

Identification of Antioxidant compounds in red raspberry (*Rubus Idaeus*) Fruit in Kurdistan region (north Iraq)

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Abstract: The six different antioxidant compounds in red raspberry (*Rubus idaeus*) Fruit which grows in sulaimani city- Kurdistan region –north Iraq (season 2011) were detected by using HPLC technique including neoxanthin, lutein, anthocyanin, ellagitanins, B-carotene and violaxanthin the amount of them were 21.6060mg/ml, 110.8226mg/ml, 291.7407mg/ml, 205.2504mg/ml, 261.6247mg/ml and 129.3089mg/ml respectively

Keywords: Antioxidant, red raspberry, HPLC analysis, lutein, anthocyanin, B-carotene, violaxanthin, neoxanthin, ellagitanins

I. Introduction:

Red raspberry is a plant which stand about 6 feet tall. It is often found growing wild in hedges. It may be vining or shrubby but tends to grown in thickets, it belongs to the genus (*Rubus*), the genus *Rubus* is a member of the rose family (*Rosaceae*). It is cultivated and grows wild in sulaimani city- Iraq(1). A local name of red raspberry fruit in Kurdish is *tirk*(1).

Antioxidant substances are phytochemicals, which are found naturally in plants that help fight disease in humans, these antioxidant delay aging and prevent disease by destroying free radicals from harming your DNA and causing cellular damage and death(2). Toxicity of these compounds and the growing interest in safe nutritional additives by consumers has created the necessity to identify natural and safe source for food antioxidants(3). Raspberries are an important commercial fruit crop. Widely grown in all temperate regions of the world (4.) Raspberries contain significant amount of poly phenol antioxidants such as anthocyanin pigments linked to potential health protection against several human diseases (5). The aggregate fruit structure contributes to its' nutritional value as it increases the proportion of dietary fiber, placing it among plant foods with the highest fiber contents known up 20% fiber per total weight(6). Raspberry are a rich source of vitamin C with 30mg per serving of 1 cup (about 50% daily value), manganese (about 60% daily value), content of B vitamins 1-3, folic acid, magnesium, copper and iron are considerable in raspberries(6). Raspberries rank near the top of all fruit for antioxidant strength, particularly due to their dense contents of ellagic acid (from ellagitannins), and anthocyanins. It has an ORAC value of about 4900 per 100 gram(7). Neoxanthin is a carotenoid and xanthophylls in plants, it is intermediate in the biosynthesis of the plant hormone abscisic acid. It is produced from violaxanthin by the action of neoxanthin synthase(8). Lutein from Latin *luteus* meaning yellow is xanthophylls and one of 600 known naturally occurring carotenoids. It is synthesized only by plants and like other xanthophylls is found in high quantities in green leafy vegetables such as spinach and kale(9). It is antioxidant, which belongs to the carotenoid family. Although lutein is not categorized as a vitamin, dietary lutein is believed to be an essential nutrient for normal vision, studies have also indicated that lutein improves heart health, reduces diabetes and anti-cancer properties(10). Anthocyanins belong to group of flavonoids polyphenolic molecules they can be found in tissues of plants, including leaves, stems, roots, flowers and fruits. Studies have shown that anthocyanin may act as anti-cancer by inhibit promotion and progression of tumor cells by stopping the growth of pre-malignant cells(11) results demonstrate the strong antiproliferative activity of violaxanthin on one human mammary cancer cell line, and suggest that studying the pharmacology of violaxanthin and pharmacomodulated derivatives on cancer cells may allow potent antiproliferative drugs to be obtained(12).



Fig(1) Red raspberry (*Rubus Idaeus*) fruit

II. Material and methods

Red raspberry fruit was harvested by hand in its optimum state for two consecutive seasons in 2011 in Sulaimani city -Iraq. After a morphological and chemical characterization, the sample was prepared for determination of antioxidant compounds, elemental analysis and protein determination.

