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TECHNOLOGICAL AND BIOLOGICAL STUDIES ON RED PIGMENTS IN RED BOUGAINVILLEA AND MATHIOLA.

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ABSTRACT

The natural pigments considered the more safety in public health as natural colorants in food instead of the harmful synthetic colorants in this study, red Anthocyanins pigments were extracted from red bougainvillea (Bougainvillea commerson ex) and mathiola (Matthiola incane) petals as a natural colorant by the following methods:

(1) by using ethanolic solution acidified by Hcl.
(2) by using Distilled water
(3) by using Distilled water acidified by 0.1% citric acid.
(4) by using Distilled water acidified by 0.3% citric acid.

It could be noticed that the first method is the most effective in the extractions of the pigments but the last method is the most economically.

On the other hand, in biological experiment, there is no any bad effect on the liver and the kidney functions and there is no any case of death after 45 days of injection of rats with these anthocyanins extracts. too, noticed that there is a gradually decrease of anthocyanins content by storage for 3months.

***Conclusively,** anthocyanins pigments were extracted from red bougainvillea and mathiola petals as natural colorants of foods instead of the harmfull synthetic colorants. In biological experiments , it were safe which there is no any bad effect on the liver and the kidney functions and there is no any case of death during 45 days. Too, noticed that there is a slight gradually decrease of anthoyanins content by storage for 3 months.*

Key worsds: Technological & Biological Studies, Red Pigments, Red Bougainvillea, Mathiola**.**

INTRODUCTION

Interest in anthocyanins as natural colorants and antioxidants ingredients has recently increased due to their color characteristics and potential health benefits (Walkowiak - Tomczak and Czapski, 2007).

The anthocyanins were extracted studied by the researchers, Abd El-Latif *et al.* (1992) were extracted anthocyanins from hibiscus and pomposia petals and the flesh of pomposia fruits, while Monica Giusti and Wrolstanad, (1996) were extracted anthocyanins from red radish. They found that, acylation increased anthocyanin resistance to acid hydrolysis. Labib (1996) was extracted anthocyanins from roselle leaves and studied its stability during storage. He was found a gradually decrease of retention percent of the extracted anthocyanins during storage at 20°C for 15days.

In recent years, the market of food colors industry has rapidly increased and it is expected to continue growing 10% to 15% annually (IFT 2016). Regarding food color industry trends, The use of natural food pigments (also known as natural colorants) has increased in food and beverages as substitutes for their synthetic counterparts . This is mainly due to the growing awareness of environmental hazards and the potential side - effect impacts of the chemicals used in synthesis of food colorants (Carocho *et al.,* 2014). In addition, to satisfy consumers who demand natural ingredients, major food, and beverage companies have committed to removing artificial substances, including synthetic colors, from their products. This marketing strategy is in line with the so. called clean label trend.

Commonly used natural food pigments include anthocyanins, cartenoides, betalains and chlorophylls, among others. In addition to their food application, The consumption of these natural colored compounds has been associated with a reduction of non communicable diseases such as cancer, diabetes and obesity (Lix *et al*., 2016; Cooprstone and Schwartz, 2016; Rodriguez *et al*., 2016).

Several studies have demonstrated the potential use of anthocyanins as food and beverage colorants (Aguilera *et al*., 2016; Chung *et al*., 2016) anthocyanins have been used as food pigments in wide variety of products; for instance, dairy products such as cream cheese, fermented milk, milkshakes as well as in low- PH beverages and also in solid food matrices such as pancakes and omelets (Kitts and Tomiuk, 2013; de Mejia *et al*., 2015; Shin *et al.,* 2015; Pineda-vadillo and *et al*., 2017).

Therefore, this work was aimed to evaluate the extraction of anthocyanin by different methods; to study the effect of these extracts on the

rats, and the effect of PH, temperature and storage conditions on anthocyanin stability.

MATERIALS AND METHODS

Materials:

a- The flowers of red bougainvillea were picked from the farm of El-Kassassin horticultural research station.

b- The flowers of mathiola (El-Manthour) were picked from the farm of Faculty of Agriculture, Zagazig University, Egypt. The petals were separated by hands after the flowers were weighed to obtain of the ratio of petals to the all weight of the flowers.

Methods:

A-The extraction and preparation of the pigment:

1- Anthocyamins pigments were extracted according to the method described by Fuleki and Francis (1968) using acidified ethanol solution at ratio (85/ 15) ethanol 95% Hcl 1.5N. The extract filtered at paper of whatman No. 1 was transferred into 500ml volumetric flak and make up to the volume using the solution of the extraction.

