

THE ANTIBACTERIAL ACTIVITY OF COLD AQUEOUS AND PIGMENT OF HIBISCUS ROSA SIENSIS EXTRACTS AGAINST GRAM POSITIVE AND NEGATIVE BACTERIA

* Zeenah Weheed Atwan

** Fatima Saiwan

*Department of Biology , College of Science, University of Basrah , Basrah, Iraq

**Department of chemistry , College of Science , University of Basrah,- Basrah, Iraq

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ABSTRACT

The antibacterial activity of aquaeous and purified pigment extracts of Hibiscus rosa siensis in concentration (20,50,100,200,250 mg) were tested against reference strains of Staphylococcus aureus and Esherichia coli . The preliminary qualitative tests showed that the two extracts have flavonoids, carbohydrates and glycosides ,while alkaloids found only in the aquaeous 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 20 mg/ml.

INTRODUCTION

The use of medicinal plants or their active compounds in the prevention and treatment of chronic disease is based on experience from traditional systems of medicine from various ethnic societies , during the past decade , a large number of natural products and dietary compounds have been evaluated as potential chemo preventive agents (1).

Many people are interested in having more autonomy over their medical care . A multitude of plant compounds (alkaloids , polyphenols "flavonoids" , saponins and terpenoids) is really a vailable over the counter from herbal supplier and natural –food stores, and self medication with these substances in common place, the use of plant extracts , as well as other alternative forms of medical treatment (2).

The selection Bent El-Kunsil (Hibiscus rosa siensis) an evergreen shrub which usually attains height , growing to 2.5 m by 2.5 m , but in some subtropical countries

it may grow to a small tree ,flowers axillary bright rosa-red it is in leaf all year. The flowers are hermaphrodite. (3).

Anthocyanin pigment (are major natural phenol compounds of flower petals) is present flower on chemical analysis , the following substances have been determined : Water , calcium, thiamine, riboflavin and ascorbic acid . (4; 5). The red flowers is reported to be eaten used for darkening and the flowe sap is used for colouring hair , eyebrows , food and liquors as well as treatment of dysentery , lung and urinary aliment. (6)

MATERIALS AND METHODS

Plant Material:

Bent El Kunsil (*Hibiscus rosa siensis*) obtained from Maaqel gardens .It classified in Plant Taxonomy lab \\ College of Science Basrah University, the flowers cleaned and allowed to dry at room temperature . The dried flowers blended by using (Electrical Mill blender) . The powder of flowers kept until required.

Chemical and Materials :

All chemicals were of purity analytical grades :hydrochloric acid (analar) , ethyl acetate , lead acetate and methyle alcohol from (BDH) ; acetone, 95% ethanol from Baghdad factory for drugs and cosmetics (Whatmann 540) filter paper.

Instruments

JASCO UV- visible spectrophotometer

Infra Red spectrophotometer PYE-UNICAM SP – 3-300S

Extraction and Pigment Isolation

red flowers (20 gm) were extracted by soaking in 300 ml of cold water for 6 hours , the extract filtered, to the filtrate 2% aqueous lead acetate consecutively (7) , The product salt was converted into chloride by dissolving in 25 ml acetone and 5 ml 2 N HCl and filtered through Whatmann No.540 .The filtrate placed in Petri dishe at room tempreture till dry .The weight of amorphous red powder formed was 0.856 gm.

Preliminary Qualitative Tests

Preliminary tests were carried out on the aqueous extract and the isolated pigments.

Thin Layer Chromatography

(TLC) were carried out on the aqueous extract and isolated pigment using sec-butanol- acetic acid water (4:1:1.2)

Infra red and UV- visible spectroscopy

IR spectra using PyE-UNICAM-30300S Infra red spectrophotometer and Uv-visible spectra on JASCO UV- in showed in fig (1), (2), (3) and table (3). (12; 13)

Antibacterial Activity

Reference strains (Staphylococcus aureus ATCC25923 and Escherichia coli ATCC25922) were brought from Immunology Laboratory –Department of Biology- College of Science-Basrah University one strain from each species , a bacterial suspension prepared in a concentration of (1*10⁶) in comparison with Mcfarland tube .Agar Diffusion Method used which depended on the formation of wells with 5 mm in diameter by cork poorer on Muller-Hington agar medium, 100 µl of aqueous and pigment extract placed in the wells. The plates incubated at 37 °C for 24 hours(7).(8)

Results

Aqueous extract contained flavonoids , carbohydrates Glycosides , alkaloids and amino acids , while the pigment contained only the three first compounds as shown in table 1.

