Isolation of New Pigment from Liflaf (*Ipomoea Purpuea L.*) flowers

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Abstract:

A new pigment was isolated from aqueous extract of lifaf flowers (*Ipomoea purpurea L.*). The pigment was characterized by conventional characterization methods *i.e* UV, IR, TLC, PC, and molecular weight determination.

الخلاصة

تم عزل صيغة من المحتوى المائي لازهـار نبـات الـبلاب Liflaf (*Ipomoea purpurea L.*) وشملت الدراسة التعرف على المحتوى الكيميائي للمستخلص المائي لازهار هذا النبات من خـلال تقنيـة كروماتوغرافي الصفائح الرقيقة TLC وبعض الاختبارات الكيميائية ، وتم اقتراح صيغة كيميائيـة لهـذه الصيغة من خلال نتائج استخدام تقنيات الـ UV ، IR ، TLC ، PC وتعيين الوزن الجزيئي .

Introduction:

Anthocyanins are a major natural phenol compounds. They are water soluble glycosides and acyl glycosideds of anthocyanidins, which are polyhdroxyl and polymethoxyl derivatives of 2-phenyl benzo pyrylium (flavylium cation). Anthocyanins belong to alarge and wide spread group of plant constituents collectively known as flavonoids. The most common naturally occurring anthocyanins are 3-0-glycosides or 3.5-di-o-glycosides[(1)].

Natural anthocyanins are prescribed as medicine in many countries, they have been reported to have positive effects in treating various micro circulation diseases resulting from capillary fragility such as preventing cholesterol induced atheresel-erosis, inhibiting platelets

aggregation and improving visual function. Anthocyanins are also potent antioxidants in vitro against various reactive oxygen species[2][3].

The anthocyanin (ACN) pigments responsible for red and purple radishes have been characterized by different researchers[4][5] In south Africa the Zulus use it as a purgative and as anti-syphilitic[6].

Our objective was to isolate the pigment from the flowers of liflaf (*Ipomoea purpurea L.*) which is used as medicinal plant.

1. Materials and Methods:

1.1 Plant Material:

The flowers of liflaf were collected, 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.

1.2 Chemicals and Materials:

All chemicals were of purely analytical grades. Methyl alcohol, sulphuric acid (Analar), sodium hydroxide, 2,4-dinitro phenyl hydrazine,Ammonium hydroxide, magnesium turninges were purchased from Fluka; Iodine, Glucose, silica gel plates, silica gel powder, chlorofrom and ferric chloride were purchased from Merk; P-anisaldehyde, ninhydrine and sodium carbonate from RDH; phthalic acid, acetic acid, lead acetate, n-butanol, acetone, sodium bicarbonate, hydrochloric acid (Analar), α-naphthol from H and W; 95% ethanol from Baghdad factory for drugs and cosmetics. Copper sulphate, sodium citrate and folin cioalteus were purchased from Ajax.

1. 3 Extraction Method:

20.000gm of purple flowers powder were extracted by soaking in 250ml of cold water for 24 hours, the extract was filtered through (whatmann No.541) filter paper. The filtrate was concentrated using arotavapor at 50°C. The filtrate was used in petridish at room temperature until dry. The weight of amorphous purple powder formed was 6.196gm[7].

1. 4 Isolation of Pigments:

10.000gm of purple flowers powder were extracted by soaking in 100ml cold water for 5hours, the extract was filtered through (whatmann No.541); 2% methanolic lead acetate was added to the filtrate until formation of flocculant and blue precipitate, the precipitate was separated by (whathmann No.540) and washed with

water, methanol and ethyl acetate consecutively[8].

The product salt was conveted into chloride by dissolving in (25ml acetone and 5ml 2N HCL) and filtered through (whatmann No.542). the filtrate was placed in petridish at room temperature until dry. The weight of amorphous red powder formed was 1.025gm[8].

