

THE ANTIBACTERIAL ACTIVITY OF *IPOMOEA PURPUREA* AND ANTHOCYANINE PIGMENT EXTRACTS AGAINST GRAM POSITIVE AND NEGATIVE BACTERIA

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ABSTRACT

In this study ,the antibacterial activity of aqueous and purified pigment extracts of *Ipomoea purpurea* were tested against reference strains of *Staphylococcus aureus* and *Escherichia coli* . The preliminary qualitative tests showed that the two extracts have flavonoids, carbohydrates and glycosides ,While alkaloids found only in the aqueous extract ,Thin Layer Chromatography (TLC) showed the presence of anthocyanin pigment , both extracts gave a clear activity against the tested strains with a minimal Inhibitory Concentration reached to 25mg/ml.

INTRODUCTION

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. In which research on plants that form a part of our normal diet has been compiled irrespective of activity and the second on phytochemical studies which are associated with pharmacological activity (1).

Finding healing powers in plants is an ancient idea. People on all continents have long applied poultices and imbibed in lusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory. There is evidence that Neanderthals living 60,000 years ago present –day Iraq used plants such as hollyhock. (14).

We reinforce on the flowers of Liflaf (*Ipomoea purpurea* L.). It is a herbaceous trailing or twining annual which attains a height up to 3m. Leaves broadly ovatecordate. Peduncle few-

flowered. Corolla funnel-shaped, light blue, purple, pink and diversely Variegated Flowering from July to September (2;3)

The plant has applications in medicine in South Africa where the Zulus use it as a purgative and as an anti-syphilitic chemical analysis reveals the presence of soft resin, essential oil, tannin and other colouring matter, this resin is the active principle responsible for the therapeutic properties. *Ipomoea* extracts also used in the reduction of glucose concentration in blood (11) , anoter uses to treat ring worm, an ametic in arsenic and opiate poisonings, it is believed to benefit the kidney, spleen, and stomach disturbance experimentally hypoglycemic elsewhere it's used in folk remedies for tumors of the mouth and throat. It's also used in treatment of diarrhea and stomach distress. (4)

MATERIALS AND METHODS

Plant material

The flowers of liflaf were collected, cleaned and allowed to dry at room temperture. The dried flowers were blended by use (Electrical mill blender), the flowers powder were kept until required.

Extraction method

20.000gm of purple flowers powder were extracted by soaking in 250ml of cold water for 24hours, the extracted was filtered through filter paper (whatmanNo. 541), a rotorevaporator at 50C was used to concentrate the solution. The filterate left in Petridish at room temperture Until it dry. The weight of amorphous purple powder that formed was 3.196gm.

Isolation of pigment

10.000gm of purple flowers powder soaked in 100ml of cold water for 5hours, the extracted filtered through filter paper (whatman No. 541) , 2% aqueous lead acetate then added until the formation of flocculent and blue precipitate, the precipitate separated by filter paper (whatman No 540) and washed with water, methanol and ethyl acetate consecutively (5)

The salt that produced converted to chloride by dissolving in (25ml acetone and 5ml 2NHCl) and filtered through filter paper (Whatman No 542). The filterate placed in petridish at room temperature until it dry. The weight of amorphous red powder that formed was 1.025gm .

Preliminary qualitative test

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

Thin layer chromatography

(TLC) were carried out on the aqueous extract and on the isolated pigment by using (EhOAc- HOAc-HCO₂H-H₂O) {5:1.1:1.1: 0.5}.(6)

Infra red and UV-Visible Spectroscopy

Uv-Vis spectra on JASCO UV- and IR spectra using pye- unicam-3-300S infra red-spectrophotometer in were shown in fig (1), (2), (3) respectively and table (3).

Antibacterial Activity

Reference strains (*Staphylococcus aureus* ATCC25923 and *Eschericia coli*) were gained from Immunology Laboratory –Department of Biology-College of Science-Basrah University ,Agar Diffusion Method was used which depended on the formation of wells with 5 mm in diameter by cork porer on Muller-Hington agar medium. Then 100 µl of the extract put in the wells. The plates incubated at 37 for 24 hours(7).

