THE ANTIOXIDANT AND ANTICANCER ACTIVITIES OF STRAWBERRY SEEDLING *(Fragaria X ananassa c .v. Sweet Charlie)* EXTRACTS.

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ABSTRACT

Studies were conducted to evaluate the antioxidant and anticancer activities of strawberry seedlings shoots extracts (SS) and strawberry seedlings roots extracts (SR) using different solvent systems. SS and SR extracts had high antioxidant activity % on linoleic acid system, where butanol fraction (BUF) of SR had the most antioxidant activity (IC 50=28.32 μg /ml) In comparison with BHT (IC 50=31.5 μg/ml) and BHA (IC 50=33.75 μg /ml).

SS AND SR extract had high scavenging activity on di phenyl picryl hydrazyl (DPPH) radical, where the highest scavenging activity was observed in butanol fraction (BUF) of SS (93.55%) followed by ethyl acetate fraction (EAF) of SS (93.16%), EAF of SR (93.08%) and BUF of SR (92.79%) at concentration 25 μg /ml while ascorbic and BHT had lower scavenging activity (90.30% and 90.80% respectively)

The observed data revealed a positive relationship between antioxidant activity and the extracts content of total phenols and flavonoids and also between reducing power value.

The obtained result showed that SS fractions had a higher anti cancer activity than SR fractions where EAF of SS had the highest anticancer activity (81.70%) while EAF of SR had (53.18 %) at

concentration 10 μg/ml. No relationship between anticancer activity and antioxidant activity of extract.

Key words: Antioxidant Activity, Scavenging Activity Lipid Peroxidation, Anticancer Activity, Strawberry.

INTRODUCTION

Green nature full of several biologically active compounds. Plants produce a great variety of organic compounds known "Natural products". Most of the natural products can be classified into three major groups, terpenoids, alkaloids and phenolic compounds .Phenolic compounds are well distributed and have multifunctional activities in plant kingdom.

Phenolic compounds presented in strawberry include anthocyanins (the most important group), flavonols, catechins and proanthocyanidins (Torronen *et al.,* 2002)

The highest phenolic content was found in *Fragaria vesca* while lowest content was measured for white strawberry (*F. Chiloensis*). Total anthocyanin and total flavonoids contents in the samples investigated were lower for white strawberry and higher in *F. ananssa*. Total flavonoids content showed a better correlation than total anthocyanines with free radical scavenging effect of the extracts measured by mean of the DPPH discoloration assay (Cheel *et al.* (2007).

Methanolic extract 60% of *Rubus* (cloud berry and raspberry) which contain ellagitannins as the main phenolic compounds gives 97% and 96% antioxidant activities respectively .While methanolic extract of *Fragaria* (strawberry), which contain the ellagitannins as the second largest group after anthocyanins gives 60% antioxidant activity(Kahkonen *et al* (2001) .Also ellagitanins are dietary polyphenols containing ellagic acid (EA) subunits with antioxidant and cancer chemopreventive activity that might contribute to heath benefits (creda *et al* (2006)

Strawberry cultivers extracts (Earliglow, Annapolis, Evangeline, Allstar, Sable, Sparkle, Jewel and Mesabi) inhibit the proliferation of HEPG2 liver cancer cell where Earliglow exhibiting the highest antiproliferative activity and Annapolis exhibiting the lowest. No relationship was found between antiproliferative activity and antioxidant content (Mayers *et al*. 2003).

Fragaria virgeniana fruits extract inhibited the proliferation of A549 human lung epithelial cells to a significantly greater extent (34%inhibition) than the extracts from fruit of either *F .chiloensis* (26%) or *F .ananassa* (25%) (Wang *et al* (2007)

Therefore, the present study is conducted to shed some lights on strawberry shoots and roots extract as a source of phenolic compounds especially flavonoids to investigate their function as antioxidants and anticancer agents.

MATERIALS AND METHODS

Plant materials:

Fresh strawberry seedling (*Fragaria ananassa*) *c.v. sweet charlie* were obtained from the Strawberry and Non Traditional Crops Improvement Center, Faculty of Agriculture, Ain Shams University.

Preparation of methanolic extract (crude extract [CE]) and fractions:

Fresh seedlings of strawberry were washed thoroughly with tap water, cleaned, divided into shoots and roots, then freeze dried immediately. Then the dry material was ground using coffee grinder, powdered strawberry shoot and root were macerated in methanol 80% (1:3 w/v) for 24h. The methanolic extracts were filtered and evaporated to dryness under vacuum, the residue (I) were named crude extract (CE).

The crude extracts (CE) were dissolved in distilled water and then partitioned with methylene chloride (6 times × 200ml). The

methylene chloride layers, dehydrated with Na_2SO_4 were evaporated to dryness. The residue (II) were named methylene chloride fraction (MCF).

The remaining water layers then were partitioned with ethyl acetate (6 times × 200ml). The ethyl acetate layers dehydrated with Na_2SO_4 were evaporated to dryness. The residue (III) were named ethyl acetate fraction (EAF).

The remaining water layers then were partitioned with n-butanol (6 times × 200ml). The butanol layers dehydrated with Na_2SO_4 were evaporated to dryness. The residue (IV) were named butanol fraction (BUF), the remaining water layer were evaporated to dryness. The residues (V) were named aqueous fraction (AF).

All determination were carried out on all fractions I, II, III, IV and V.

Determination of total phenol content:

Total phenol content was determined in all fractions I, II, III, IV and V by the colometric method at 725nm using the Folin-ciocalteus reagent according to Shahidi and Naczk (1992).

Determination of total flavonoids content:

Total flavonoids contents were measured by the aluminum chloride colorimetric assay according to Marinova *et al.* (2005) method.

