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OPTIMIZATION EXTRACTION OF *BOUGAINVILLEA GLABRA* VIOLET BRACTS PIGMENT

MYASAR **K**H. **I**BRAHIM[*] and **D**R. **M**AHER **K**HALID[**]

[*]Dept. of Basic Science, College of agricultural Engineering Sciences, University of Duhok, Kurdistan Region- Iraq

[**]Dept. of Chemistry, Faculty of Science, University of Zakho, Kurdistan Region- Iraq

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ABSTRACT

ABSTRACT: *Bougainvillea glabra* violet bract flower was selected to explore natural pigment. Various solvent combinations were used to extract the pigments from the flowers. UV–Vis spectroscopy were used to investigate the suitable solvent and the optimum portion ratio for pigment extraction by maceration. The results of mass of floral bract to (50%) ethanol solvent portion ratio in a constant volume (20 ml) was found to be (0.1 gm /20 ml), measured at λ_{max} 548 nm and the maceration extraction time for pigment extraction from violet bracts was 72 h.

Different neutral, basic and acidic organic solvent solutions (ethanol, methanol, 2-propanol, diethyl ether, ethyl acetate, 2- butanol, chloroform and acetone), shows different result of extraction for the pigments. The combination 1:1 methanol: water ratio was found to be a suitable combination solvent for pigment extraction and distilled water solvent give the best results to protect the pigment violet color for *Bougainvillea g.* bracts from conversion in the period for 15 days.

***KEYWORDS*:** Bougainvillea, Pigment, Extraction, Food Industry, Natural Colorants.
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INTRODUCTION

Increases in health damage reports and toxic quality of manufactured colorants are driving the food factories across stratify natural colorants to a rising the number of treated food productions**(Santos, Albuquerque, and Meireles, 2011).** The utilization of natural dyes as light-absorb pigments in Dye-sensitized solar cells for the conversion of solar energy into electrical energy. **(Calogero *et al.*, 2010; Assous *et al.*, 2014; Dumbrava *et al.*, 2012; Dumbrava *et al.*, 2008).**The natural pigments were extracted from the fruit, flower, leaves, seeds, and roots. natural organic pigments are Environmental friendly, which can be extracted with minimal chemical treatments. it considered as an appealing alternative because of the other considerable advantages, such as cheapness, simple accessibility, and not poisonous. **(Hernández-Martínez *et al.*, 2013; Singh *et al.*, 2014)**. *Bougainvillea g*. is a variety of brilliant blossoming plants having a place with the Nyctaginaceae family. It is a prevalent decorative plant in many zones with warm climatic conditions. *Bougainvillea g.* likewise called as paper bloom having glossy green and fuchsia or purple shaded bracts**(Alvarez Perez Gil *et al.*, 2012).**It is also called a paper flower having shiny green and magenta or purple - colored bracts. It is flower bracts are rich in betalain shades which can be utilized as a dye in sensitized solar cells therapeutic and sustainable uses**(Kumar *et al.*, 2017).** Betalains (structure illustrated in supplementary (Fig. 1s)) are a class of pigment present in plants of the order Caryophyllales, which are likewise found in some higher fungi, displace the anthocyanins in fruitiness and blooms of most groups of the plant kingdom. Betalains are divided into two kinds, namely, betacyanins, which incorporate the red-violet betalain colors, and the betaxanthins, which are yellow-orange betalain shades**(Kumar et al., 2017)(Kumar et al., 2017)(Strack *et al.*, 2003).** Betalains heads are essentially accountable for the color of the bract, especially betacyanins**(Moreno *et al.*, 2008; Piattelli, 1981)**. Betacyanins are water- soluble red-violet shading color hold nitrogen in its framework**(Moreno *et al.*, 2008)**.From the alimentation side of view, betalains act as a family of phytochemicals with a limited event in the diet since the plant food sources of them are quite reduced. Only red beet, swiss chard,

