Isolation and Identification of pigment from Bent EL-Kunsil (Hibiscus rosa – Sinensis) flowers

Fatima S. Sebah
Department of Chemistry , College of Science,University of Basrah, Basrah Iraq

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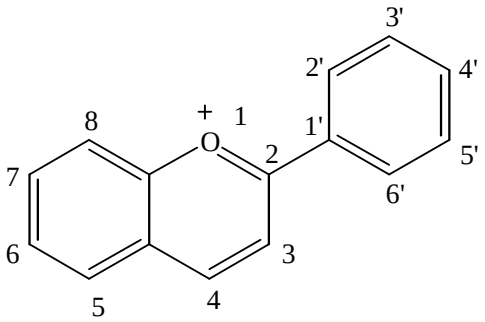
Abstract:

A new pigment was isolated from aqueous extract of Iraqi red flowers Bent EL-Kunsil (Hibiscus rosa – Sinensis) , the pigment was characterized by conventional characterization methods i.e. UV.,IR, TLC ,PC and molecular weight determination .

Key words:- Anthocyanins , TLC , pigment, Test catechol , organic layer

Introduction:-

The flavonoids are a very large and wide spread group of plant constituents. The water soluble anthocyanins which are responsible for the various shades of red and blue colors of many fruits, they are one of the major flavoniod classes [1]. They are glycosides and acyl glycosides of anthocyanidins . Which are poly hydroxyl and poly methoxyl derivatives of 2 phenyl benzo pyrylium (flavylium cation).[2,3].



flavylium cation

The interest in identifying sources of anthocyanin pigment by the food .Pharmaceutical and cosmetics industries is based on consumer demand for natural instead of synthetic colorants [4,5]. The major impoirtance of anthocyanin comes from their potential application as nature colorants to replace synthetic dyes in foods. Other important a spects of anthocyanin are medicinal because of their biological activities . Natural anthocyanins are prescribed as medicine in many countries . They have been reported to have positive effects in treating various microcirculation diseases resulting from capillary fragility [6]. Our objective was to isolate the pigment in the flowers of Bent El-Kensal(Hibiscus rose – Sinensis) , which give flower throughout the year and it is used in a variety of applications [7,8].

Materials and methods :

Plant material:

Bent El-Kunsil (Hibiscus rosa –sinesis) were obtained from Maaqel gardens . They have been classified in college of science labs, the flowers cleaned and allowed to dry at room temperature .The dried flowers were blended by using (Electrical mill blender). The powder of the flowers were kept until required .

Chemical and materials :

All chemicals were of purety analytical grades: Methanol , sulphuric acid (Analar) , sodium hydroxide ,2,4- dinitrophenyl hydrazine ,ammonium hydroxide , magnesium turnigs were purchased from Fluka ; Iodine , glucose , silica gel plates , chloroform and ferric chloride were purchased from Merck ; p-anisaldehyde , ninhydrine and sodium carbonate from RDH ;phthalic acid ,acetic acid ,lead acetate ,n-butanol , acetone, sodium bi carbonate ,sec –butanol , hydrochloric acid (Analar),ethylacetate ,ribose ,xylose , fructose were purchased from BDH ; α – naphthal and aluminium chloride from H&W; Ethanol from Baghdad factory for drugs and cosmetics ; Copper sulphate ,sodium citrate and folin cioalteus were purchased from Ajax.

Instrument:-

JASCO uv-visible spectrophotometer.
pye –unicam-30-300s infrared-spectrophotometer.
PH-meter HI 8417HANNA Instrumental.

Extraction and Isolation pigment :-

20 gm of red flowers powder were extracted by soaking in 300 ml of cold water for 6 hours . The extract was filtered through (whatmann No.540) , filter paper .To the filtrate 2%aqueous lead acetate was added until the formation of blue precipitate , the precipitate was separated by (whatmann No. 540) , filter paper and washed with water , methanol and ethyl acetate consecutively[9].