1. 5 Preliminary Qualitative Test:

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

1. 6 Thin layer chromatography:

TLC using plates of silica get was carried out on the aqueous extract and on the isolated pigment using (EtOAc-HOAc-HCO_2H-H_2O) (5:1. 1:1. 1:0.5)[2].

1. 7 Acid Hydrolysis:

0.050gm of the isolated pigment were placed in round bottom flask, 10ml of 2N HCL was added and the solution was refluxed at 100°C for one hour. After cooling, the solution was poured into separating funnel[1]. Chloroform (10ml) was added and the solution was shaked to separate the organic layer, the process was repeated three times with 10ml chloroform and the organic layer were collected and condensed to 5ml by rotary evaporator at 40°C.

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

1. 8 Identification of Sugar Moieties:

Examination of the sugar components in aqueous layer was carried out. Preliminary test was made using molisch's, Benedict, Barfoed's, Brel's and seliwonoff's test (Table 3). A further test has been done on TLC by applying (5drops) of the aqueous layer on silica gel plate (10x6 cm) and the plate was run with n-butanol-HOAc.H_2O (4:1:5) as eluant for 60min. standard sugar was applied as well at the same plate as shown in Table (4).

1. 9 Test of Organic Layer (Aglycone component):

The glycone component was examined after hydrolysis of the pigment with 2N HCl. The resultant hydrolysate was spotted directly on paper chromatography (whatmann No.3) (3x12cm). Three eluated system were used, conc. HCl-HCO_2H-H_2O (2:5:3) , n-butanol-HOAc-H_2O (4:1:5) and conc. HCl-HOAc-H_2O (3:30:10) for 45 min, the paper were dried and examined under uv at 366 nm. The results are shown in Table (5).

1. 10 Infrared and uv-visible Spectroscopy:

IR spectra using pye-unicam-3-3005 infrared spectrophotometer and uv-visible spectra on JASCO UV-Visible spectrophotometer were recorded as shown in Figs. (1),(2),(3) and table (6).

2 . Results and Discussion:

The preliminary tests of aqueous extract of liflaf (Ipomoea purpurea L.) and the isolated pigment shows the presence of flavonoiod as glycoside table (1). The TLC shows presence of anthocyanin pigments, which changes their color by changing the PH values Table (2). The same results were obtained by other authors[9][10][11].

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

Test sample	Flalvonoid test	Carbohydrate test	Glycosides test	Alkolid test	Amino acid test	Saponin test	
						Foam test	5% $HgCl_2$
Aqueous extract	+	+	+	+	+	-	-
Pigment	+	+	+	-	-	-	-

Table (2): TLC for pigment and aqueous extract.

Test sample	UV. Lamp	visible	ninhydrin	Drang drof	40% H_2So_4	$FeCl_3$ + $K_3Fe(CN)_2$	10% NH_4OH	dil. HCl	vanilin
Rf Aqueous extract	0.20 0.41	0.20	0.13 0.31 0.49	0.95	0.20	0.20	0.20	0.20	0.20
Pigment	0.20	0.20	-	-	0.20	0.20	0.20	0.20	0.20

In the aqueous fraction (after acid hydrolysis) one type of sugar was identified. The TLC of the aqueous fraction and the standard sugars showed a very high agreement between R_f of aqueous layer and the glucose which indicates that the sugar was glucose, Table (3 and 4). As illustrated by other outhers, the major sugar appeared in the

anthoyanin pigment of lifaf (Ipomoea purpurea L.) is the glucose[9][10][12].

Table (3): preliminary test of aqueous fraction.

Barfoed's test	Molish ch's test	Benedicts test	Biel's test	Seliwonoff's test
+	+	+	-	-

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

Aniline hydrogen phthalate	Glucose	Aqueous fraction
Brown	RF=0.42	RF=0.43

In the organic layer, The aghycone was identified as pelargonidin based on PC comparisons with anthentic anthocyanin[1], Table (5).

Table (5): PC results for organic layer.