myasar.khalil@uod.ac, maher-333@hotmail.de

amaranthus, cactus pear, pitaya, and some tubers and their derived products provide betalains to our diet**(Frank *et al.*, 2005; Stintzing and Carle, 2004; Campos *et al.*, 2006; Kluger *et al.*, 2006).** However, the use of betanin as food and the plant betalain- enriched extract in functional foods increases the consumption of this type of phytochemicals **(Gliszczyn´ska-S´wigło *et al.*, 2006; Piga, 2004)**. Anthocyanins and betalains play an important part both in plant physiology, visional attractiveness for pollinators and seed dispersers**(Belhad *et al.*, 2017),** however in food fundamentally locate its aesthetic estimate. Betacyanins are known to show two absorption maxima, one in the visible region between 270 and 280 nm due to the cyclo- Dopa structure and the second one in the visible region between 535–538 nm relying upon the used solvent**.** In general, the stability of betalains in fabricating production is influenced by considerable pigment characteristics and extrinsic agents such as pigment content, degree of glucosylation or acylation, matrix constituent, chelating agents, antioxidants, temperature, pH, oxygen, light, water activity, and nitrogen atmosphere**(Von Elbe *et al.*, 1974; Jackman& Smith 1996; Herbach *et al.*, 2006; Schwartz *et al.*, 2008; Cai *et al.*, 2005; Boo, Hee Ock *et al.*, 2012).**In addition, pigment concentration and the individual betalain framework, pH and water activity will have significant effects on pigment stability. Orderly to enclose optimum pigment and color retention, the individual time-temperature conditions throughout fabrication must be accurate planning**(Von Elbe *et al.*, 1974; Cai *et al.*, 1998; Han *et al.*, 1998; Cai *et al.*, 2003; Herbach *et al.*, 2004; Herbach *et al.*, 2006; Cardoso-Ugarte *et al.*, 2014)**. Besides, external factors throughout the store such as temperature, light and oxygen exposure need to be taken into account**(Bensen, *et al.*, 2003; Azeredo *et al.*, 2009).**

In this context, the main objective of the present investigation is to illustrate the impact of the solvent on the stability of the pigment and to explore the suitable solvent to lower the ratio of color conversion of pigment through the use of different solvent combinations.

2. MATERIALS AND METHODS

1.1 Chemicals and reagents

Ethanol, methanol, 2-propanol, diethyl ether, ethyl acetate, 2- butanol, chloroform and acetone supplied by Scharlau company. Formic acid, acetic acid and sodium carbonate ware analytical grade.

1.2 Instruments

The absorption spectra of the dyes were determined using an Jenway 7315 spectrophotometer, and Jenway 3510 PH meter for measuring the acidity of the solution.

1.3 Collection of floral bracts

The bract (aerial part, flowers) of the plants were collected from the college of agriculture, the University of Duhok during the summer of 2018 and identify as *Bougainvillea g.* by Prof. Dr. Saleem Esmael Shahbaz. A voucher specimen (3634) was deposited at the Duhok university province herbarium (DUPH).

1.4 Experimental procedures

1.4.1 Dry Grinding Process

The flowers were cut approximately in to 12 mm in size, cleaned by using distilled water, and dried at room temperature. The dried bracts were crushed into a fine powder using a coffee grinder and kept in a cool dark place until it used in the extraction process.

1.4.2 Pigment extraction process

The objective of the research to provide a simple and reliable method to extract *Bougainvillea g.* pigment. In order to achieve this goal, we started with one of the simplest methods to extract pigments by soaking.

1.4.3 Effect of amount of powdered plant material

Pigment extracts were performed as described (Figueroa et al., 2014). A Series of (0.033, 0.05, 0.066, 0.1, 0.133, 0.186, 0.2, 0.3, 0.4 gm) of powdered plant material was macerated with 20 ml of ethanol (50%) for 72 h at 20 ^{o}C. The extract was filtered after 72 h. The filtrate was kept in a cool dry place to measure the absorbance every 24 h at the maximum absorbance (λ_{max}) 397 and 548 nm respectively to investigate the pigment stability.

1.4.4 Effect of solvents

To investigate the effect of solvent on the extraction of *Bougainvillea g.* pigment. 0.1 gm of dry powdered bract was macerated with 20 ml of different types of organic solvent for 72 h. After filtration, the PH of each filtrate was measured as shown in (Table 1) and the filtrate was kept in a cool dry place to measure the absorbance every 24 h.

Table (1): Solvent groups and the pH of the solvent mixture used in the extraction of *Bougainvillea g.* pigment.

group	abbreviation	Solvent	pH
G1	W	water	N
	Eta	ethanol absolute	N
	50% E	1 ethanol: 1water	N
	Et 1/3	1 ethanol: 3 water	N
	Et 3/1	3 ethanol: 1 water	N
G2	M1/1	1methanol: 1water	N
	M1/3	1methanol: 3water	N
	M3/1	3methanol: 1water	N
G3	2-but	2- butanol	N
	DEE	diethyl ether	N
	2-P	2- propanol	N
	EA	ethyl acetate	N
	AC	acetone	N
	Cl	chloroform	N
G4	W0.1	0.01M Na2CO3	11.16
	W1	0.1 M Na2CO3	11.35
	W5	0.5 M Na2CO3	11.25
	E0.1	1 ethanol :1 (0.01 M Na2CO3)	9.3
	E1	1 ethanol :1 (0.1 M Na2CO3)	11.25
	E5	1 ethanol :1 (0.5 M Na2CO3)	11.3
G5	W5	95 water: 5 formic acid	2.65
	F5	34 ethanol: 68 water:5 formic acid	2.65
	W1	1 formic acid: 99 water	3
	E5	100 ethanol: 95 water:5 formic acid	3.2
	W0.1	99.9 water: 0.1 formic acid	3.5
	E1	100 ethanol: 99 water:1 formic acid	3.6
	E0.1	100 ethanol: 99.9 water: 0.1 formic acid	3.8
G6	A10	100 ethanol: 95 water: 5 acetic acid	2.3
	A5	95 water: 5 acetic acid	2.5
	E10	100 ethanol: 90 water: 10 acetic acid	2.86
	E5	90 water: 10 acetic acid	3.02