The product salt was converted into chloride by dissolving in (25 ml acetone and 5 ml 2N HCl)and filtered through (Whitman No. 542).The filtrate was placed in Petri dish at room temperature until dry .The weight of amorphous red powder was 0.856 gm, Percentage yield 4.28%.

Preliminary qualitative test :-

Preliminary tests were carried out on the aqueous extract and on the isolated pigment as shown in table (1).

Acid hydrolysis :-

0.100 gm of the isolated pigment were placed in round bottom flask , 10 ml of 2N HCl was added and the solution was poured into separating funnel (3) . Chloroform (10ml)was added and the solution was shacked to separate the organic layer , the process was repeated three times with 10ml chloroform and the organic layers were collected and condensed to 5ml by rotary evaporator at 40˚C .

The aqueous layer was neutralized by adding 2N NaOH and evaporate to 5ml by rotary evaporator at 60˚C.

Identification of sugar moieties :-

Examination of sugar components in aqueous layer was carried out. Preliminary test was made using Molish's , Bendict , Barfoed's ,Biel's and Seliwonoff's tests (Table 3). A further test has been done on TLC by applying (5drops) of aqueous layer on silica gel plates (10×5cm) and the plate was run with n-Butanol-HAC-H_2O(4:1:5) as elute for 60 min . Standard sugars were applied well at the same plate as shown in table (4).

Test of organic layer (Aglycone component):-

The aglycone component was examined after hydrolysis of the pigments with 2N . HCl. The result hydrolysat was spotted directly on paper chromatography(3×12cm).Three eluted systems were used . Conc. HCl –HAC-H_2O (3:30:10), Conc.HCl- HCO_2H –H_2O (2:5:3)and the (n-Butanol- HAC –H_2O(4:1:5) for 45min , the paper were dried and examined under UV at 366 nm. The results are shown in table (5).

Test of catechol on the isolated pigment :-

0.01gm of the isolated pigment were dissolved in 10 ml 0.01%HC l methanol, pH was measured and the spectra was recorder from (400-600)nm wavelength.To 5ml of the solution 5٪ alcoholic $AlCl_3$ was added and the spectra (400-600)nm was recorded as will .The results are shown in fig (1).

Infrared and UV-Visible spectroscopy:-

IR spectrum using pye –unicam-30-300s infrared-spectrophotometer and uv-visible spectra on JASCO uv-visible spectrophotometer were recorded.as shown in fig. (2,3) ,(4) and table (6).

Results and discussion :

The preliminary tests of aqueous extract of red flower Bent El-Kunsil (Hibiscus rosa-sinesis) and the isolated pigment shows the presence of flavonoid as glycoside table (1). The TLC show the presence of anthocyanin pigments table (2)which change their color by changing the pH values .The same results were obtained by other authors [2,10,- 12].

Table (1): Results of preliminary qualitative tests for pigment and aqueous extract.

Test / *Sample*	*Flavonoid test*	*Carbohydrates test*	*Glycoside test*	*Alkaloides test*	*Amino Acide test*	*Saponin test*	
						Foam test	*5%$HgCl_2$*
Aqueous extract	+	+	+	+	+	-	-
Pigment	+	+	+	-	-	-	-

Table (2):TLC for pigments in (Sec- butanol – acetic acid –water(4:1:1.2))as mobile phase.

Test / Sample	UV-lamp	Iodine	Foline	Vaniline	10%HCl	P-anisaldehyde	10% NH_4OH
Rf pigment	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Test / Sample	Day light	Ninhydrine	2% Lead acetate	5%$AlCl_3$	$FeCl_3$ + $K_3Fe(CN)_4$	Drangdroff	40% H_2SO_4
Rf Pigment	0.56	–	0.56	0.56	0.56	–	0.56

In the aqueous fraction (after acid hydrolysis) on type of sugar was identified ,table (3). The TLC of aqueous fraction and the standard sugars showed a very high agreement between Rf of the aqueous and the glucose which indicates that the sugar was glucose, table (4). As illustrated by authors ,the major sugar appeared in the anthocyanin pigments of red Bent El-Kunsil (Hibiscus rosa –sinesis) is the glucose [10,13-15].