Determination of proanthocyanidins:

Proanthocyanidins was measured according to the method of Bahorun *et al* (1994).

Determination of reducing power:

The reducing power was determined according to (Mau et al. 2004). Each extract 0.5 to 0.25 μg /ml in methanol (2.5ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml

of 1% potassium ferricyanide and the mixture was incubated at 50°C for 20 min. after that 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 200g for 10 min. the upper layer (5 ml was mixed with 5 ml of deionized water and measured at 700 nm against blank. A higher absorbance indicates a higher reducing power. Ascorbic acid, butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) were used as controls.

Determination of antioxidant activities:

An antioxidant activity in a linoleic acid system:

An antioxidant activity assay was carried out by using linoleic acid system (Osawa and Namiki, 1981) where, 10, 20, 30, 40 and 50 μg /ml of each fraction were added to a solution of linoleic acid (0.13ml), 99.8% ethanol (10 ml) and 0.2M phosphate buffer (pH 7.0, 10ml). Total volume was adjusted to 25 ml by distilled water. The reaction mixture was incubated at 40°C and the degree of oxidation was measured by using the thiocyanate method according to Misuda *et al.* (1966). By sequentially, adding ethanol (10ml 75%), ammonium thiocyanate (0.2 ml, 30%) sample solution (0.2ml) and ferrous chloride solution (0.2ml) (20 mM in 3.5% HCl) to the mixture.

After the mixture was stirred for 3 min., the peroxide value was determined by reading the absorbance at 500 nm, and the antioxidant activity % can be calculated according to the following equation:

$$\text{Antioxidant activity \%} = \left[100 - \frac{\text{Absorbance increase of sample}}{\text{Absorbance increase of standard}}\right] \times 100$$

From this equation we can express the antioxidant activity.

Scavenging activity of DPPH radical

The hydrogen atom or electron donation ability of the corresponding extract was measured from the bleaching of a purple colored methanolic solution of DPPH according to Gulluce *et al.*

(2004). This spectrophotometric assay uses the stable radical 2,2′-diphenyl picryl hydrazyl (DPPH) as a reagent.

10µg, 20, 30, 40 and 50 µg/ml of each fraction in methanol was added to 2 ml of 0.004% (2mM) methanolic solution of DPPH. After 30 min. of incubation period at room temperature, the absorbance was read against the blank at 517 nm. Inhibition of free radical DPPH (1%) was calculated according to the following equation:

$$\% \text{ Scavenging activity } = \left[\frac{A_{control} - A_{sample}}{A_{control}} \right] \times 100$$

Anticancer activity (cytoxicity activity) against tumor cell lines HEPG2

Cytotoxicity was determined in National Cancer Institute, Cairo University. Using the methods of Skehan *et al.* (1990)

*HEPG2 cells (liver carcinoma cell line) were plated in 96-multi mell plate (10^4 cells/well) for 24 hours before treatment with the compound (s) to allow attachment of cell to the wall of the plate. Different concentration of each extract and its fractions (0, 1, 2.5,5 and 10µg/ml) were added to the cell monolayer triplicate wells were prepared for each individual dose. Monoplayer cells were incubated with the compound (s) for 24 hrs at 37 c and inatomosphere of 5% CO_2.After 48 hours, cells were fixed, washed and stained with sulfo-rhodamine – B- stain.Excess stain was washed with acetic acid and attached stain was recoverd with tris edta buffer.color intensity was measured in an elisa reader and calculated the surviving fraction.

* The anti cancer activity % was calculated from the following equation:

Anti cancer activity %={(1-surviving fraction/1) x 100}

Statistical analysis

IC 50 value of extract were calculated from linear regression analysis by SPSS program. Also means from three replicates were separated by the least significant difference (LSD) test at P< 0.05 with SAS soft ware (SAS Institute cary ,NC)

RESULTS AND DISCUSSION

1. Total phenol content

The data presented in Table (1) showed total phenol content (g/100g dry extracts) in strawberry seedlings shoots (SS) and strawberry seedlings roots (SR) extracts. In general there were significant different between all strawberry extracts in comparison with LSD 0.05 values (Table 1).

Table (1) Total phenol % in strawberry seedlings shoots and strawberry seedlings roots extracts.

	TOTAL PHENOL % g/100g (C)					
extract (S)	CE	MCF	EAF	BUF	AF	Mean
SS	26.19	11.96	43.13	37.34	32.78	30.28
SR	30.93	22.49	39.96	43.65	10.20	29.45
Mean	28.56	17.23	41.55	40.5	21.49	29.86

LSD 0.05 for S = (0.54) and SC (1.21)

There were significant different between SS fractions in comparison with LSD 0.05 value (1.21) EAF contain higher total phenol %(43.13%) followed by BUF (37.34%), CE (26.19%), AF (32.78%) and MCF contain lower total phenol % (11.96%) Table (1). Also, there were significant different between SR fractions in comparison with LSD 0.05 value (1.21). BUF contain higher content of total phenol % (43.65%) followed by EAF (39.96%), CE (30.93%),

MCF (22.49%) and AF contain lower total phenol % (10.2%) Table (1).

2. Flavonoid content.

Table (2) showed the flavonoid content (g/100g dry extract) in strawberry seedling shoot (SS) and strawberry seedlings roots (SR) extracts. In general there were significant difference between all extracts and fractions in flavonoid content in comparison with LSD 0.05 value (Table 2). The data revealed significant difference between SS fractions in flavonoid content in comparison with LSD value (1.09), where BUF contain the highest flavonoid content (44.64%), followed by EAF (43.15%), CE (21.92%), MCF (19.73%) and AF contain the lowest flavonoid content (11.45%).