Water is distilled water, N is neutral, the solvent ratio used by volume (ml)

2 RESULTS AND DISCUSSION

The effect of the mass of powdered plant material to solvent ratio was investigated. (Fig. 1) illustrates increasing the absorption spectrum by increasing the weight of the powder in a fixed volume of solvent. Weights of more than 0.1 g gave absorption higher absorption than the range allowed in the instrument, so a mass of 0.1 g in 20 ml of solvent was selected as the best mass of solvent ratio for the extraction of pigment, and therefore was selected to be used to determine the appropriate solvents that give maximum absorption and more dye extracted.

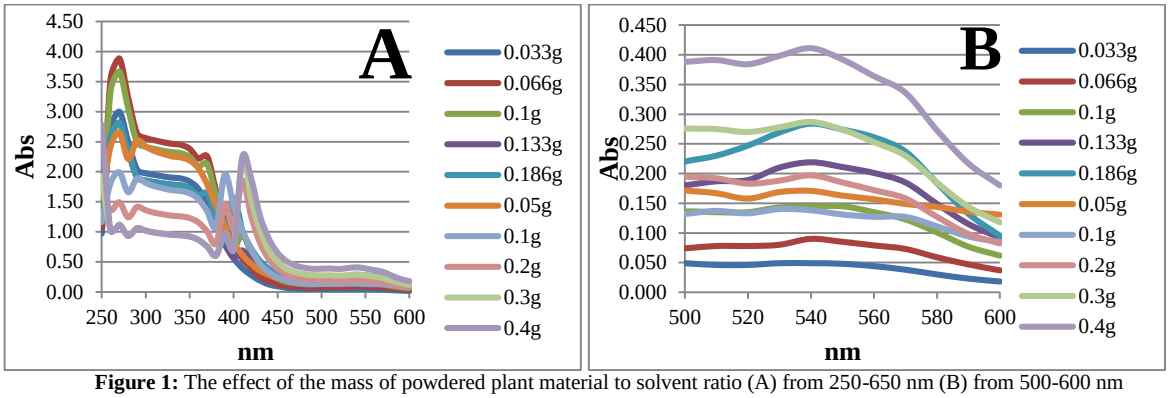

Figure 1: The effect of the mass of powdered plant material to solvent ratio (A) from 250-650 nm (B) from 500-600 nm

The effect of the solvent used in the extraction process is important. Recently, different types of organic solvents have been used to extract natural dyes from different parts of the plant. The type of solvent affects the absorption spectrum of the dyes as well as the bonding between the dyes and the solvent (Zhou &Gao, 2011; Al-Alwani et al., 2015).

Figs. 2-7 shows the UV–Vis absorption spectra of the *Bougainvillea g.* flower dye extracts at different solvent groups (G1-G6) and the maximum absorbance illustrated in table 2. Absorbance peaks around 300 and 535 nm are characteristic absorptions for red–violet betalain group and betacyanin.

All the extractions have a maximum peaks extracts between 323-432 nm in the UV-range with maximum absorbance at 350 nm, and another broad peak between 531-562 nm resulting from π - π* transitions. The measurements depended on the second peak in the visible range for pink color.

For the solvents in G1 (Fig. 2).The uses of pure water as a solvent shows the maximum absorbance (0.422) in 551 nm. Methanol and water were used to extract the pigments. The Vis absorptions spectrum of the extract is shown in G2 (Fig. 3). Different absorbance results were obtained with the use of methanol and water. The suitable solvent to extract pigment was (1 methanol: 1 water) (M1/1) and the best extraction time is 24 h. with the absorbance of (0.534).

The different pure organic solvents in G3 were unsuitable for the extraction of dyes (Fig. 4) except for acetone showed low efficiency where the absorption intensity of the extract was (0.268), half of the absorption intensity by using pure water as a solvent.

In order to study the effect of alkalinity of solvents on the absorbance and on the stability of pigment, sodium carbonate was added to the mixture of water and ethanol. The G4 solvents as shown in (Fig. 5) changed the violet color to yellow directly after adding sodium carbonate, and the maximum absorbance intensity decreased to (0.238).