2- Anthocyanins pigments were also extracted using distilled water. The extract was filtered on paper of whatman No. 1 and make up to the volume of 500ml in volumetric flask.

3- Anthocyanins pigments were also extracted using The acidified distilled water using citric acid at. The ratio 0.1%. The extract was filtered on paper of Whitman No. 1 and make up to 500ml in volumetric flask.

4- Anthocyanins pigments were also extracted using the acidified distilled water with 0.3% citric acid. The extract was filtered on the paper of whatman No. 1 and make up to 500ml in a volumetric flask. The extracts were concentrated under vaccum at temperature not excced 60°C +1°C

B-Anthocyanins content was obtained calorimetrically by spectrophotometer spectronic 21 (at 535nm).

C- Anthocyanins content was obtained by spectrophotometer at different PH and different temperature.

D-The moisture content of the flowers and extracts were determined by the method described by Rangana (1979). the total soluble solids were determined by the Abe refractometer as reported in the AOA C (1990).

E- The effect of the extracts on rats was determined according the method of Merier and Theakston (1986). The white rats at 150gm weight were divided to 4 groups on the base of the selected treatments. The white rates were injected with definite volume of the extract as the following:

1- The first group was injected with 0.1ml of the extract/ 100 gm of rat weight body.
2- The second group was injected with 0.3ml of the extract/ 100 gm of rat weight body.
3- The third group was injected with 0.5ml of the extract/ 100gm of rat weight body.
4- The fourth group was injected with 1.0ml of the extract/ 100gm of rat weight body.

After 45days of the treatment, the liver function, the kidney function and the death rate were determined for treated rats.

F-Storage of anthocyanins pigments. anthocyanins extracts were filled in white bottles and stored for 3 months at room temperature.

The spectronic determinations were adopted to obtain of anthocyanins pigments content during storage.

RESULTS AND DISCUSSION

Some physical characteristics and anthocyanin content of red bougainvillea and mathiola:

Data represented in Table (1) indicated that, the petals of red bougainvillea flowers were represented 78.43% of the flowers weight, while the petals of mathiola flowers were represented 51.33% of the flower weight.

The weight of 10flowers of red bougainvillea and mathiola were 4.08 and 1.5gm respectively while the petals weight were 3.20 and 0.77 (gm) respectively it could be noticed that red bougainvillea flowers and petals weight are bigger than that in mathiola flowers and petals.

The color of the anthocyanins extracts of both red bougainvillea and mathiola petals were red when it were extracted by using the acidified ethanol solution.

Table (1): Some physical characteristics and anthocyanin content of red bougainvillea and mathiola.

The main items	**Red bougainvillea**	**Mathiola**
Flower color (visual)	Red	Red
The weight of 10 flowers (gm)	4.08	1.5
The weight of 10 flowers petals (gm)	3.20	0.77
Petals/ Flower (%)	78.43	51.33
Anthocyanin content extracted using acidified ethanol (mg/ 100gm)	88.40	112.30
The color of the ethanolic extract	Red	Red

Effect of extraction solutions on anthocyanin content.

The data represented in Table (2) showed that the extraction with the Acidified ethanol solution had the highest content of anthocyanins extracted from both of red bougainvillea and mathiola petals. It could be noticed that the distilled water with 0.3% citric acid was more effective in anthocyanins extraction than the extraction by the distilled water with 0.1% citric acid both in red bougainvillea and mathiola petals and economically applicable process in industry scale and commercially, These notice are in agreement with those reported by Abd El-Latif (1992) and Labib (1996).

Table (2): Effect of extraction solutions on anthocyanin content (mg/100gm).

Extraction Solution	**Anthocyanin (mg/100gm)**	
	Red Bougainvillea	**Mathiola**
Acidified ethanol	88.39	110.23
Distilled water	39.99	102.71
Distilled water with 0.1%citricacid	59.37	104.31
Distilled water with 0.3%citricacid	63.41	107.32

Biological effect.

Data represented in Table (3) showed the effect of the injection of the white rats at the weight of 150 gm with anthocyanins extracts on rats liver (G.O.T) and (G.P.T) kidney function (creatinine and uric acid) and the rate of the death. It could be noticed that the treatments of these rates with the

extraction at rates of 0.1, 0.3, 0.5 and 1.0 ml/100gm of the rats body were not affected on any treated rats which (G.O.T) were 35/ml, (G.P.T) were 32u/ml, creatinine were 0.8mg/dI and uric acid were 5 mg/dl for all treatments and all these results were in normal level. These results were obtained after 45 days of the injection.