Table (2) Flavonoid % in strawberry seedling shoots and strawberry seedling roots extracts.

	Flavonoid % g/100g (C)					
extract (S)	CE	MCF	EAF	BUF	AF	Mean
SS	21.92	19.73	43.15	44.64	11.45	28.18
SR	53.39	43.08	65.03	62.06	11.46	47.00
Mean	37.65	31.41	54.10	35.53	11.46	37.59

LSD for S (0.49) and SC (1.09)

On the other hand, there were significant difference between flavonoid content in SR fractions, where EAF contain the highest flavonoid content (53.39%), followed by BUF (62.06%), CE (53.39%), MCF (43.08%) and AF contain the lowest flavonoid content (11.46%) in comparison with LSD value (1.05).

3. Proanthocyanidin content (P)

Table (3) showed the proanthocyanidin content (g/100 g dry extract) in strawberry seedlings shoots (SS) extracts and strawberry seedlings roots (SR) extracts. The observed of results showed significant difference between all extracts and fractions in proanthocyandin content in comparison with LSD 0.05 value (Table 3).

Table (3) Proanthocyanidin % in strawberry seedlings shoots and strawberry seedlings roots extracts.

	Proanthocyanidin % g/100g (C)					
extract (S)	CE	MCF	EAF	BUF	AF	Mean
SS	0.13	0.32	2.33	2.21	0.06	1.01
SR	0.86	1.06	2.05	3.39	0.11	1.49
Mean	0.50	0.69	2.19	2.08	0.09	1.25

LSD 0.05 S=0.06 SC =0.13

There were significant difference between all SS fractions except EAF and BUF had no significant difference between them in comparison with LSD 0.05 value (0.13). EAF contain higher proanthocyandin content (2.33%) followed by BUF (2.21), MCF (0.32%), CE (0.13%) and AF contain the lower proanthocyanidin content (0.06%). Also, there were significant difference in proanthocyandin content between all SR fractions in comparison with LSD 0.05 value (0.13). BUF contain higher proanthocyandin content (3.39%) followed by EAF (2.05%), MCF (1.06%), CE (0.86%) and AF contain lower proanthcyanidin content (0.11%).

4. Reducing power

4. 1. Reducing power of strawberry seedlings shoots (SS) extracts

Data represented in Table (4), and Figure (1) showed the reducing power of strawberry seedlings shoots extracts. In comparison of reducing power of fractions there were significant difference between all fraction in comparison with LSD value (0.04) EAF had the highest reducing power (2.21) at the concentration 250 µg/ ml followed by BUF (1.76), CE (0.92), AF (0.89) and MCF had the lowest reducing 0.88 at the same concentration. Also, EAF had the lowest IC 0.5 value (24.18µg/ ml) followed by BUF (28.28µg/ ml), CE (58.91µg/ ml), MCF (92.84µg/ ml) and AF (144.77µg/ ml). On the other hand, in comparison of reducing power of SS extracts and reducing power of standard substances ascorbic, BHA and BHT (table 5), we found that IC0.5 value of EAF (24.18µg/ ml) and BUF (28.28µg/ ml) were lower than IC0.5 value of BHA (33.38µg/ ml) and BHT (56.97µg/ ml) while it was higher than IC 0.5 value of ascorbic acid (6.91µg/ ml). From this data we concluded that EAF and BUF of strawberry seedling shoot extracts (SS extracts) had high reducing power equal or high than standard substances (ascorbic, BHA and BHT) at some concentration.

Table (4) Reducing power of strawberry seedlings shoots extracts

extract (S)	concentration µg /ml (C)						
	50	100	150	200	250	Mean	IC 0.5
CE	0.72	0.80	0.84	0.88	0.92	0.83	58.91
MCF	0.57	0.64	0.69	0.78	0.88	0.71	92.84
EAF	0.77	1.38	1.93	2.13	2.21	1.68	24.18
BUF	0.81	1.14	1.52	1.61	1.76	1.37	28.28
AF	0.18	0.34	0.53	0.65	0.89	0.52	144.77
Mean	0.61	0.86	1.10	1.21	1.33	1.02	

LSD 0.05 S= (0.04), and SC (0.9)

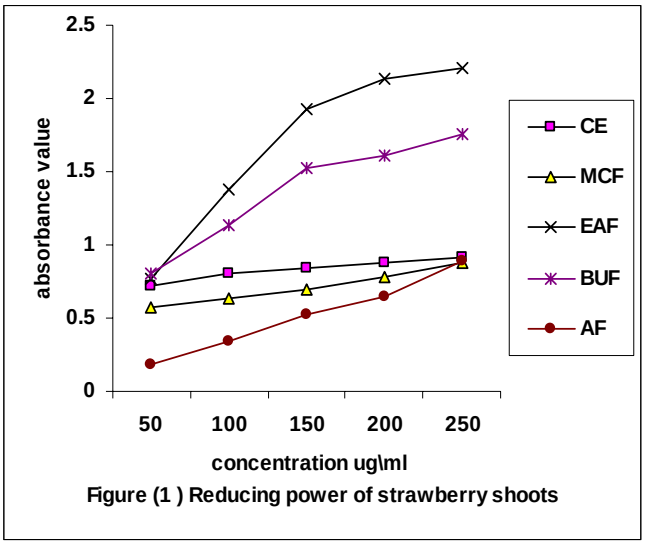

Figure (1) Reducing power of strawberry shoots

Table (5) Reducing power of Ascorbic, BHA and BHT.

extract (S)	concentration µg /ml (C)						
	50	100	150	200	250	Mean	IC 0.5
Ascorbic	0.93	1.84	2.17	2.32	2.38	1.93	6.91
BHA	0.74	1.18	1.82	2.11	2.38	1.65	33.38
BHT	0.49	0.9	1.23	1.51	2.04	1.23	56.97
Mean	0.72	1.31	1.74	1.98	2.27	1.6	

LSD 0.05 S=0.06 and SC =0.13

4.2. Reducing power of strawberry seedlings roots (SR) extracts

Data represented in Table (6) and Figure (2) showed the reducing power of strawberry seedlings roots (SR) extracts.