The effect of acidic media on the extraction was investigated in the groups' G5 and G6(Figs. 6,7). In group G5, a mixture of ethanol, water, and formic acid was used in a different ratio. G6 used acetic acid instead of formic acid. In the two groups maximum absorbance showed (0.177) at PH 3 and (0.176) at PH 3.8 respectively.

The lowest absorption intensity is observed for dye in acidic media indicating as a result of degradation of betanin in a very strong acidic environment. Dye extract at a pH of 3 displays broad absorption peak in the 480–550 nm range resulting from π - π* transitions due to the mixed contributions of the yellow-orange betaxanthins (480 nm) and of the red-purple betacyanin (540 nm). From the data of the experiments we may concluded that in general the mixture of three solvents (containing formic acid) enhances the absorbance of the extract and decrease the stability of the pigment**(Garcı´aBarrera *et al.*, 1998).** It could be noticed that the stability of pigment high at acidic pH values ranged from 2.65 to 3.8, while the color conversion was accorded at pH above 7**(Stintzing *et al.*, 2002, Jackman& Smith, 1996).**

At neutral media, the extract has the highest absorption spectrum indicating a wider range of red, orange, yellow, and blue light can be absorbed. Absorbance peaks around 300 and 535

myasar.khalil@uod.ac, maher-333@hotmail.de

nm are characteristic absorptions for the red-violet betalain group, betacyanin.

For the purpose of studying the stability of the dye, the absorption of the extract was read every 24 hours for 60 days. The results are shown in Table 2.

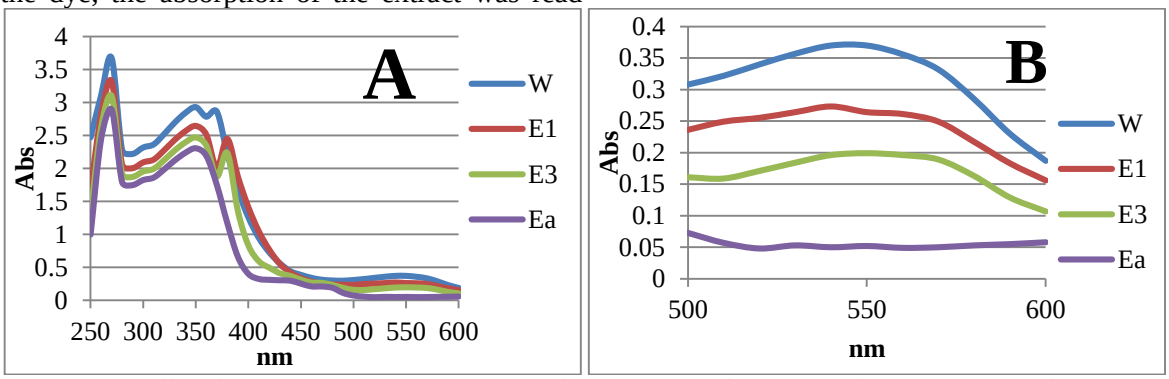

Figure 2: The effect of the solvents in (G1) on the extraction of the bougainvillea flower dye (A) from 250-650 nm (B) from 500-600 nm

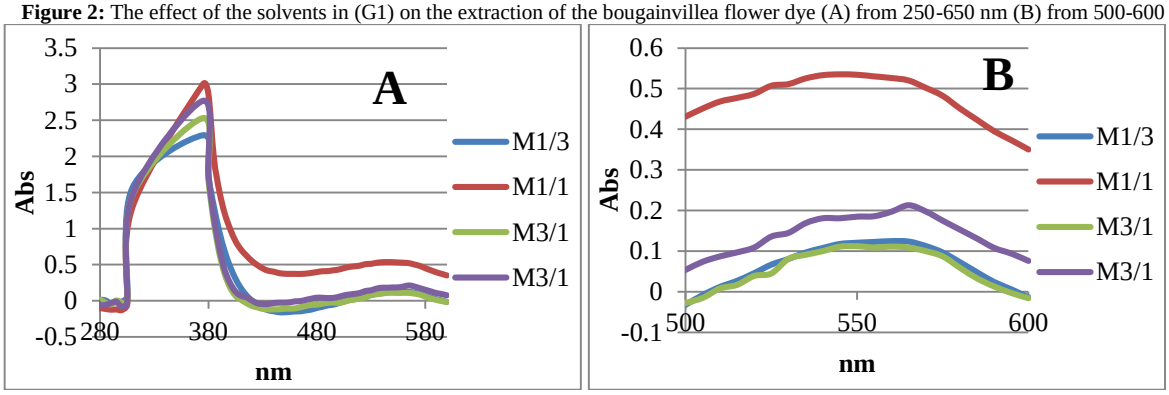

Fig. (3): The effect of the solvents in (G2) on the extraction of the bougainvillea flower dye (A) from 250-650 nm (B) from 500-600 nm

Two steps of extraction used in (G3) in which different individual pure organic solvent was used (Table 1). The organic extracted pigment did not show any absorbance, therefore the dried residue of bracts were extracted again by solvent ratio of 1:3 (ethanol: water)(1/3E). Fig. 4 shows the effect of second extraction of pigment and acetone was the best solvent to use before using the solvent combination (1/3 E).