RESULTS

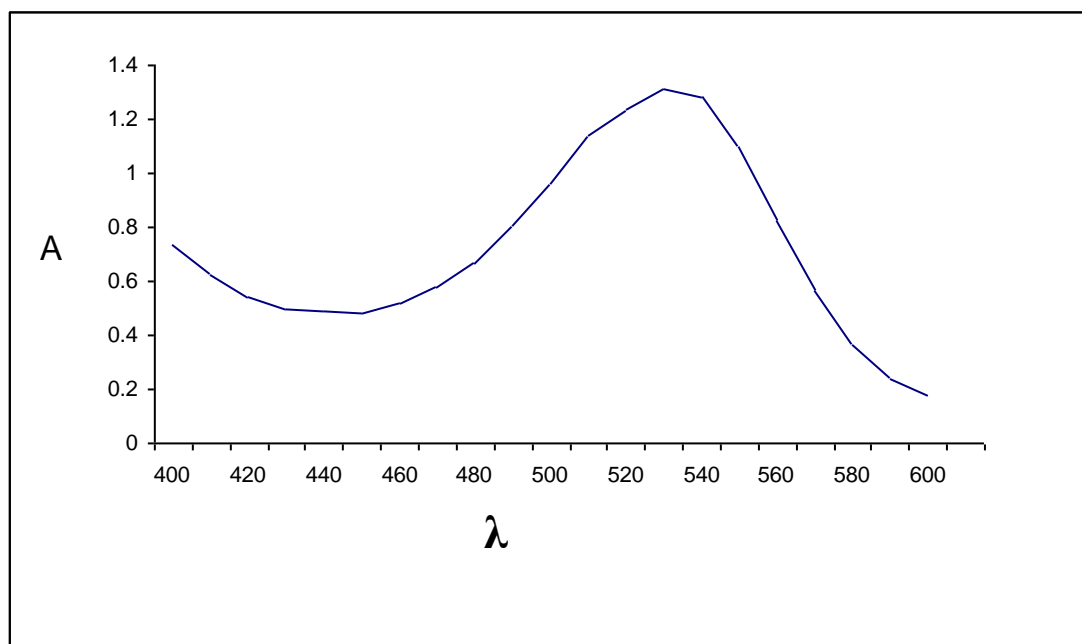
Table(1): Results of preliminary qualitative test for pigment and aqueous extract

Test sample	Flavonoid test	Carbohydrate test	Glycoside 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	Visibl e	Ninhydin	Drangdrof	40% H2SO4	FeCl3+ K3Fe(CN)6	10% NH4OH	Dil. HCl	Vanillin
Aqueous extract	0.2	0.2	0.13	0.95	0.2	0.2	0.2	0.2	0.2
	0.41		0.31						
			0.49						
Pigment	0.2	0.2	-	-	0.2	0.2	0.2	0.2	0.2

Table (1) shows the preliminary tests of aqueous extract of (*Ipomoea purpurea* L.) and the isolated pigment the results shows the presence of flavonoid as glycoside. The TLC shows the presence of anthocyanin pigments table (2) which changes their color by changing the pH value. (8; 9;10; 11) .