Table (6) Reducing power of strawberry roots

extract (S)	concentration µg /ml (C)						
	50	100	150	200	250	Mean	IC 0.5
CE	0.68	0.77	0.83	0.90	1.04	0.84	64.89
MCF	0.47	0.77	0.81	0.84	0.88	0.76	84.55
EAF	0.62	0.82	0.93	1.14	2.04	1.11	62.34
BUF	0.81	1.87	1.98	2.25	2.40	1.86	12.98
AF	0.24	0.45	0.67	0.80	0.95	0.62	120.17
Mean	0.57	0.94	1.04	1.19	1.46	1.04	

LSD 0.05, S= (0.04) and SC= (0.09)

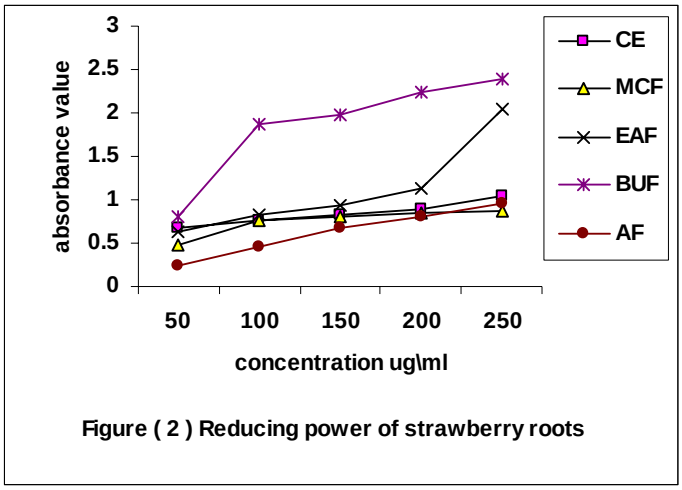

Figure (2) Reducing power of strawberry roots

In comparison between fractions in reducing power value, there were significant differences between all fractions in comparison with LSD value (0.04). Where BUF had the highest reducing power (2.4) at the concentration 250 µg/ml, followed by EAF (2.04), CE (1.04), AF (0.95) and MCF was 0.88 at the same concentration. Also, BUF had the lowest IC0.5 value (12.98µg/ ml) followed by EAF (62.34µg/ ml), CE (64.89µg/ ml), MCF (84.55µg/ ml) and AF (120.17µg/ ml).

In comparison with reducing power of SR extracts of strawberry (table 6) and reducing power of standard substance ascorbic, BHA and BHT (table 5), we found IC0.5 value of BUF (12.98µg/ ml) was lower than IC 0.5 value of BHA (33.38µg/ ml) and BHT (56.97µg/ ml) while it was higher than IC 0.5 value of ascorbic acid (6.91µg/ ml). These results are in harmony with those concluded by Tsao *et al* (2003) on strawberry extracts.

In comparison between reducing power value (table 4 and 5) and extracts contents of total phenols (table 1), flavonoids (table 2) and proanthocyanidins (table 3), the obtained data revealed positive relationship between total phenols and flavonoids content of extracts and reducing power value, where extracts which contain high amounts of total phenols and flavonoids had high reducing power value. While

no relationship between content of proanthocyanidin and reducing power value. These results are in agreement with those obtained by Bahorun *et al* (1994) on *Crategus monogyna*

5. Antioxidant activity

5.1. Antioxidant activity on linoleic acid system

Antioxidant activity of strawberry seedlings shoots extracts (SS) on linoleic acid system.

Data represented in Table (7) and Figure (3) showed the antioxidant activity % of. Strawberry seedlings shoots extracts on linoleic acid system. In comparison between SS extracts, there were significant difference between all fractions of SS in antioxidant activity % in comparison with LSD value (0.71), where BUF had the highest antioxidant activity % (75.96%) at concentration 50 µg/ml followed by EAF (73.19%), CE (35.39%) AF (22.87%) and MCF had the lowest value (15.77%) at the same concentration. Also, BUF had the lowest IC50 value (34.57µg/ ml) followed by EAF (35.52µg/ ml), CE (71.72µg/ ml) AF (105.1µg/ ml) and MCF (171.71µg/ ml).

Table (7) Antioxidant activity of strawberry seedlings shoots extracts on linoleic acid system.

extract (S)	concentration µg /ml (C)						
	10	20	30	40	50	Mean	IC 50
CE	16.08	21.19	26.05	27.72	35.39	25.29	71.72
MCF	10.22	11.79	13.86	14.27	15.77	13.18	171.71
EAF	18.21	25.77	43.27	52.10	73.19	42.51	35.52
BUF	22.38	29.62	36.52	55.71	75.96	44.04	34.57
AF	8.18	9.62	18.67	20.50	22.87	15.97	105.1
Mean	15.01	19.60	27.67	34.06	44.64	28.20	

LSD 0.05 for S =0.71 and SC =1.58

In comparison between antioxidant activity % of SS and antioxidant activity of standard substances ascorbic, BHA and BHT (Table 8), we found that BUF of SS had antioxidant activity 75.96% higher than BHA (74.39%) and BHT (73.8%) while it was lower than

ascorbic 96.06%. On the other hand, EAF had antioxidant activity (73.19%) equal antioxidant activity of BHT (73.8%) while it was lower than ascorbic (96.06%) and BHA (74.39%) at the same concentration 50 µg/ml. Also, IC 50 value of BUF (34.57µg/ ml) and EAF (35.52 µg/ ml) were higher than ascorbic (19.90µg/ ml) and BHA (31.5µg/ ml). These results were in harmony with the findings of Tsao *et al* (2003) on strawberries extracts.

