3.1	5.1
Coumarin	2.1	--
Querctin	12.2	1.0

The forementioned results concerning the identification of the chemical constituents in ethyl acetate and butanol fractions of strawberry were in agreement with the findings of Ren *et al*. (2003), Siriwoharn and Wrolsted (2004) and Seeram *et al.* (2006).

Data recorded in table (4) represented the anticancer activity of the fractions of strawberry fruits extracts. Regarding cruds extract (CE) 80% methanolic extract, the anticancer activity increased from 69.4% to 78.0% with increasing the concentration from 1 to 10 µg/ml respectively .

Table (4): Anticancer activity of strawberry extracts on HEPGR.

Extracts	Concentration µg/ml .			
	1	**2.5**	**5**	**0**
CE	69.4	71.8	75.0	78.0
EAF	22.4	33.6	40.7	81.7
BUF	41.0	59.1	67.2	70.1

In the case of ethyl acetate fraction (EAF), the anticancer activity increased from 22.4% to 81.7% with in creasing the concentration from 1 to 10 µg/ml respectively. Concerning butanol fraction (BUF), there were gradual increases in anticancer activity from 41.0% to 70.1% with increasing the concentration from 1 to 10 µg/ml respectively. Generally the results indicated that the anticancer activity of the previous fractions increased with increasing concentrations. Duthic *et al*. (2000) and Nijveldt *et al*. (2001) revealed that flavonoids as antioxidants can inhibit carcinogenesis and the antioxidant potential may be anticarcinogenic.

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الأنشطة المضادة للسرطان لبعض المكونات الكيميائية من ثمار الفراولة

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أجريت هذه الدراسة لتقييم نشاط فاكهة الفراولة كمضاد للسرطان . حيث تم عمل مستخلص ميثانولي (٨٠%) ثم تم تبخير الميثانول ثم أخذ الجزء المتبقي (المستخلص الخام) (CE) وتم تجزئته باستخدام المذيبان ايثيل أستات والبيوتانول وبالتالي ثم الحصول علي جزء الايثيل استيات وجزء البيوتانول بالإضافة للمستخلص الخام .

ولقد تم تقدير كل من الرطوبة ، والسكريات ، والألياف وقيمة الأس الإيدروجينى وفيتامين C وأيضا أملاح البوتاسيوم والفوسفور والكالسيوم والمغنسيوم و الصوديوم وكانت القيم المتحصل عليها هي ٨٨,٩ % ، ٦,٢% ، ٢,٢% ، ٤,٢ ، ٦٠ (ملجرام / ١٠٠جم) ، و ١٦٠ (ملجرام /١٠٠جم) و ٢٣ (مليجرام / ١٠٠جم) ، و٢٢ (ملجرام / ١٠٠جم) و ١٢ (ملجرام /١٠٠جم) ، و ٣ (ملجم / ١٠٠جم) علي الترتيب . كما أحتوي المستخلص الايثانولي علي ٤٣,١٣% ، و ٤٣,١٥% ، ٢,٣٣% من الفينولات والفلافونويدات والبروانثوسيانيدين علي الترتيب أما مستخلص البيوتانول فقد احتوي علي ٣٧,٣٤% ، و ٤٤,٦٤% ، و٢,٢١% علي الترتيب وى حين أحتوى المستخلص الخام علي ٢٦,١٩% ، ٢١,٩٢% ، ٠,١٣% علي الترتيب .

وقد أوضحت النتائج أن كل مستخلصات ثمرة الفراولة لها أعلي فاعلية كعوامل مضادة للسرطان حيث يتراوح نشاطها من ٧٠,١% إلي ٨١,٧% وذلك عند تركيز ١٠ ميكروجرام/ مل .