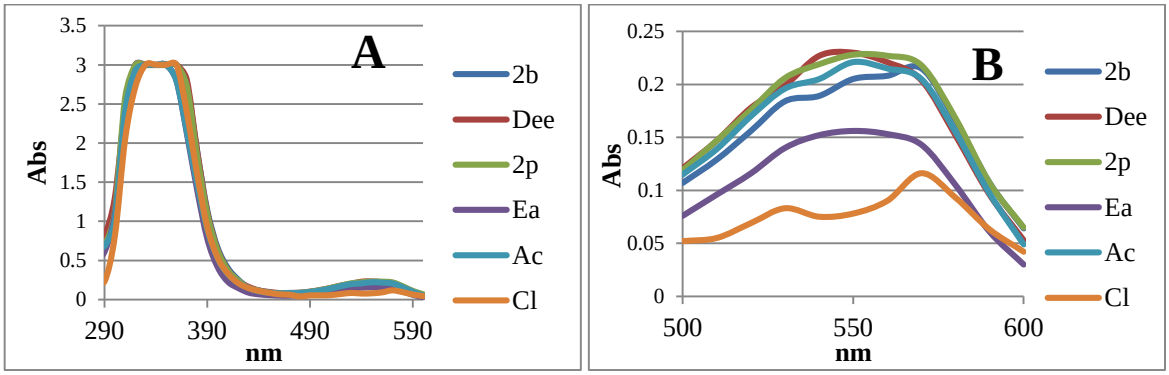

Fig. (4): The effect of the solvents in (G3) on the extraction of the bougainvillea flower dye (A) from 250-650 nm (B) from 500-600 nm

In order to study the effect of alkalinity of solvents on the absorbance and on the stability of pigment, sodium carbonate was added to the mixture of water and ethanol. The G4 (Fig. 5) shows a change of violet color to yellow direct by adding sodium carbonate, the maximum absorbance (0.238) at 548 nm for yellow color, in other word the basic solvent decreases the intensity of the absorbance of the extract and starts the color conversion of the pigment. Alkaline conditions may cause aldimine bond hydrolysis(**Schwartz & von Elbe, 1983; Von Elbe *et al.*, 1974; Attoe& von Elbe, 1981; Cai *et al*, 1998; Peattelli and Imperato, 1970).** Optimal pH range for maximum betanin stability is 5–6 **(Huang & von Elbe, 1985, 1986, 1987; Castellar *et al.*, 2003; Vaillant*et al.*, 2005**).

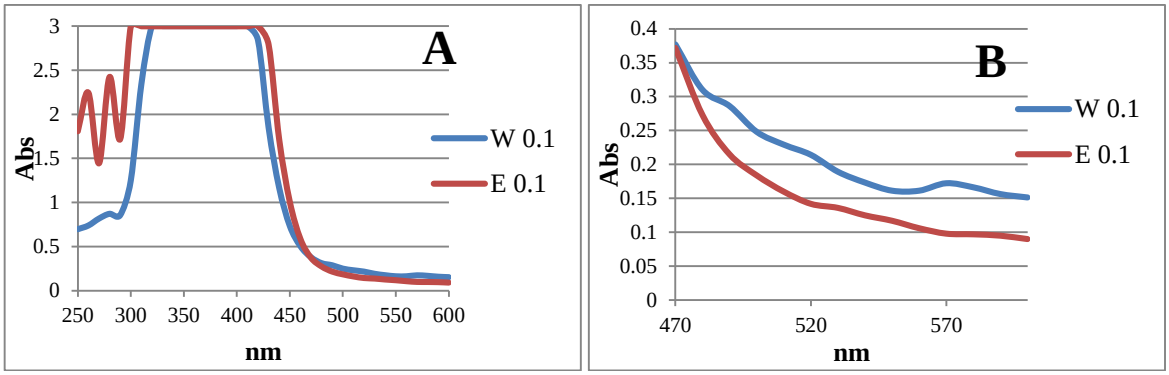

Fig. (5): The effect of the solvents in (G4) on the extraction of the bougainvillea flower dye (A) from 250-650 nm (B) from 500-600 nm