On the other hand, there is no any bad effect on the liver, the kidney and the rate of death.

Effect of processing temperature:

Data represented in Table (4) showed that the effect of temperature on anthocyanins content (mg/100gm) on different pH at wave length (535) by spectrophotometer.

It could be noticed that, there are as light gradually decrease of the anthocyanins content by the affect of increasing temperature in both pigments of red bougainvillea and mathiola petals. The decrease of anthocyanins pigments content may be due to the conversions of anthocyanins by the effect of temperature and acidity. This results are in agreement with those which reported by Labib (1996).

Table (4): Effect of processing temperature on anthocyanins content (mg/100gm) at different pH for 5 minutes.

Temp. / pH	50˚C		70˚C		100˚C	
	R	**M**	**r**	**M**	**r**	**m**
8.1	86.23	104.50	84.37	98.31	80.91	94.20
5.6	88.41	106.70	87.00	99.10	82.00	96.35
3.1	91.27	108.34	91.20	100.30	85.60	98.38

r: is red bougainvillea, m: is mathiola

Effect of storage period (weak) at room temperature (25+2˚C)

The represented data in Table (5) showed the effect of storage at room temperature for 12 weeks on anthocyanins pigments contents in red bougainvillea petals and mathiola petals.

It could be noticed that there are a slight gradually degradation of the anthocyanins content in both extracts of red bougainvillea and mathiola petals. The decrease of anthoeganins pigments content is may be attributed to color oxidation by passing time in storage but this decrease had not effect on the color of the pigment in both red bougainvillea and mathiola petals extracts.

Table (5): Effect of storage period (weak) at room temperature (25+2˚C) on anthocyanin content of acidified ethanolic extracts.

Storage period (week)	Anthocyanin content of acidified ethanolic extract (mg/100gm)	
	Red Bougainvillea	Mathiola
0	88.43	108.03
1	88.24	108.00
2	87.30	107.20
3	86.55	106.34
4	86.10	104.22
5	84.39	102.33
6	82.94	102.13
7	81.70	101.95
8	79.34	100.41
9	79.10	100.20
10	78.25	99.9
11	77.00	99.2
12	76.31	99.1

Conclusively, anthocyanins pigments were extracted from red bougainvillea and mathiola petals as natural colorants of foods instead of the harmfull synthetic colorants. In biological experiments , it were safe which there is no any bad effect on the liver and the kidney functions and there is no any case of death during 45 days. Too, noticed that there is a slight gradually decrease of anthoyanins content by storage for 3 months.

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دراسات تكنولوجية وبيولوجية على الصبغات الحمراء فى بتلات الجهنمية الحمراء والمنثور

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أن الصبغات الطبيعية هى الأكثر أماناً من ناحية الصحة العامة كملون طبيعى للأغذية بدلاً من الملونات الصناعية الضارة بالصحة وفى هذا البحث تم استخلاص صبغة الأنثوسيانين الحمراء من بتلات الجهنمية الحمراء والمنثور وذلك بالطرق الآتية:-

١ ـ الاستخلاص بالكحول الإثيلى المحمض بحامض الهيدروكلوريك.

٢ ـ الاستخلاص بالماء المقطر.

٣ ـ الاستخلاص بالماء المقطر والمحمض بحامض الستريك ١و٠ %.
٤ ـ الاستخلاص بالماء المقطر والمحمض بحامض الستريك ٣ و٠ %.

من النتائج المتحصل عليها وجد أن الطريقة الأولى أفضل فى الاستخلاص ولكن الطريقة الأخيرة أكثرها اقتصاديا.

أما التجربة البيولوجية فقد ثبت أنه لا يوجد أى تأثير ضار على وظائف الكلى أو الكبد أو موت الفئران محل التجربة والتى تمت على مدار ٤٥ يوم ، كما لوحظ وجود نقص تدريجى طفيف لمحتوى الصبغة بالتخزين لمدة ثلاثة شهور.

التوصية: تم استخلاص صبغة الأنثوسيانين الحمراء من بتلات الجهنمية الحمراء والمنثور كملون طبيعي للأغذية بدلا من الملونات الصناعية الضارة بالصحة العامة وقد ثبت أنها امنة بيولوجيا بتجربتها على فئران التجارب على مدار ٤٥ يوم ولم تعطي أي تأثير ضار على وظائف الكلى أو الكبد أو موت الفئران وقد لوحظ وجود نقص طفيف لمحتوى الصبغة بالتخزين لمدة ثلاثة شهور.