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

test sample	Flavonoid test	Carbohydrate test	Glycoside test	Alkaloid test	Amino acid test	Saponin test	
						Foam test	5% Hg Cl2
Aqueous extract	+	+	+	+	+	-	-
pigment	+	+	+	-	-	-	-

While the presence of anthocyanin pigment is shown in table 2 .

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

test	UV lamp	Iodin	Folin	vanilin	10%HCl	FeCl ₃ K ₃ Fe(Cn) ₄	P- ansaldehy de	dranbdrof f	10%NH ₄ OH
Sample									
Pigment	0.56	0.56	0.56	0.56	0.56	0.56	o.56	—	0.56
sample	40% H ₂ SO ₄	Daylight	Ninhydi n	2% Lead acetate	5% AlCl ₃ +UV lamp				
	0.56	0.56		0.56	0.56				

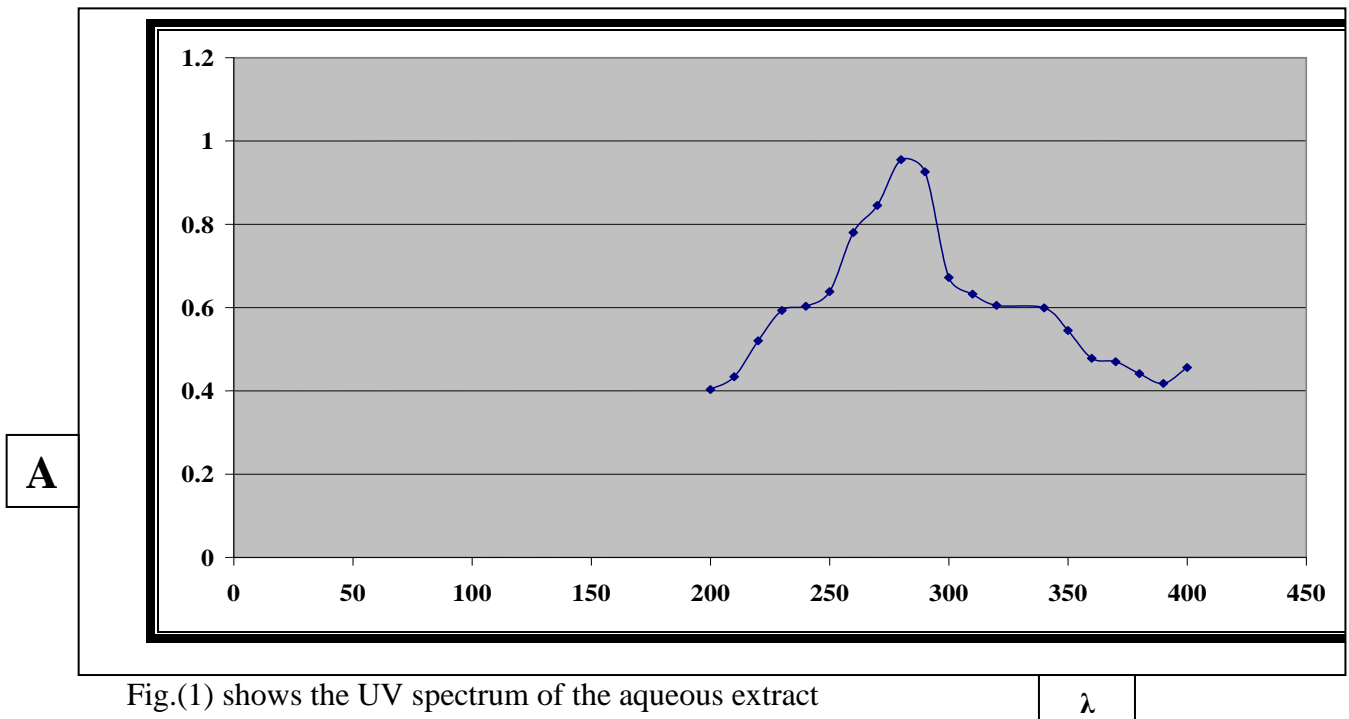


Fig.(1) shows the UV spectrum of the aqueous extract

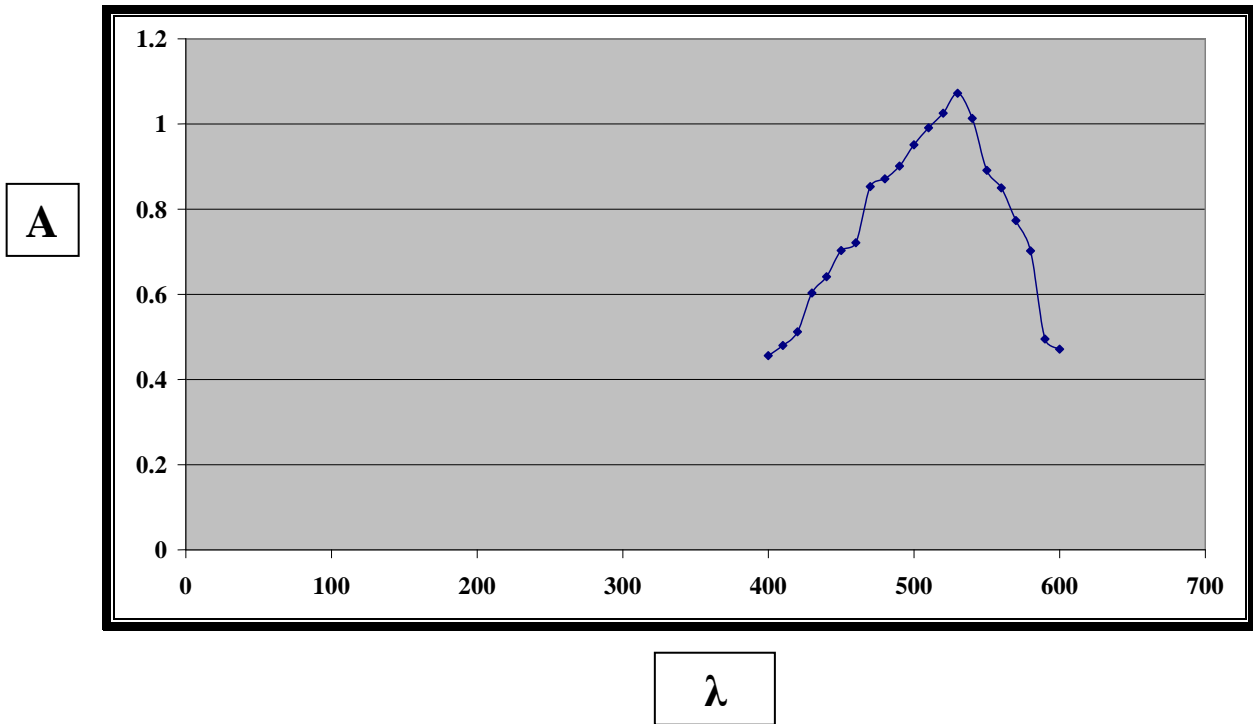


Fig.(2) shows the UV spectrum of the pigment extract

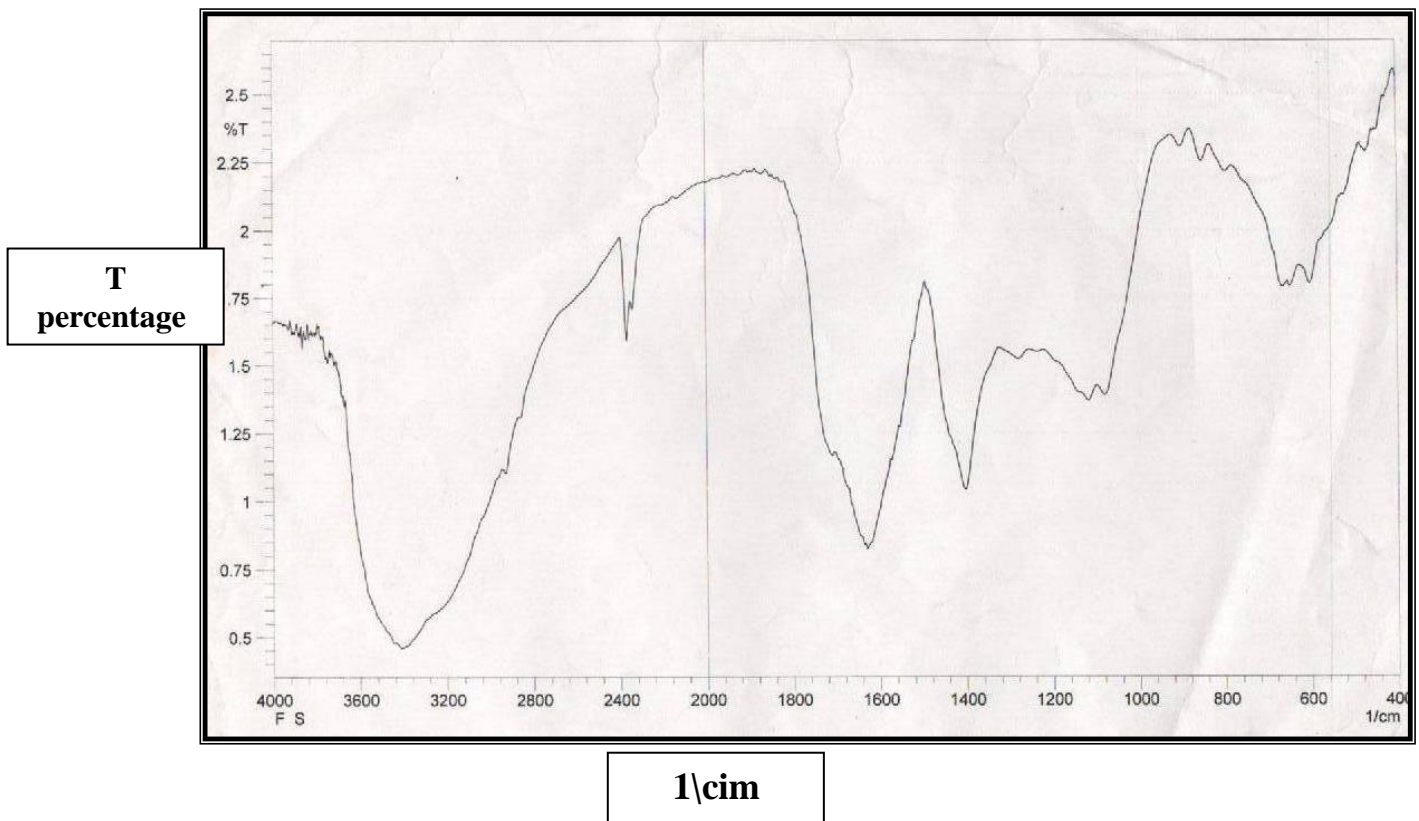


fig (3) shows the full scan of IR spectrum of the pigment

Table (3) full scan of IR spectrum 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-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-