**Fig.1.** The visible spectrum of the pigment

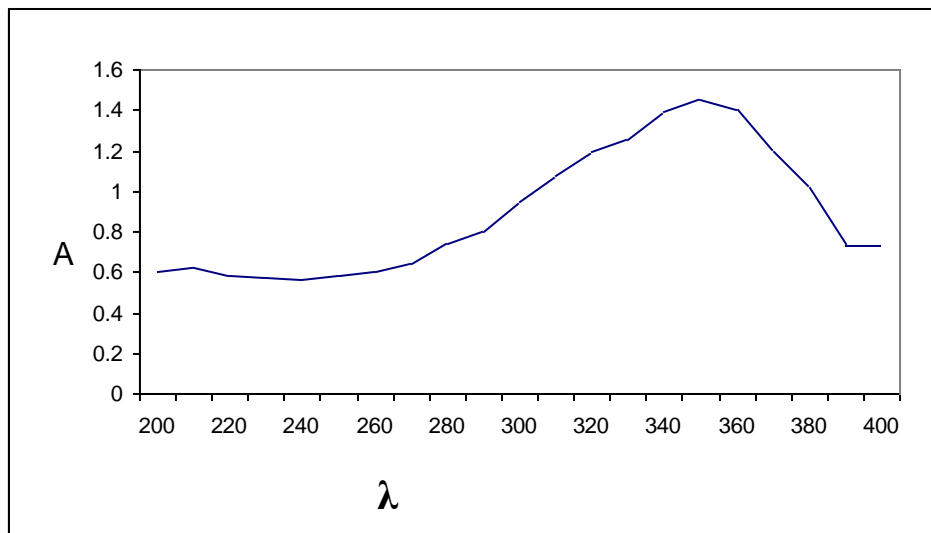


Fig.2:the UV spectrum of the piment

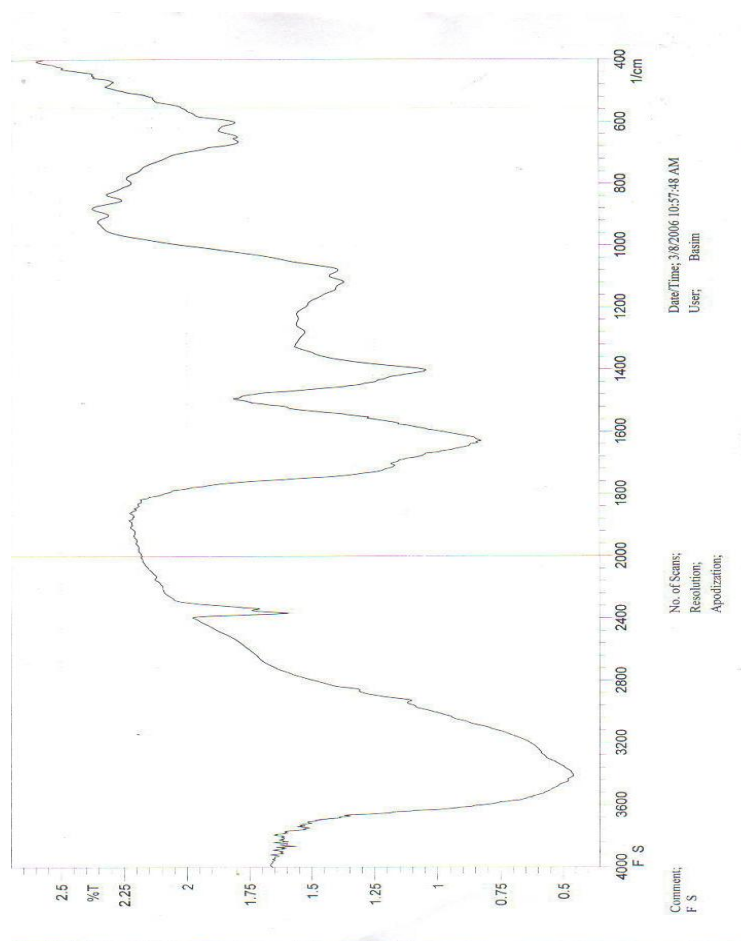


Fig.3; Full scan of IR spectrum of the pigments

Fig(1,2) shows the UV spectrum. Fig(3)and table (3) shows the full scan of IR spectrum of the pigments. The UV spectrum shows maximum absorption at 350nm due to $n \rightarrow \pi^*$ transition due to non-bonding, the visible spectrum also shows max absorption at $\lambda = 530\text{nm}$ due to the transition of $\pi \rightarrow \pi^*$ (15;16). \longrightarrow