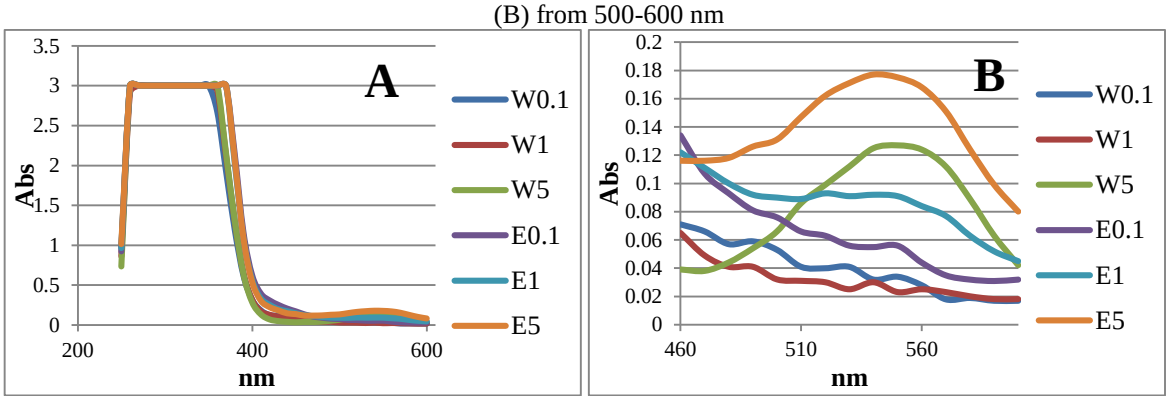

Fig. (6): The effect of the solvents in (G5) on the extraction of the bougainvillea flower dye (A) from 250-650 nm (B) from 500-600 nm

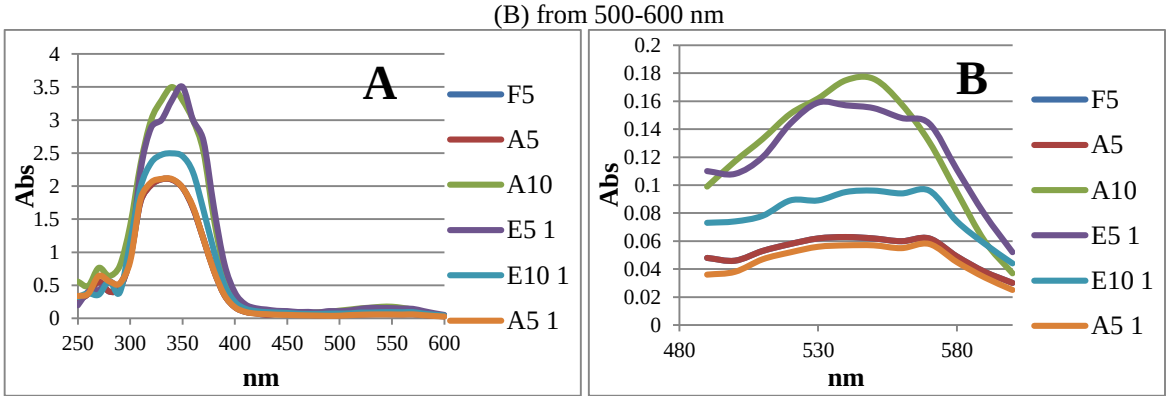

Fig. (7): The effect of the solvents in (G6) on the extraction of the bougainvillea flower dye (A) from 250-650 nm (B) from 500-600 nm

The stability of pigment in different solvents G1-G6 shows in (Table 2). In G1(Fig. 8) various series of ethanol to water ratio were used as a solvent mixture. The uses of only water as a solvent shows the maximum absorbance (0.422) at 548 nm in day 5, after 5 days the absorbance of the extract decreases, which indicate that the stability of the extraction using water as solvent give maximum absorbance in 5-15 days, but after this period the pigment lose its stability and begins to convert.

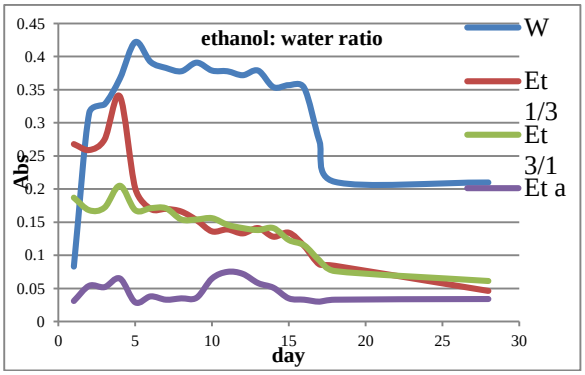

Fig. (8): Absorbance (Abs) at 548 nm of Pigment in different ethanol to water ratio at different time intervals.