UV.	Visible color	n. butanal-HOAc-H_2O (4:1:5)	conc. H_2O-HCl-HCO_2H (2:5:3)	conc. HCl-HOAc-H_2O (3:30:10)
Darkness	Red	R_F=0.82	0.34	0.67

Fig.(1,2) shows the visible and UV. spectrum, Fig.(3) and Table (6) shows the full scan of IR spectrum of the pigment. The UV. spectrum shows maximum absorption at 350 nm, n□π* transition due to non-bonding, the visible spectrum also shows max absorption at λ=530 nm due to the transition of π □ π* [13][14].

Table (7) shows some chemical and physical test of the pigment, The moleular weight of the pigment were determined by hygroscopic method[15] and found to be 623.6 g/mol.

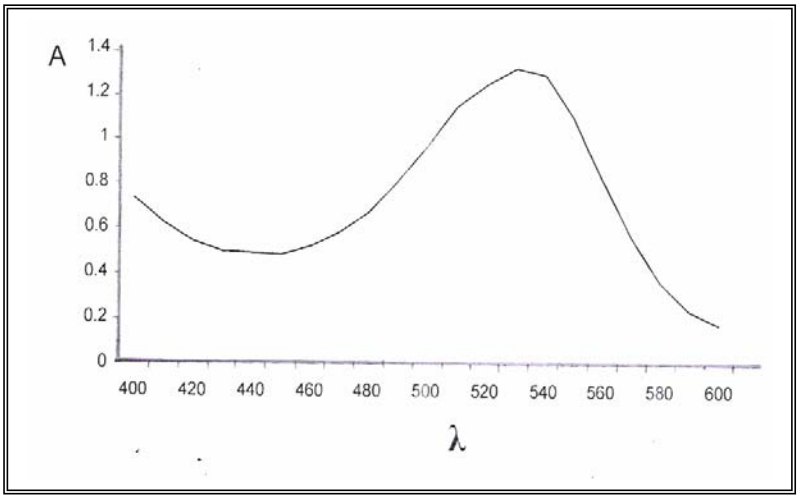

Fig . (1): The visible spectrum of the pigment

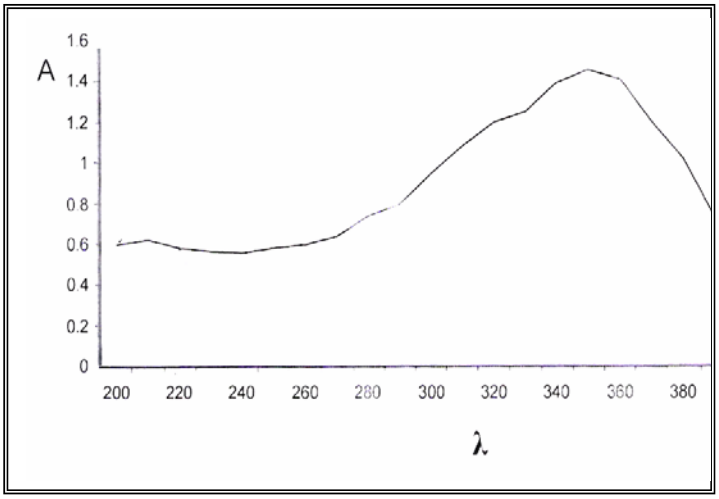

Fig . (2): The UV spectrum of the pigment

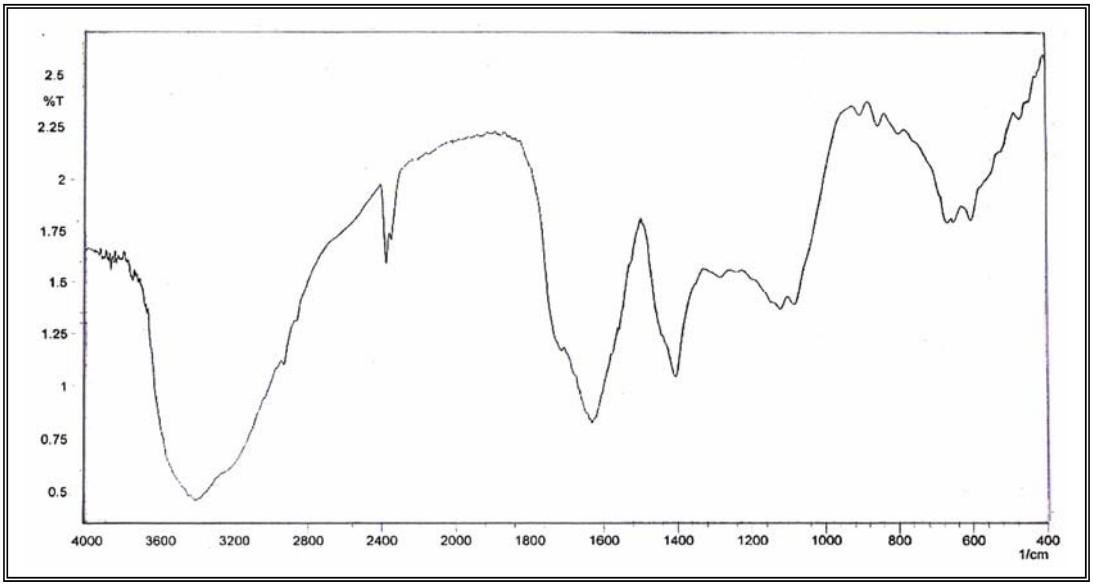

Fig. (3): Full scan of IR spectrum of the pigments

Table (6): IR data of the pigment.

Band Frequency cm^{-1}	Band Shape	Bond	Function Group
3400-3100	***br.***	**O-H**	**Alcoholic , Phenolic**