Table (3):Preliminary test of aqueous fraction.

Seliwonoff's test	Barfoed's test	Biel's test	Benedict test	Molisch's test
–	+	–	+	+

Table(4):TLC result aqueous fraction of pigment.

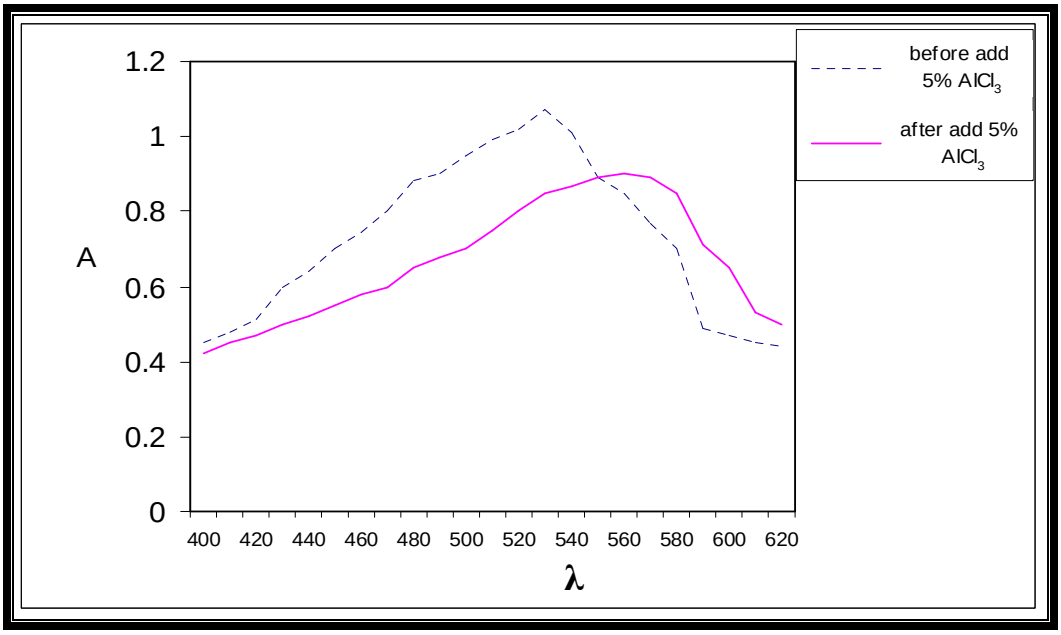
Aniline hydrogen Phthalate	Glycose	Aqueous fraction
Brown	Rf=0.33	Rf=0.32

In the organic layer , the aglycone was identified as cyanidin , based on PC comparisons with authentic anthocyanidin, table(5).

Table (5):PC results for organic layer.

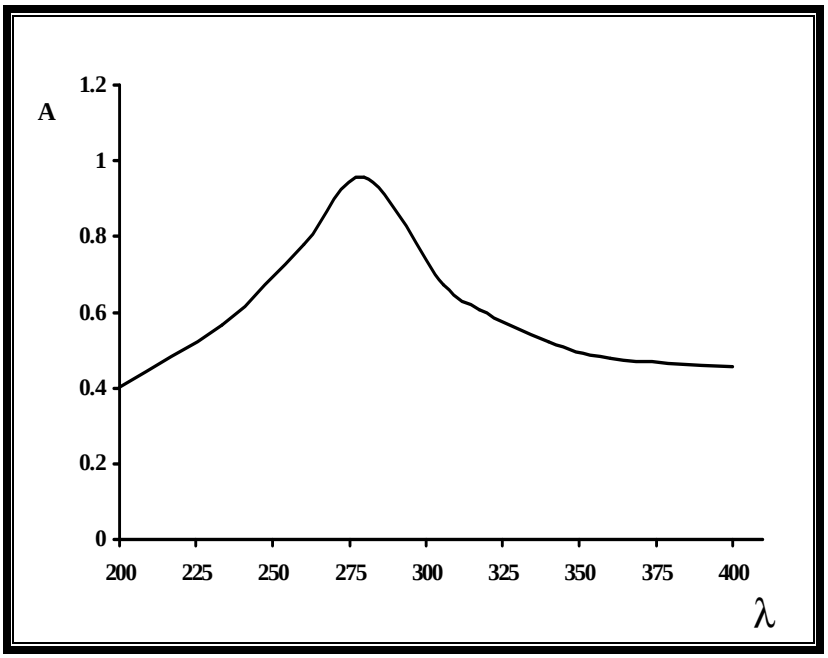
Organic layer	*Conc.HCl-HAc-H2O(3:30:10)*	*Conc.HCl-HCO_2H (2:5:3)*	*BAW(4:1:5)*
Magenta	*Rf=0.48*	*Rf=0.21*	*Rf=0.68*