Table (2): Wavelength and intensity of the main absorbance peaks of the UV-Vis spectra shown in Figs. 2-7.

group	abbreviation	Solvent	λ1 , λ2 nm	abs	Stabilityday
G1	W	water	351, 551	-, 0.37	15
	Eta	ethanol absolute	351, 551	-, 0.052	*
	50% E	1 ethanol: 1water	351, 542	-, -	15
	Et 1/3	1 ethanol: 3 water	351, 542	-, 0.273	15
	Et 3/1	3 ethanol: 1 water	351, 543	-, 0.198	15
G2	M1/1	1methanol: 1water	-, 545	-, 0.535	1
	M1/3	1methanol: 3water	-, 562	-, 0.127	6
	M3/1	3methanol: 1water	-, 548	-, 0.115	1
G3	2-but	2- butanol	427, -	-, -	4
	DEE	diethyl ether	423, 539	-, 0.058	2
	2-P	2- propanol	431, 539	-, 0.041	4
	EA	ethyl acetate	432, 539	-, 0.055	2
	AC	acetone	431, 538	-, 0.011	2
	Cl	cloroform	431, 534	-, 0.037	2
G4	W0.1	0.01M Na2CO3	-, 531	-, 0.189	3
	W1	0.1 M Na2CO3	-, -	-, -	3
	W5	0.5 M Na2CO3	-, -	-, -	3
	E0.1	1 ethanol :1 (0.01 M Na2CO3)	-, -	-, -	1
	E1	1 ethanol :1 (0.1 M Na2CO3)	-, -	-, -	1
	E5	1 ethanol :1 (0.5 M Na2CO3)	-, -	-, -	1
G5	W5	95 water: 5 formic acid	-, 537	-, 0.123	1

	F5	34 ethanol: 68 water:5 formic acid	-, 550	-, -	2
	W1	99 formic acid: 1 water	-, 536	-, 0.034	1
	E5	100 ethanol: 95 water:5 formic acid	-, 539	-, 0.177	1
	W0.1	water: 0.1 formic acid	351, 544	-, 0.038	1
	E1	100 ethanol: 99 water:1 formic acid	-, 538	-, 0.091	1
	E0.1	100 ethanol: 99.9 water: 0.1 formic acid	-, 538	-, 0.058	1
G6	A10	100 ethanol: 95 water: 5 acetic acid	-, 549	-, 0.176	4
	A5	95 water: 5 acetic acid	328, 550	-, 0.062	2
	E10	100 ethanol: 90 water: 10 acetic acid	344, 547	-, 0.1	2
	E5	90 water: 10 acetic acid	-, 546	-, 0.159	2

Water is distilled water, N is neutral, the solvent ratio used by volume (ml), * there is no extracted pigment appear therefore no stability time.

In the G2 (Fig.9) shows different stability results were obtained with the use of methanol and water. The stability of (M 1/3) is 6 days while 24 h for others.

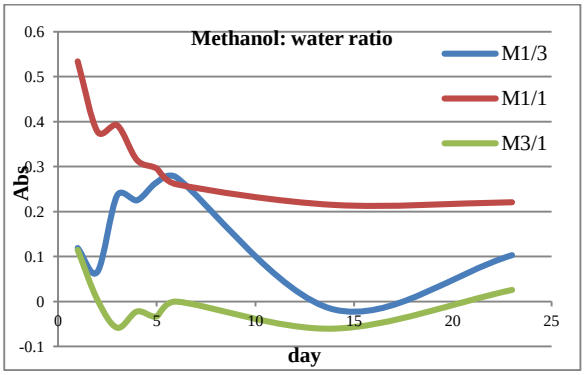

Fig. (9): Absorbance (Abs) at 548 nm of Pigment in different methanol to water ratio at different time intervals.

The G3 (Fig. 10) shows the effect of second extraction of pigment and the stability of pigment is 48 h and DEE shows long time stability.

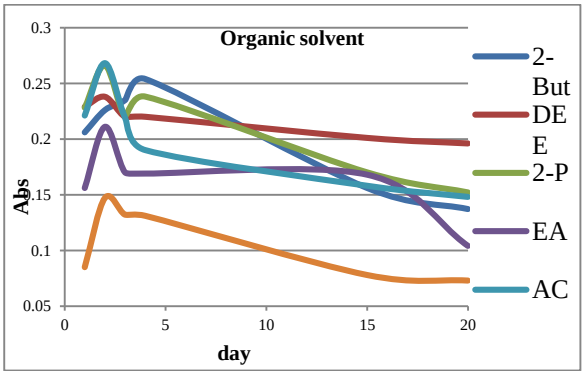

Fig. (10): Absorbance at 548 nm of Pigment in ethanol: water 1:3 after use different organic solvent at different time intervals.

The G4(Fig. 11) shows a change of violet color to yellow direct by adding sodium carbonate, the stability of yellow color was not more than 72 h. and after that the pigment lost its stability.