1-Extraction Procedure of antioxidant compounds(13)

Active ingredient were extracted according to the procedure reported by Lakshimin anayana et al (13) which were modified according to our fast FLC fast liquid chromatographic separation which briefly described as follow ;the sample powder 5 mg were extracted with ice –cold acetone until the sample become colorless (final volume 100 ml) ,the crude extract (50 ml) were taken in separating funnel ,100 ml of the petroleum ether added after mixing well , the upper layer were separated ,the extract were repeated three times. Total volume (200ml) the extract was dried over anhydrous sodium sulphate 20 mg and filtered through whatmann no.1 Filter paper. The filtrate were evaporated to dryness in rotary evaporator (Buchi Switzerland) at 30 C^o and re-dissolved in known volume of hexane and aliquot of 20µ/ml were used for HPLC analysis.

2- Determination of element concentration

10gm of sample was weighted in to a porcelain crucible , placed in a muffle furnace temperature was increased to 600^oC and held at the temperature for 6h., the sample was removed from the furnace and weighted, the determine percentage of ash for sample was (0. 2)% . The residual ash was dissolved in 1:1 nitric acid filtered and the volume was completed to (100ml) distilled water, the solution was injected to OES-ICP Perkin Elmer 2100 for determine

3- Determination of protein by Micro Kjeldhal (14)

Preparation of solution

A- 10 N NaoH:- (40g)of NaoH was dissolved in 100 ml distilled water

B-Boric acid solution : Dry boric acid (2g)was dissolved in(100ml) of distilled water in a volumetric flask (100ml)and mixed well

C-Mixed indicator :- Methyl red indicator(0.016g)and bromocresol green indicator (0.099)g were dissolved in absolute ethanol(100ml) and mixed well

D-(2ml)of solution in step C was added to the solution in step B, the volume completed with distilled water and the PH of the solution was adjusted to (5) exactly

Procedure

According to the Schuffelen and co-workers (14) the raspberry fruit (rubs idea) (0.5g) was digested by digestion system with concentrated Sulfuric acid (10ml) and hydrogen peroxide 10 ml , until a clear solution was obtained , then the volume was completed with distilled water to 100ml , 10ml of digested was transferred to a micro Kjeldahi system ,solution number A 10ml was added and the liberated ammonia was combined to a conical flask that contain 25ml solution number D. The colour was changed to green .The latter solution was titrated with Sulfuric acid 0.0462 N

The total nitrogen and protein percentage were calculated according to equation (1) and (2)

%N=(ml acid of sample – ml acid of blank) x0.05x 100x100x14 /wt of sample x10x1000 ----1

%protein=% Nx protein Coefficient -----2

III. Result and discussion

The HPLC analysis of antioxidant compounds in red raspberry fruit are present in table(1)fig(2), from the result six different antioxidant compounds are indicated includes violxanthin ,lutein , anthocynins , ellagitanins and B-carotene . red raspberry fruit contain high amount of anthocyanins 291.7407%,β-carotein 261.6247%,ellagitanins 205.2504%,violanthin 129.3089%,lutein 110.8226% and neoxanthin 21.6060%.

Elemental analysis have been done by inductive couple plasma(ICP) techniques (OES-ICP Perkin Elmer 2100) for red raspberry fruit table(2) which a high amount of K,P,Ca and Mg elements have been detected.

The percentage of protein by Micro Kjeldhal method in this fruit is 1.2 per 100 gram and glucose, sucrose and fructose was determined

IV. Conclusion

The red raspberry fruit in sulaimani city –Kurdistan region- Iraq is a rich source antioxidant compounds like violxanthin ,lutein , anthocynins , ellagitanins and B-carotene . The content of anthocyanin is very high in the raspberry fruit , all these components are powerful anti-oxidants and are beneficial in preventing cardiovascular diseases like Angina ,arteriosclerosis and present of anthocyanin,ellagitanins can reduce LDL oxidation .Also Research on anthocyanins also shows that they reduce the coagulation of blood platelets, inhibiting formation of blood clots involved in stroke, pulmonary embolism, peripheral vascular

Identification of Antioxidant compounds in red raspberry (Rubus Idaeus) Fruit in Kurdistan

disease and heart attack, promote higher levels of “ good” cholesterol (HDL), and inhibit oxidation of “ bad” cholesterol (LDL).