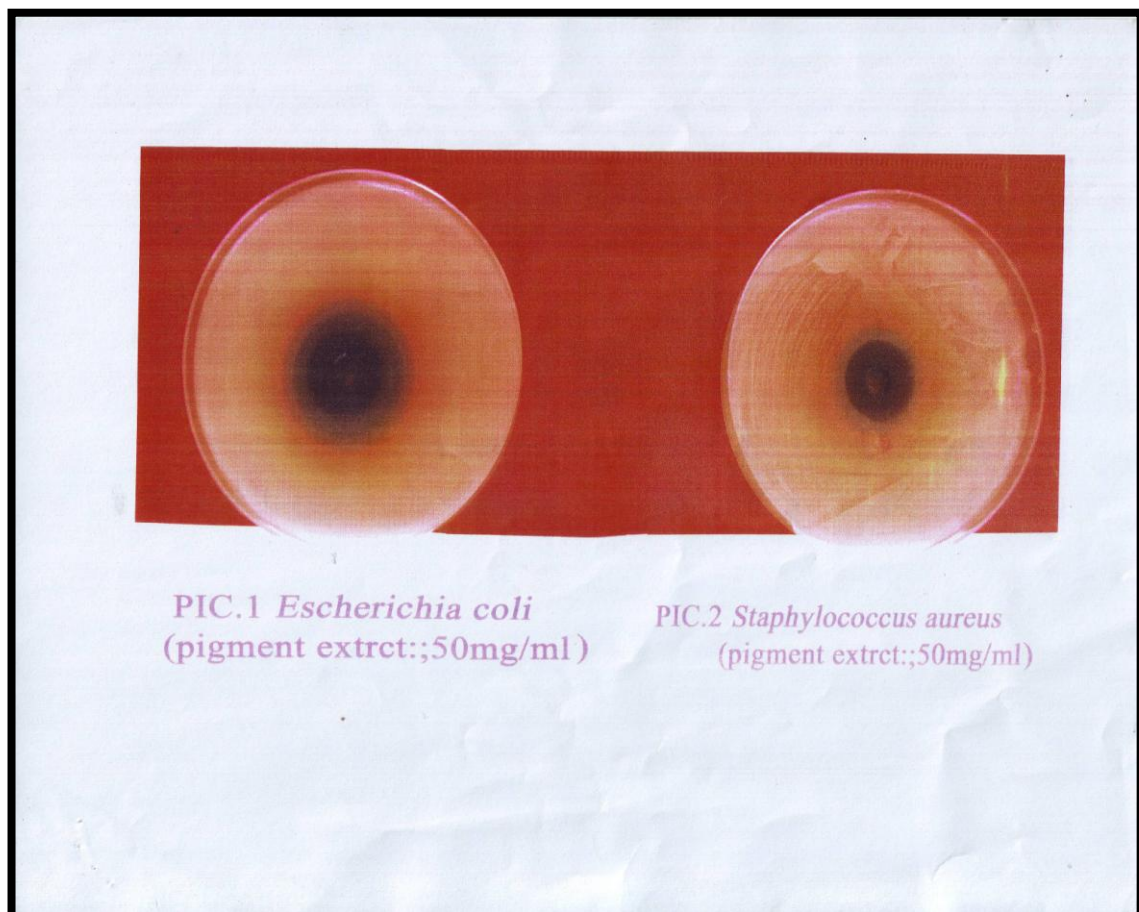
Antibacterial activity

Table (4) : The minimal Inhibitory concentration of the aqueous extract

concertation	Escherichia coli inhibition zone in mm	Staphylococcus aureus Inhinition zone in mm
20 mg \ml	12	15
50 mg\ml	15	18
100 mg\ ml	20	18
200 mg\ml	23	15
250 mg\ml	23	14

Table (5) : The minimal Inhibitory concentration of the pigment extract

concentration	Escherichia coli inhibition zone in mm	Staphylococcus aureus Inhibition zone in mm
20 mg \ml	1.5	1.2
50 mg\ml	1.5	1.3
100 mg\ ml	2.5	1.8
200 mg\ml	2.8	2
250 mg\ml	2.8	2.2



DISCUSSION

The preliminary tests of aqueous extract and isolated pigment from flower Bent el-Kunsil (*Hibiscus rosa siensis*) showed the presence of flavonoids as anthocyanin pigments table (2) which change their color by changing the pH values. The same results were obtained by other authors (8; 9).

UV spectrum showed maximum absorption at 280 nm due to $\lambda \rightarrow \lambda^*$ transition which is considered as characteristic feature of the unsaturated double bond. The visible spectrum also showed max-absorption at $\lambda = 530$ nm due to the transition of $n \rightarrow \pi^*$. (10,11)

Hibiscus is well known about his bactericidal activity for more than 40 years, his coloring matter used as a bactericidal agent against *Mycobacterium tuberculosis*, it was added to the broth and prevented the bacterial growth (14), Farnesyl acetate is the major component of seed oil of Hibiscus, In addition, compounds such as farnesene, farnesyl acetate, 2,3-dihydrofarnesol etc. were identified, seed oil had antibacterial activities. (15)