Antibacterial activities

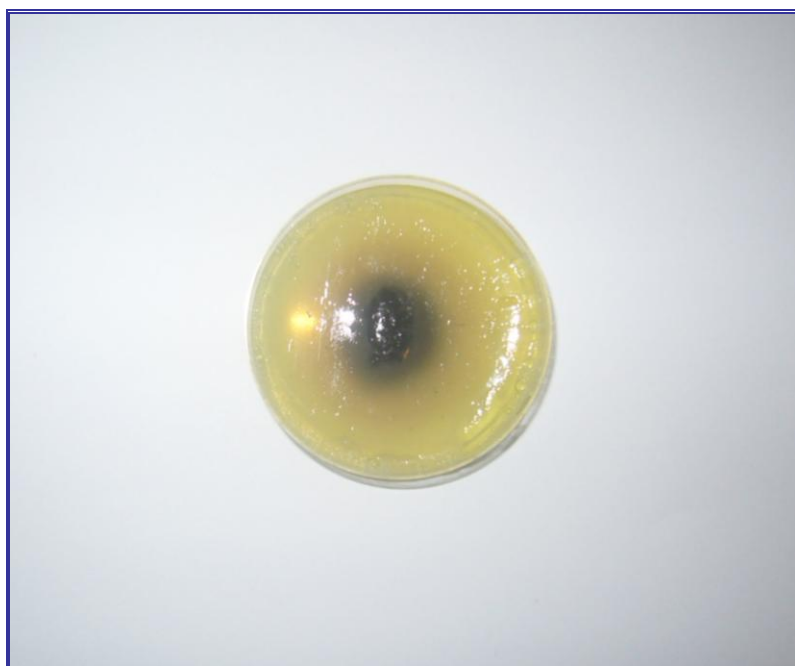
The activity appeared in inhibition zones as shown in pic.1,2,3 and their diameters in table 4



Fig.1 ;The antibacterial activity of the crude extract against *S. aureus* (Reference strain)



Fig2;. The antibacterial activity of the crude extract against *E. coli* (Reference strain)



Fig;3. The antibacterial activity of the pigment against *E.coli* (Reference strain)

Table (4) antibacterial activities in vitro for aqueous extract of pigment.

Minimal inhibitory concentration (MIC) mg/ml	The inhibition zone <i>S. aureus</i>	The inhibition zone <i>E.coli</i>
300	5	2
250	4	5
200	4	4
150	3	5
100	4	2
75	3	1
50	1	1
25	1	1

DISCUSSION

This obvious activity of *Ipomoea purpurea* against gram positive and negative bacteria may belong to its possession of flavonoids and alkaloids which have antimicrobial properties against a wide array of microorganisms. This activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, may also disrupt microbial membranes (13) or to obtain protein which inhibits the growth of several bacteria such as (*Agrobacterium tumefaciens*, *Serratia*, *Pseudomonas aureofaciens*) and fungi (*Phytophthora cinnamomi*, *Fusarium oxysporum* and *Nectria hematococcus*, *Rhizoctonia solani*) ocatin displays substantial amino acid sequence similarity with widely distributed group of intracellular pathogenesis related proteins with unknown biological function (17).

An antibacterial composition of this protein having at least one alkyl glycoside, at least one saccharide, and at least one therapeutic agent, wherein the alkyl glycoside has an alkyl chain length from about 12 to about 14 carbon atoms and wherein the saccharide has antibacterial activity, thereby providing for a composition having antibacterial property, this activity may also be due to the presence of glycolipids which gave activity

against many virulent bacteria ,for example *Ipomoea leptophylla* showed activity against *Mycobacterium tuberculosis*.(18)

Methylene chloride and methanol extracts of genus *Ipomoea* antibacterial and antifungal properties it had been tested by disk diffusion method (19)

تأثير للمستخلص المائي ومستخلص الانثوسيانين لنبته

Ipomoea Ipomoea purpurea

تجاه عزلات مرجعية لـ *Escherichia* و *Staphylococcus aureus*

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الخلاصة

في هذه الدراسة تم اختبار الفعالية المضادة للجراثيم للمستخلص المائي ومستخلص الانثوسيانين لنبته *Ipomoea purpurea* تجاه عزلات مرجعية لـ *Escherichia coli* و *Staphylococcus aureus* ، اظهرت الاختبارات الاولى امتلاك كلا المستخلصين لمركبات الفلافونيدات والكاربوهيدرات والكلايكوسيدات، اما القلويدات فقد ظهرت فقط في المستخلص المائي، واطهر اختبار كروماتوغرافيا الطبقة الرقيقة وجود صبغة الانثوسيانين ، اعطى كلا المستخلصين فعالية واضحة مضادة للجراثيم وكان التركيز المثبط الأدنى 25 ملغم/مل.