Many methods are used to ensure that the pigment contains organic part (cyandin), a specific test for catecol group in cyandin is done by adding 5% $AlCl_3$ solution shift the visible spectrum ,indicate presence of catechol group fig.(1).

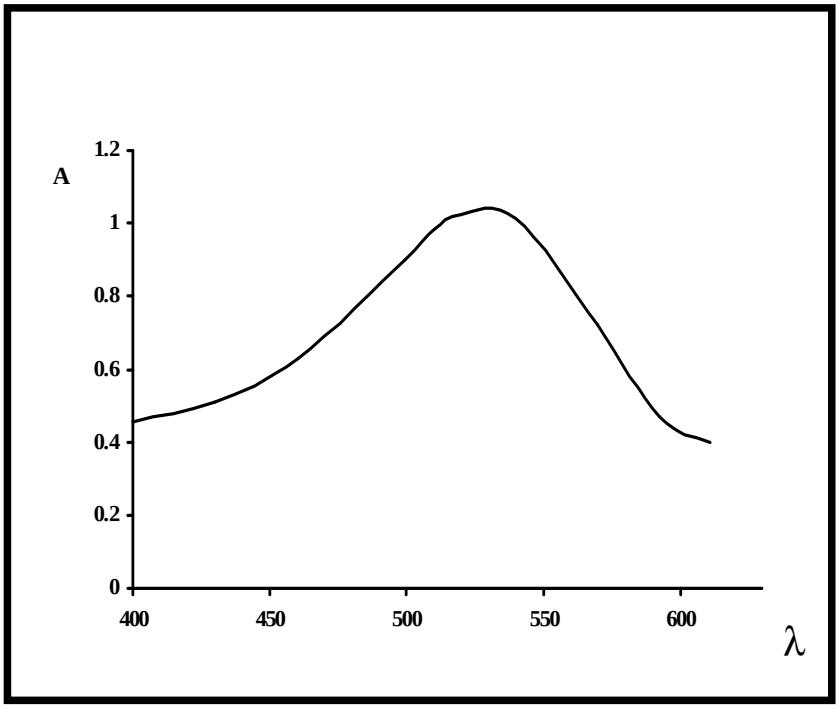


Fig(1): 5% $AlCl_3$ solution shift the visible spectrum of the pigment

Fig (2,3) shows the uv-visible spectrum fig(4) and table (6) shows the full scan of IR spectrum of the pigments, the uv spectrum shows maximum absorption at (280)nm due to $\pi \rightarrow \pi^*$ transition which is the characteristic of unsaturated double bond ,the visible spectrum also shows max absorption at λ=530nm due to the transition of $n \rightarrow \pi^*$[16,17].