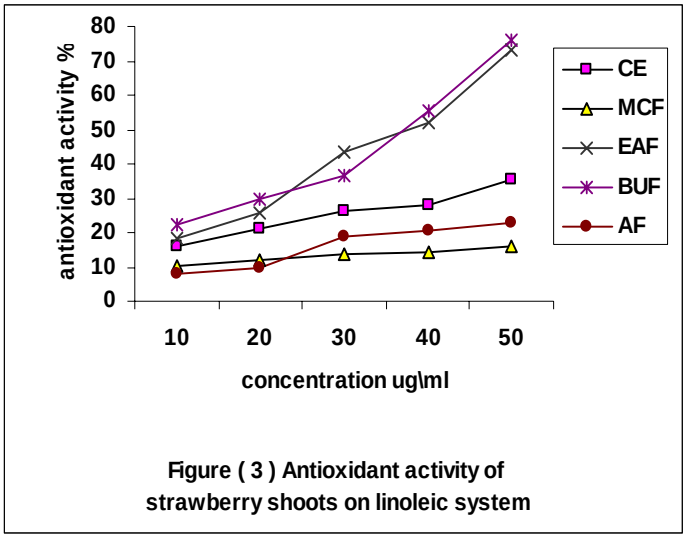

Figure (3) Antioxidant activity of strawberry shoots on linoleic system

In comparison between antioxidant activity % and the content of total phenol, flavonoids and proanthocyanidins, we found that there were positive relationship between total phenol and flavonoids contents and antioxidant activity %. No relation between antioxidant activity and proanthocyamidin content. These results were in agreement with those obtained with Costantino *et al* and Bahorun *et al* 1994).

Table (8) Antioxidant activity of Ascorbic, BHA and BHT on linoleic acid system

extract (S)	concentration µg /ml (C)						
	10	20	30	40	50	Mean	IC 50
Ascorbic	26.73	56.90	89.30	93.25	96.06	72.45	24.78
BHA	19.79	27.68	40.41	61.32	74.39	44.72	21.72
BHT	18.27	44.10	46.07	61.85	73.80	48.82	19.98
Mean	21.60	42.89	58.59	72.14	81.42	55.33	

LSD 0.05 S=0.71 and SC=1.59

Antioxidant activity of strawberry seedlings roots extracts (SR) on linoleic acid system

Data represented in Table (9) and Figure (3) showed the antioxidant activity % of strawberry seedlings roots extracts.

Table (9) Antioxidant activity of strawberry seedlings roots extracts on linoleic acid system

extract (S)	concentration µg /ml (C)						
	10	20	30	40	50	Mean	IC 50
CE	14.87	23.34	29.08	30.17	29.89	25.47	75.08
MCF	9.87	11.36	12.06	13.64	14.09	12.2	194.06
EAF	24.88	34.62	47.53	52.5	79.36	47.78	32.24
BUF	23.4	33.92	54.15	64.29	90.35	53.22	28.32
AF	4.59	4.77	8.65	9.03	10.23	7.46	249.23
Mean	15.52	21.6	30.29	33.92	44.79	29.23	

LSD 0.05 S=1.13 and SC=2.52

In comparison between antioxidant activity of SR extracts, there were significant difference between all fractions in comparison with LSD value (1.13), where BUF had the highest antioxidant activity % (90.35%) at concentration 50 µg/ml followed by EAF (79.36%), CE (29.89%), MCF (14.09%) and AF had the lowest antioxidant activity (10.23%) at the same concentration. Also, BUF had the lowest IC 50 value (28.32µg/ml) followed by EAF (32.24µg/ml), CE (75.08µg/ml), MCF (194.06µg/ml) and AF (249.23µg/ml).

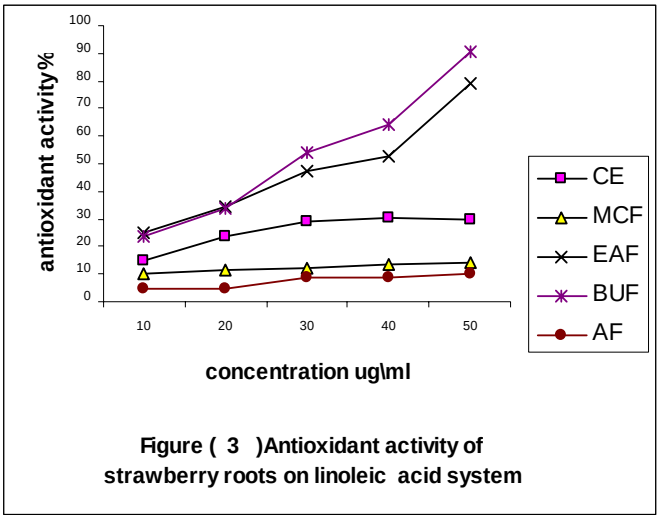

Figure (3)Antioxidant activity of strawberry roots on linoleic acid system

In comparison between antioxidant activity of SR extracts and standard antioxidants ascorbic, BHA and BHT, we found that IC 50 value was increased in the following order ascorbic (19.90µg/ml) < BUF of SR (28.32µg/ml) < BHT (3.50µg/ml) < EAF of SR (32.24µg/ml) < BHA (33.75µg/ml). These results are in harmony with those obtained by Tsao *et al* (2003) on strawberries extracts. From these results we concluded that BUF and EAF of strawberry roots extracts was good antioxidant agent.