REFERENCES

- 1- Dahanukar , S. ; Kulkarni,R. and Rege , N.(2000). "Pharmacology of medicinal plants and natural products " , Indian J. of pharmacology , Vol. 32 , P.81-118.
- 2- Chakaravarty ,H. (1976) . " Plant wealth of Iraq " Ministry of agriculture , Baghdad,Iraq.
- 3- Al- Rawi ,A. (1967) "Medicinal plants of Iraq." ,Ministry of agriculture , Baghdad , Iraq.
- 4- Ody ,P.(1993) " The whole of medical plants " , Academica International , Beirut, Lebanon.
- 5-Ishkura,N. and Hayashi,K.(1962). Anthocyanins in red roots of a radish .Bot.Mag.,Tokoy,Vol.75:28-36

- 6-Hammouri, M.; Al- Samdi ,M. ;Bataineh, M. and Ou, b.,(2001)."Separation and characterization of an anthocyanin , cyanidin-3-O-arabinosyl glucoside from petals of Pheasants Eye (*Adonis aestivalist L.*) ", International J. of biochromatography, Vol.6 , P.173-183.
- 7-WHO,World Health Organization.1987.Manual for laboratory Investigation of Acute Enteric Infection.CCD 183:83-3.
- 8-Yoda ,K. ; Yokoi ,M. and Shigihara ,A. (1995) "Acylated cyanidin glycosides in the violet –blue flowers of *Ipomoea purpurea* " , Phytochemistry ,Vol. 40 ,P.1283-1289.
- 9-Saito ,N,Tatsuzawa,F. and Yokoi,M.(1996)."Acylated pelarpgonidin glycosides in *Ipomoea Purpurea* " , Phytochemistry ,Vol.43 ,P.1 365-1370.
- 10-Nikolin A.; Nikolin ,B. and Jankovi ,M. (1978),M.(1978). "*Ipomoea* Purpuroside ,a new glycoside from *Ipomoea purpurea*", Phytochemistry ,Vol . 17, P. 451-452.
- 11- Tatsuzawa , F.;Al-Norio,S.;Kichiji,K.; Tide,S.and Honda ,T. (1998). "Acylated cyanidin – cyanidin-3-sophorosides in the brownish red flowers of *Ipomoea Purpurea*", Phytochemistry ,Vol. 49 , P .875 -885.
- 12-Teh ,S. and Francis , F.(1988) "Stability of anthocyanins from zebrine pendula and *Ipomoea tricolor* in amodel Beverage " .Journal of food science ,Vol. 53, No. 5.
- 13- **Cowan,M.M.(1999)**,plant products as antimicrobial agents.Clin.Microb.Rev.12.4:564-582.
- 14- Cowan,M. (2005). Plant products as antimicrobial agents. Clinic. Microbiol. Vol.12.4.:1-3
- 15- Sherba,A.K. ; Al-Rawi,J.M. ; Iraqi,M.A. (1985). Absorption spectroscopy of organic molecules.Printed in Mousel University, translated from spectroscopy of organic molecuoles.
- 16- Silvesterstone,R. Bassler,G and Moril,T. (1981). Spetroscopic identification of organic compounds,4th, John Wiley and Sons.Inc.USA.
- 17- Flores,T. ; Alape-Giron,A. ; Flores-Diaz,M. and Flores,E.H. (2002). Ocatin. A novel tuber storage protein from Andean Tuber Crop Oca with antibacterial and antifungal activities. Plant.Physiol.128(4):1291-1302.

- 18-Goun,E. ; Cunningham,G. ; Chu,D. ; Nguyen,C. and Miles,D. (2003). Antibacterial and antifungal activity of Indonesian Ethnomedical plants. *Fitoterpia*.Vol. 74(6):592-596.
- 19- David,G. and Kingstone,I. (2006). Morning glory-derived anticancer agents and novel ipomoessin compounds. Designl code 2004-2006 . Fresh Patents.com.Website Terms and Condition.