Fig(2):uv spectrum of the pigmen



Fig(3):visible spectrum of the pigment

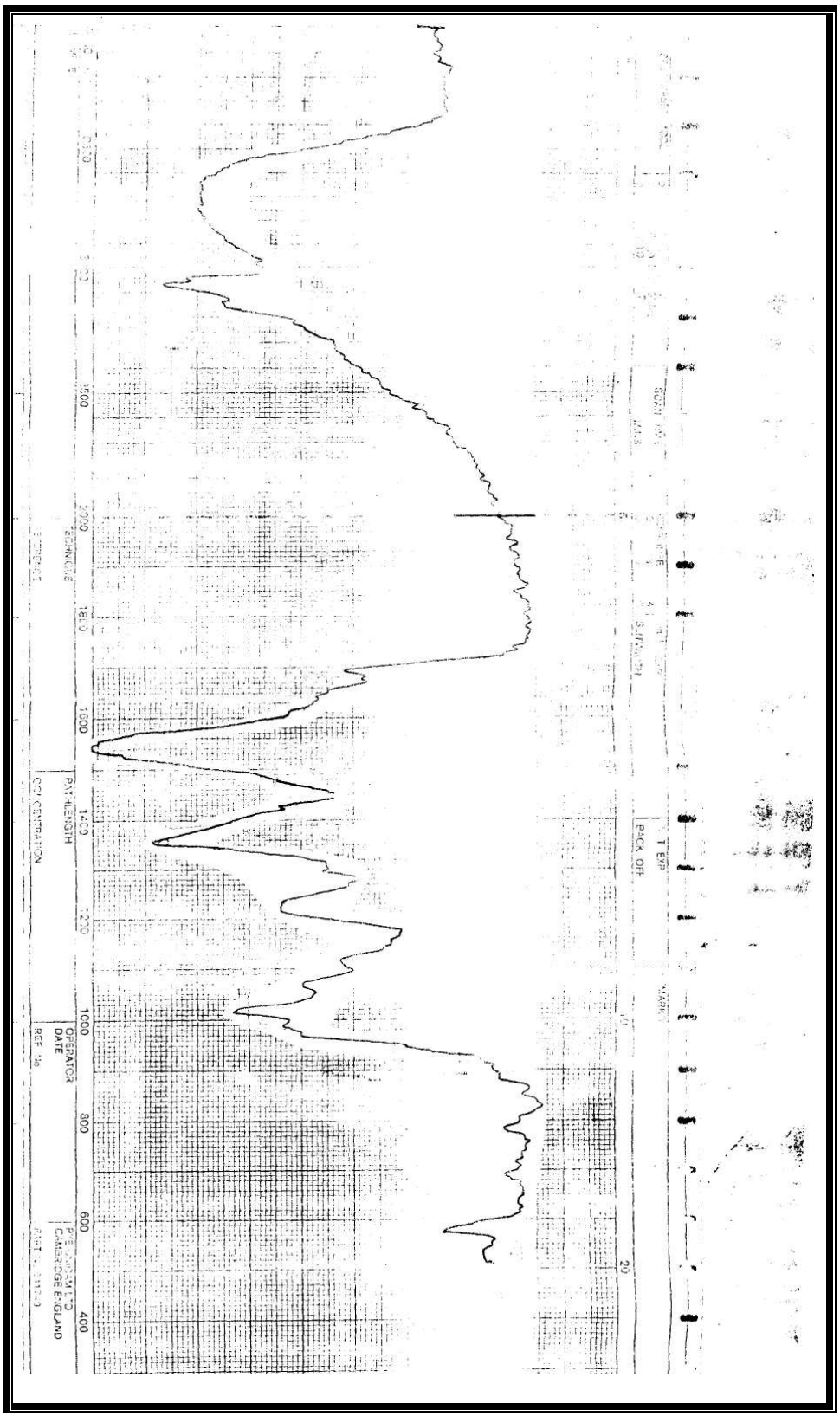
Table (6)full scan of IR spectrum of the pigment