In comparison between antioxidant activity % and the content of total phenol, flavonoids and Proanthocyanidins, we found that there were positive relationship between antioxidant activity % and the content of total phenol and flavonoid for example BUF had antioxidant activity (90.35%) at concentration 50 µg/ml and contain total phenol (43.65%) and flavonoid (62.06%) while AF had antioxidant activity (10.23%) at the same concentration and contain total phenol (10.2%) and flavonoid(11.46%). These results were in agreement with the findings of Castantino *et al* (1994) and Bahroun *et al* (1994).

5.2. Scavenging activity on DPPH radical

Scavenging activity of strawberry seedlings shoots (SS) extracts on DPPH radical

Data represented in Table (10) and Figure (4) showed scavenging activity of strawberry seedlings shoots extracts on DPPH radical.

In comparison between scavening activity of fraction there were significant difference between all fractions, where EAF and BUF had the highest scavening activity 93.16% and 93.5% respectively at concentration 25 µg/ml followed by AF (75.34%), CE (44.96%) and MCF had the lowest scavening activity (43.74%) at the same concentration. Also, EAF and BUF had the lowest IC 50 values (7.25µg/ml and 7.37 µg/ml respectively) followed by AF (16.56µg/ml), CE (23.84µg/ml) and MCF had the highest IC 50 value (27.59µg/ml). From this data we concluded EAF and BUF of SS had the best results in scavening activity on DPPH radical.

Table (10) Scavenging activity of strawberry seedlings shoots extracts on DPPH

extract (S)	concentration µg /ml (C)						
	5	10	15	20	25	Mean	IC 50
CE	27.55	33.41	41.84	43.27	44.96	38.21	23.84
MCF	13.14	20.33	31.63	36.28	43.74	29.02	27.59
EAF	60.23	70.6	88.32	91.23	93.16	80.71	7.25
BUF	55.08	77.27	86.2	90.33	93.55	80.48	7.37
AF	23.79	38.31	47.19	50.57	75.34	47.04	16.56
Mean	35.96	47.99	59.04	62.34	70.15	55.09	
LSD 0.05							

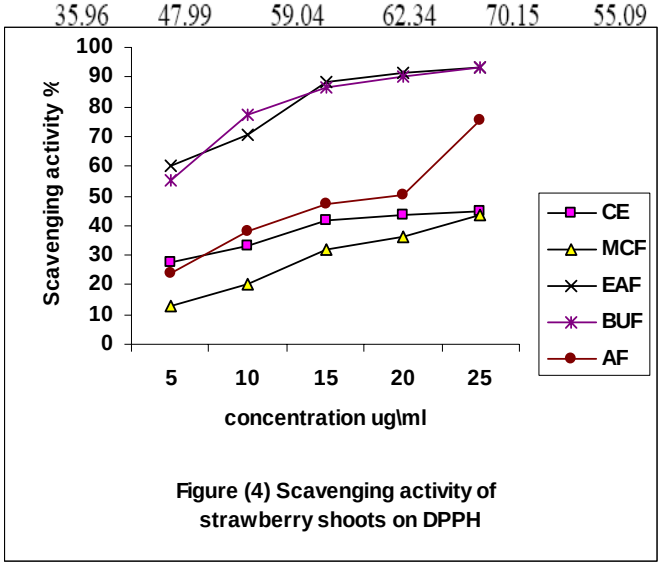

Figure (4) Scavenging activity of strawberry shoots on DPPH

Incomparison between scavening activity of SS extracts and standard substances ascorbic, BHA, and BHT Table (11) we found that IC 50 value of EAF (7.25µg/ml) and BUF (7.37µg/ml) respectively were lower than IC 50 value of Ascorbic, (10.23µg/ml), BHA (8.03µg/ml) and BHT (9.77µg/ml). From our results concluded that EAF and BUF of SS had the best scavening activity than standard antioxidant ascorbic, BHA and BHT. These results are in harmony with those concluded by Kiselova *et al* (2006) and Tsao *et al* (2003) on strawberries extracts.

Table (11) Scavenging activity of Ascorbic, BHA and BHT on DPPH

extract (S)	concentration µg /ml (C)						
	5	10	15	20	25	Mean	IC 50
Ascorbic	43.08	49.21	79.52	85.10	90.30	69.44	10.23
BHA	50.00	69.04	89.15	92.34	94.53	79.01	8.03
BHT	46.01	61.96	66.48	89.92	90.80	71.03	9.77
Mean	46.36	60.07	78.38	89.12	91.88	73.16	

LSD 0.05 S =0.69 and SC =1.53

Scavenging activity of strawberry seedlings roots (SR) extracts on DPPH radials

Data represented in Table (12) and Figure (5) showed the scavening activity of strawberry roots extracts on DPPH radical.