2840-2900	***sh.***	**C-H**	**Aliphatic**
1700	***sh.***	**C=O**	**Carbonyl group**
1660	***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-**

Table (7): chemical and physical test.

Test	Pigment
Physical principle	**Purple amorphous**
Melting point	**154-169 decomp.**
Ignition test	**Black smoke with black Carbon**
Solubility test	**The pigment soluble in water, DMSO, ethanol, acetone and insoluble in ether, hexane and ethyl acetate**
Acidity test	**pH=5.9**

From the information above it appeared that by aicd hydrolysis, the organic part was pelargonidin as compared with literature values by PC[(1)]. The sugar moieties was glucose.

Based on the obtained spectroscopic and chromatographic data, a proposed structure of the anthocyanin of liflaf (Ipomoea purpurea L.) is suggested to be pelargonidin 3,5-diglycosid. Fig.(4) suggests the chemical of the anthocyanin molecule of the pigment.

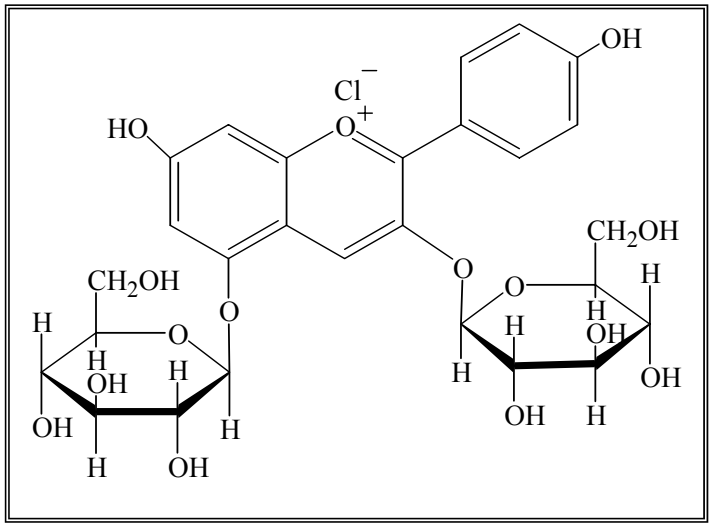

Fig. (4): pelargondin 3,5-diglucoside.

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