Band frequency cm^{-1}	*Band shape*	*Bond*	*Function group*
3400 – 3100	*Br.*	*O-H*	*Alcoholic, phenolic*
2900-2840	*Sh.*	*C-H*	*Aliphatic*
1700	*Sh.*	*C=O*	*Carbonyl group*
1660	*W-W*	*C=C*	*Aromatic (benzene)*
1580 – 1560	*S*	*C-O-C*	*Glycosidic linkage*
1400	*S, Br.*	*Bending*	*Benzene ring*
1260	*M, Br.*	*Ar – O – C-*	*Alkyl aryl ether*
1070	*S, Br.*	*-OH*	*Alcoholic C-OH*
1110 – 1170	*M*	*Bending*	*Ethers –C-O-C-*

S = Strong ; W = Weak; Br. = Broad ; Sh. = Sharp ; M = Medium

Table (7) shows some chemical and physical tests. The molecular weight of pigments were determined by grycosscopic method (18) and found to be643.5 g/mol.

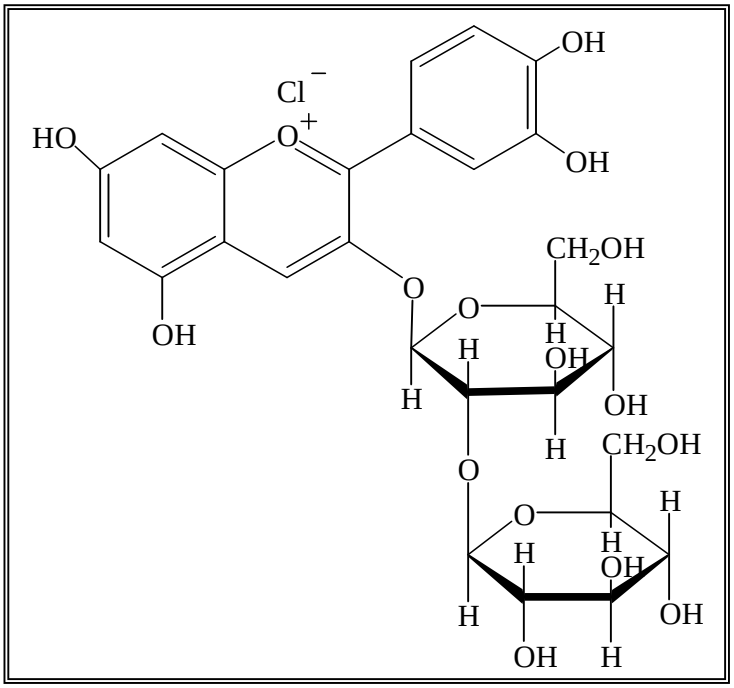
Test	*Pigment*
Physical principle	*Red amorphous*
Melting point	*145-167 decomp.*
Ignition test	*Black smoke with black carbon*
Solubility test	*The pigment soluble in water, DMSO, ethanol, acetone and insoluble ether, hexane and ethyl acetate*
Acidity test	*pH = 5.7*



Fig(4) : the full scan of IR spectrum of the pigment

From the information above it appeared that the pigment contain an organic part was cyanidin compared with literature values (3)byTLC. The sugar moieties was glucose.

Based on the obtained spectroscopic and chromatography data, a proposed structure of the anthocyanin of Iraqi red flowers Bent EL-Kunsil (Hibiscus rosa – Sinensis) is suggested to be (cyanidin -3-sophoroside),fig(5) suggested the chemical of the anthocyanin molecule of the pigment.(19,20,21,22) .



Fig(5)cyanidin -3-sophoroside

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الخلاصة :

تم عزل صبغة من المستخلص المائي لأزهارنبات بنت القنصل (Hibiscus rosa-Sinensis) وشملت الدراسه التعرف على المحتوى الكيميائي للمستخلص المائي لازهار هذا النبات من خلال تقنية كروموتوغرافي الصفائح الرقيقة TLC وبعض الاختبارات الكيميائية ، وحدد التركيب الكيميائي لهذه الصبغة باستخدام طريقة تعيين الوزن الجزيئي وتقنيات الـ UV,TLC, PC, IR.