Raspberry fruit is loaded with trace elements such as potassium, zinc, iron, copper, manganese, etc. These trace elements are important constituents of various enzymes.

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no	subjects	Rt/minute	area/standard	df	area/sample	conce.µg/ml	conce. of sample
1	Neoxanthin	1.15	12014	3	3461	25	21.6060
2	Violaxanthin	2.24	15909	3	27429	25	129.3089
3	Lutien	3.47	14777	3	21835	25	110.8226
4	β-carotene	4.47	11996	3	41846	25	261.6247
5	anthocyanins	5.31	12486	3	48569	25	291.7407
6	ellagitannins	6.46	15756	3	43119	25	205.2504

Table (1) antioxidant compounds in red raspberry fruit

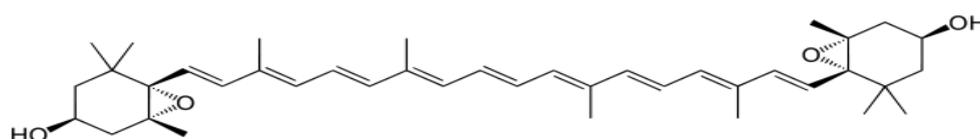
mineral	units	Value per 100gm
Ca	mg	20
Fe	mg	0.5
Mg	mg	21
P	mg	24
K	mg	140
Na	mg	1
Zn	mg	0.40
Cu	mg	o.o1
Mn	mg	0.52
Se	mg	0.20

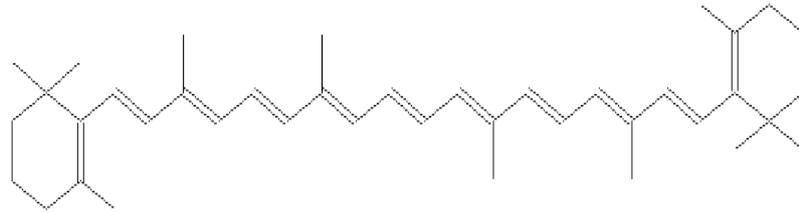
Table(2) elemental analysis of red raspberry fruit

Protein	1.2 per 100 gram
Sucrose	0.2 per 100 gram
Glucose	1.5 per 100 gram
Fructose	2.5 per 100 gram
Lactose	0 per 100 gram
Maltose	0 per 100 gram
Galactose	0 per 100 gram

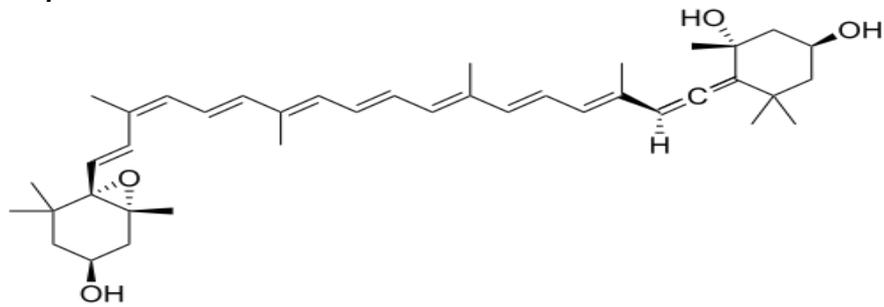
Table(3) protein and sugars content In red raspberry fruit