Hibiscus rosa-sinensis possess good antibacterial activity, its structure contains Flavanoids, apigenidine, palargonidine, cyanidine, quercitine, crisantemin, antocyanine, kaempferol, camphoral, citric and oxalic acids, tartaric acid, Juice--alkaloids, glycosides, triterpenoids, lipids, terpenes, beta-sitosterol, teraxeril, cyanidic glycosides Miscellaneous-sucrose, fructose glucose. (12)

Flavonoids are phenolic compounds containing one carbonyl group, that's why it should not be surprising that they have been found in vitro to be effective and had antimicrobial activity against a wide array of microorganisms. Their activity is due to their ability to complex with the extracellular and proteins and make a complex with the bacterial cell walls, flavonoids may also disrupt microbial membranes (2).

الفعالية الحياتية للمستخلص المائي ومستخلص صبغة الانثوسيانين لزهرة نبات بنت القنصل

Hibiscus rosa siensis

تجاه الجراثيم الموجبة والسالبة لصبغة كرام

زينة وحيد عطوان ، فاطمة صيوان

كلية العلوم ، جامعة البصرة ، البصرة ، العراق

الخلاصة

تم اختبار الفعالية المضادة للجراثيم للمستخلص المائي ومستخلص الانثوسيانين لزهرة نبات بنت القنصل *Hibiscus rosa siensis* وبتركيزات (20,50,100,200,250 ملغم/مل) تجاه عزلات مرجعية للمركبات الفلافونيدات والكاربوهيدرات والكلايكوسيدات والقلويدات ، اما القلويدات فقد ظهرت فقط في المستخلص المائي، وظهر اختبار كروماتوغرافيا الطبقة الرقيقة وجود صبغة الانثوسيانين ، اعطى كلا المستخلصين فعالية واضحة مضادة للجراثيم وكان التركيز المثبط الادنى 20 ملغم/مل.

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