Table (12) Scavenging activity of strawberry seedlings roots extracts on DPPH.

extract (S)	concentration µg /ml (C)						
	5	10	15	20	25	Mean	IC 50
CE	37.12	42.91	44.27	44.56	47.07	43.19	21.97
MCF	38.39	42.16	44.93	45.65	46.25	43.48	21.95
EAF	64.26	76.18	85.17	90.54	93.08	81.85	6.74
BUF	64.43	86.08	90.30	90.37	92.79	84.79	5.88
AF	13.46	17.30	27.07	33.84	44.44	27.22	28.89
Mean	43.53	52.92	58.35	60.99	64.72	56.10	

LSD 0.05 S =0.69 and SC =1.53

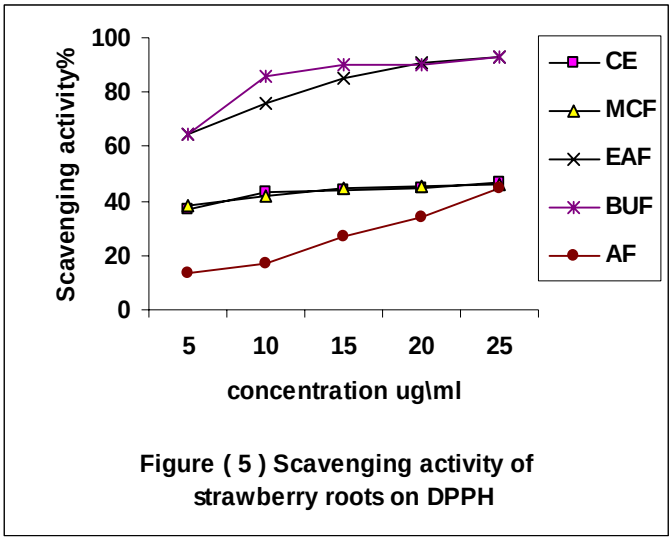

Figure (5) Scavenging activity of strawberry roots on DPPH

In comparison between scavening activity of fractions, there were significant difference between fractions in scavening activity %

in comparison with LSD value (1.23). Where EAF and BUF had the highest scavening activity (93.08% and 92.79% respectively) at concentration 25 µg/ml, followed by CE (47.07%), MCF (46.25%) and AF (44.44%). Also BUF had the lowest IC50 value (5.88µg/ml) followed by EAF (6.74µg/ml), MCF (21.95µg/ml) ,CE (21.97µg/ml), and AF (28.89µg/ml). From this data we concluded EAF and BUF of SR had the best scavening activity on DPPH than other fractions. In comparison between scavenging activity of SR extracts and standard substances ascorbic, BHA and BHT Table (12) we found IC50 value of EAF (6.74µg/ml) and BUF (5.88µg/ml) were lower than IC 50 value of ascorbic (10.23µg/ml), BHA (8.03µg/ml) and BHT (9.77µg/ml). From our results concluded that EAF and BUF of SR had the best scavenging activity than standard antioxidant ascorbic, BHA and BHT. These results are in agreement with by the findings of Kiselova *et al* (2006) and Tsao *et al* (2003) on strawberries extracts.

In comparison between scavening activity (table 11) and total phenol, flavonoid and proanthocyanidin, contents (Figure 1) we found that scavening activity increased with increasing total phenol and flavonoid contents. While no relationship between scavening activity and proanthocyanidin content. Increasing antioxidant activity by increasing phenolic compound and flavonoid content may be due to the ease with which an H atom from an aromatic hydroxyl (OH) group (presented in phenolic compound and flavonoids) can be donated to a free radical (peroxyl radicals, alkyl peroxyl radicals, and superoxide hydroxyl radicals) and the ability of an aromatic compound to support an unpaired electron due to delocalization around the π-electron system and in consequently reduce lipid peroxidation(according to Duthie *et al*. (2000).

6. Anticancer activity

Anticancer activity of strawberry seedlings shoots extracts on HEPG2

Table (13) anticancer activity of strawberry seedlings shoots extracts on HEPG2

extract(S)	Concentration µg /ml (C)					
	1	2.5	5	10	mean	IC 50
CE	68.36	72.66	75.64	77.95	73.65	1.91
MCF	68.36	72.66	75.64	77.95	73.65	2.06
EAF	21.41	33.60	40.75	81.70	44.36	5.67
BUF	40.97	55.33	54.15	70.02	55.12	4.83
AF	18.31	47.47	68.46	68.75	50.75	5.14
Mean	43.48	56.35	62.92	75.27	59.51	

LSD 0.05 S =0.81 and SC =1.62

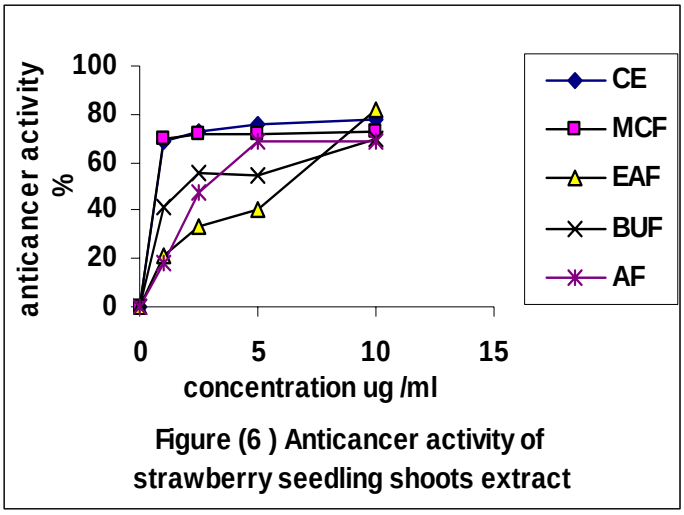

Figure (6) Anticancer activity of strawberry seedling shoots extract

There were significant difference between fraction except CE and MCF there were no significant difference between them in comparison with LSD value (0.81) Table (13) and Figure (6). In comparison between fractions at the highest concentration 10 µg/ml we found that EAF had the highest anticancer activity (81.7%)

followed by CE = MCF (77.95%), BUF (70.02%), and AF had the lowest anticancer activity (68.75%). In comparison of IC 50 value we found that CE had the lowest IC 50 value (1.91µg/ml) followed by MCF (2.06µg/ml), BUF (4.83µg/ml), AF (5.14µg/ml) and EAF had the highest IC 50 value (5.67µg/ml).