Fig(2) Chemical structure of antioxidant compounds in red raspberry fruit

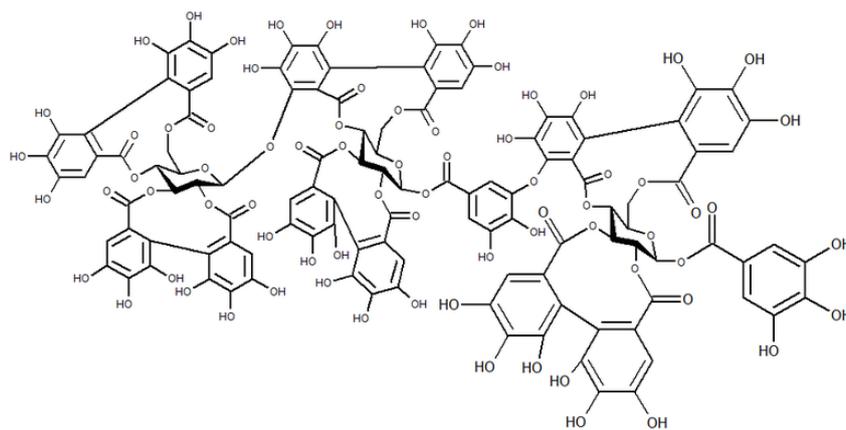




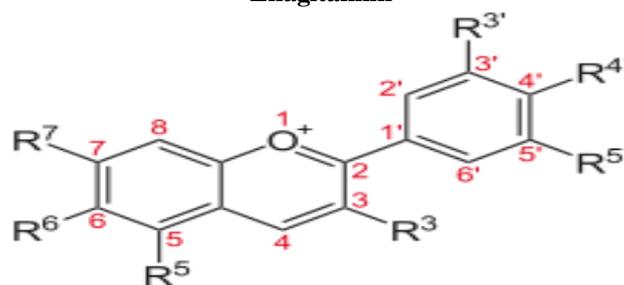
β -carotene



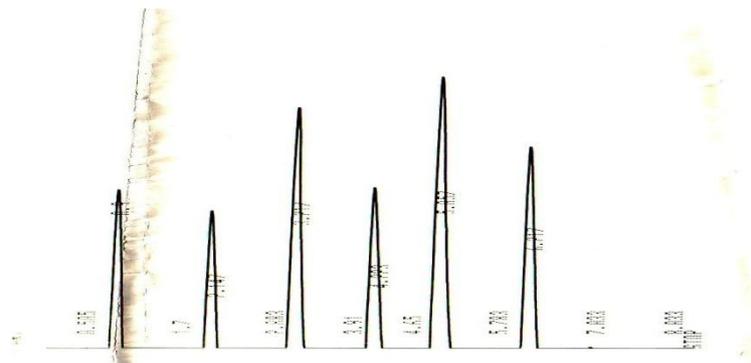
Neoxanthin



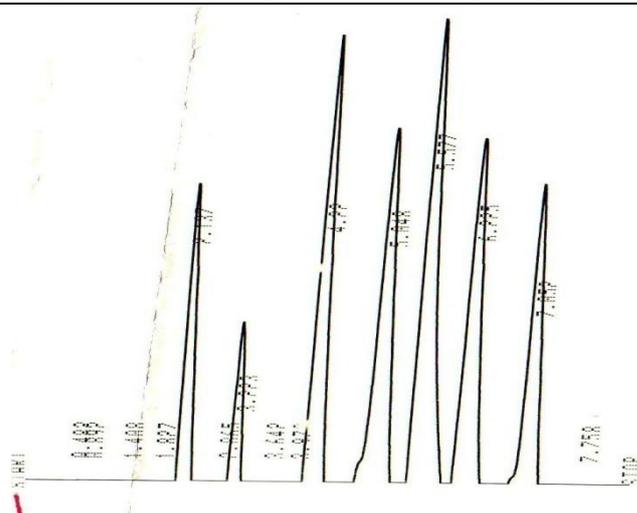
Ellagitannin



Anthocyanins



Fig(3):HPLC chromatograms of a mixture of authentic standards of antioxidant compounds



Fig(4):HPLC chromatograms of antioxidant compounds in red raspberry fruit

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