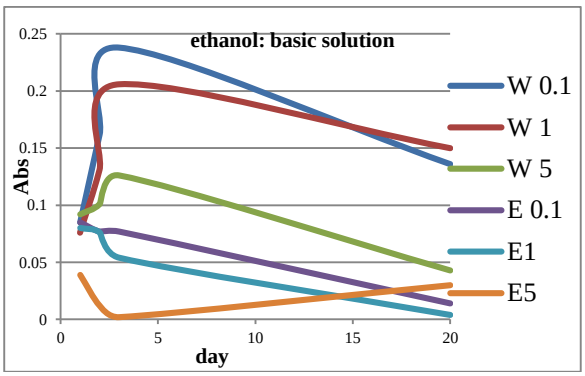

Fig. (11): Absorbance (Abs) at 548 nm of Pigment in different ethanol to sodium carbonate solvents ratio at different time intervals.

In the G5 (Fig. 12) shows the effect of solvent on pigment extraction and stability, in general the stability of pigment for most solvents are 24 h.

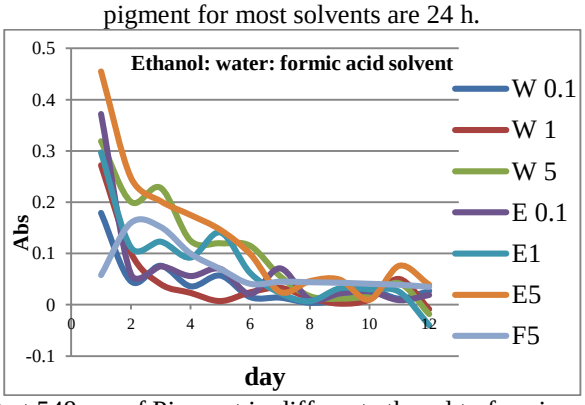

Fig. (12): Absorbance (Abs) at 548 nm of Pigment in different ethanol to formic acid solvents ratio at different time intervals.

The influence of G6 (Fig. 13) In general the results show that the ratio of (100 ethanol: 95 water: 5 acetic acid) (E5) is a best solvent mixture for extraction of the pigment with a little difference between the other solvent mixture (100 ethanol: 90 water: 10 acetic acid) (E10), hence the maximum extraction time is 2 days for (E5) with absorbance (0.197) at 584 nm, while 4 days for (E10) with absorbance (0.197).

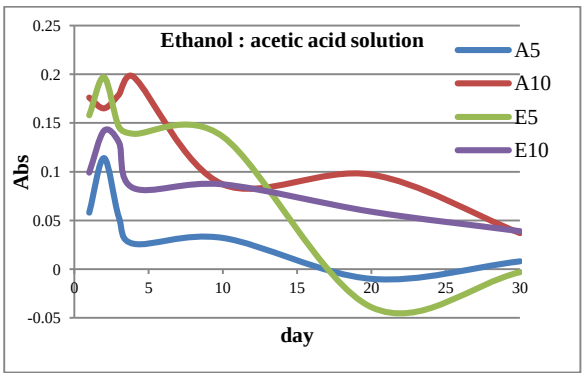

Fig. (13): Absorbance (Abs) at 548 nm of pigment in different ethanol to aqueous acetic acid ratio at different time intervals.

CONCLUSION

In the current study the effect of amount of powdered plant material was study, to optimize the best mass/ solvent ratio, different absorbance results were obtained. The optimum process conditions were found to be mass of floral bracts to solvent ratio was 0.1 gm /20 ml of 50% ethanol for pigment extraction. In the second part of current study, several solvent combinations were used to indicate the best combination of solvents mixture (Group 1-6). The results indicated that the suitable solvent for extraction of *Bougainvillea g.* pigment and to protect the violet color bracts for pigment from color conversion, is a mixture of 1:1 methanol: water ratio, with absorbance (0.534) at 548 nm, While the second solvent was water only with absorbance (0.422) at 548 nm. Adding acid or base to the solvents could not enhanced the pigment extraction and its prefer the neutral medium. The best solvent that give longer time (15 days) for stability of the pigment was water, while adding base to the mixture solvent decreases the intensity of the absorbance of the extract and convert the color from violet to golden yellow directly, that may caused by degradation of the pigment, for the reason of aldimine bond hydrolysis. Finally we conclude that the best solvent to extract bracts of *Bougainvillea g.* pigment is (1 methanol : 1 water).

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myasar.khalil@uod.ac, maher-333@hotmail.de

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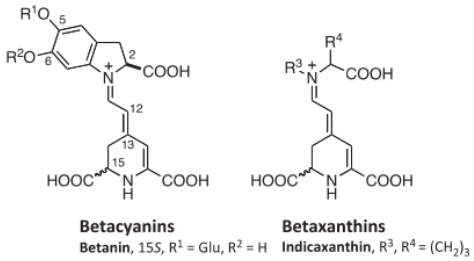

Fig 1s: structure of some betaline compounds

Supplementary