Anticancer activity of strawberry seedlings roots (SR) extracts on HEPG2

Data represented in Table (14) and Figure (7) showed anticancer activity of strawberry seedlings roots extracts.

Table (14) anticancer activity of strawberry seedlings roots extracts on HEPG2

extract (S)	concentration µg /ml (C)					
	1	2.5	5	10	mean	IC 50
CE	43.99	67.32	70.55	75.41	64.32	3.45
MCF	50.63	57.66	61.22	65.74	58.81	4.35
EAF	28.68	33.65	36.41	53.18	37.98	6.49
BUF	40.10	42.09	50.16	53.31	46.41	7.08
AF	72.27	72.84	72.45	73.72	72.82	1.79
Mean	47.13	54.71	58.16	64.27	56.07	

LSD 0.05 S =0.73 and SC =1.47

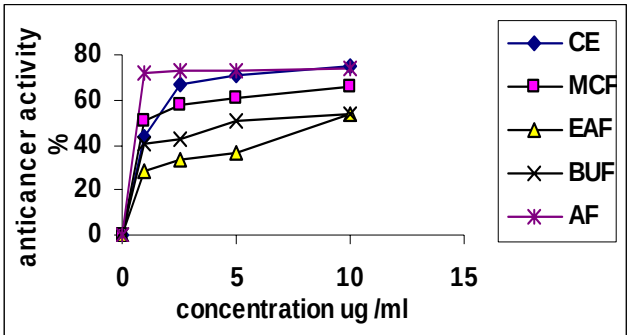

Figure (7) Anticancer activity of strawberry seedling roots extract

There were significant differences between fractions in comparison with LSD value (0.13). In comparison between all fractions at the highest concentration 10 µg/ml we found that CE had the highest anticancer activity (75.41%) followed by AF (73.72%), MCF (6.74%) and no significant difference between EAF (53.18%) and BUF (53.31%). In comparison of IC 50 value we found that AF had the lowest IC 50 value (1.79µg/ml) followed by CE (3.45µg/ml), MCF (4.35µg/ml), EAF (6.49µg/ml) and BUF had the highest IC 50 value (7.08µg/ml). No relationship between anticancer activity and the total phenols, flavonoid and proanthocyanidin contents. Also no relationship was found between anticancer activity and antioxidant activity. Our results are supported by the studies of Meyers *et al* (2003).

The anticancer activity of strawberry extracts may be due to their contents of ellagic acid , ellagitannins and flavonol glycoside (the major flavonol aglycons were quercetin and kaempferol)all this compounds had anticancer activity (according to Seeram *et al* ,2006)

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أنشطه مستخلصات بادرات الفراوله (صنف سويت شارل) كمضادات للأكسده ومضادات للسرطان

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تم دراسه تاثير مستخلصات المجموع الخضرى والجذرى ليادرات الفراوله (صنف سويت شارل) كمضادات للاكسده ومضادة للسرطان باستخدام تجارب معمليه مختلفه. اوضحت النتائج ان مستخلصات يادرات الفراوله سواء المجموع الخضرى او الجذرى لها نشاط عالى كمضادات للاكسده على نظام حمض لينوليك. حيث نجد ان جزء البيوتانول من مستخلص الميثانولى للمجموع الجذرى (له اعلي نشاط مضاد للاكسده) قيمة IC 50 (التركيز الذى يسبب نشاط 50%)=28.32 ميكرو جرام/مل) بالمقارنه بمضادات الاكسده الصناعيه BHT (IC 50=31.5 ميكرو جرام /مل) وكذلك BHA (IC50 = 33.75 ميكرو جرام/مل) كذلك نجد ان مستخلصات الفراوله سواء المجموع الخضرى او الجذرى لها نشاط كاسح للشق الحر DPPH حيث اعلى نشاط كاسح لوحظ بواسطه جزء البيوتانول من مستخلص الميثانولى للمجموع الخضرى للفراوله (93.55%) يليه جزء ايثيل اسيتات للمجموع الخضرى (93.16%) يليه جزء ايثيل اسيتات للمجموع الجذرى (93.08%) يليه جزء البيوتانول للمجموع الجذرى (92.79%) وذلك باستخدم تركيز 25 ميكروجرام/مل ونلاحظ ان كل المستخلصات السابقه لها نشاط كاسح للشقوق الحره اعلى من مضادات للكسده الصناعيه (الاسكوربيك(90.30%) يليه BHT (90.80%)). هناك علاقه طرديه بين نشاط المستخلص كمضاد للاكسده ومحتواه من الفينولات والفلافونيتيدات كذلك هناك علاقه طرديه بينهما وبين القوه الاختزاليه للمستخلص.

وبدراسه تاثير المستخلصات كمضادات للسرطان على نوع من خلايا الكبد HEPG2 نجد ان مستخلصات المجموع الخضرى لها اعلى تاثير مضاد للسرطان بالمقارنه مع مستخلصات المجموع الجزرى حيث نلاحظ ان جزء ايثيل اسيتات للمستخلص الميثانولى للمجموع الخضرى له اعلى نشاط مضاد للسرطان (81.7%) بينما جزء ايثيل اسيتات لمستخلص الميثانولى للمجموع الجزرىيعطى نشاط (53.18%)عند تركيز 10 ميكروجرام/مل. ليس هناك علاقه بين نشاط المستخلص كمضاد للاكسده ونشاطه كمضاد للسرطان.