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Investigation of DNA Protective, Anti-bacterial, and Antidiabetic Effects of Cucurbitacin E from Citrullus colocynthis L. Iraqi plant with invitro Toxicity Study

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ABSTRACT

Botanical remedies have long been used for medical purposes, and their use has grown alongside human civilization**.** *Citrullus colocynthis* plant is a member of the Cucurbitaceae family contain many secondary metabolites once of them is cucurbitacin E. Studies have revealed the significance of cucurbitacin E because of its diverse therapeutic properties like antibacterial and biological activity against bacterial combined , study antidiabetic activity and investigate the DNA protection effect of cucurbitacin E with study its effect on human erythrocyte of these plant and its metabolite (cucurbitacin E) to demonstrate its activity in this study. Extraction for *Citrullus colocynthis* fruits by hot continuous method using chloroform as solvent was done then detection of cucurbitacin E was used by High-performance liquid chromatography (HPLC), well diffusion method for antibacterial, glucose reuptake method for antidiabetic activity, Agarose gel electrophoresis was subsequently employed to evaluate the range of DNA damage also *invitro* study of the hemolytic activity assay. After successful extraction the results show the valuable antibacterial activity of cucurbitacin E detect by using bacterial isolates, and minimum inhibitory concentration of cucurbitacin E values for bacterial isolate were (0.1, 0.25, and 0.15) mg/ml for (*Staphylococcus aureus*, *Klebsiella pneumoniae* and *Bacillus subtilis*) respectively. Regarding antidiabetic effect was studying by glucose reuptake invitro assay in which metformin was used as stander and the results were the inhibition values found for cucurbitacin E, extract, and metformin were(0.108, 0.313, and 0.377mg/ml), respectively and after successful extraction the DNA damage protection assay determined the optimal concentrations (1,0.5,and0.25) mg/ml give protection %87.52, 72.21, and44.92respectivlyand cause heamolysis for red blood cell in dose dependent manner.

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1.Introduction

The desert plant *Citrullus colocynthis* is an herbaceous plant that is rich in nutrients and is essential for enhancing wellbeing. Nearly every aspect of this type of fruit harvest is beneficial to health.*C. colocynthis* have an excellent fatty acid and amino acid profile and are a significant source of protein and oil, also contains a variety of bioactive substances (secondary metabolite) including cucurbitacin, flavonoids, and polyphenols, which contribute to its therapeutic qualities [1].figure 1 show parts of plant.

Fig. 1. Citrullus colocynthis (L.) Schrad, A-B: Flowers; C-D: Fruits. Nutritional[2]

Cucurbitacin is triterpenoid compound, one of the important substances which widely exist in Cucurbitaceae family plants[3]. The primary cause of cucurbitacin's diversity is its skeleton, which is made up of 30 carbon atoms with various oxygen substituents in several locations. are categorized into 12 primary groups, have different compounds based on isomerism, deoxidization, and dihydrogen, and range from cucurbitacin A to T. [4].

Cucurbitacin types E and B are found in plants during their growth, and through secondary metabolism or other processes, they generate other naturally occurring cucurbitacin types, example Cucurbitacin B produces cucurbitacin A, C, and D, whereas cucurbitacin E forms cucurbitacin I, J, and K. [5]. *C. colocynthis* contains a high concentration of cucurbitacin E, which is bitter and toxic. Cucurbitacin E has high antifeedant effect to disrupt cellular action, and it also inhibits cancer cell proliferation chemical structure of it appeared in figure ٢

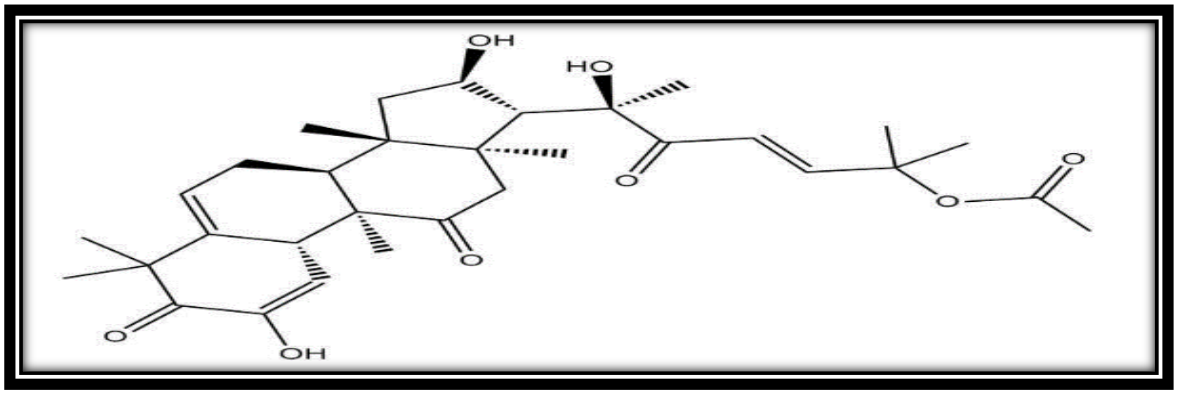


Fig. 2. Chemical structure of cucurbitacin E[6]

In many regions of the world, *C. colocynthis* is commonly utilized for a variety of illnesses, in other hand *C. colocynthis* fruit pulps contain high concentrations of Cucurbitacin E, has Significant biological actions include antiviral, antimicrobial, antihyperglycemic, anticancer, and anti-inflammatory properties [7]Multi-drug resistant bacteria have developed resistance to antimicrobial medications[8]Bacteria are categorized according to their biochemical and morphological characteristics and molecular attributes, There are two types of bacteria: gram-positive and gram-negative[9]. Cucurbitacin is an extremely bioactive compound that has the ability to form complexes with protein nucleophilic amino acids. The primary way that β-lactams work against bacteria is via inactivating penicillin binding protein (PBPs) by covalent binding. Given those details, we can also propose that cucurbitacin may function as a PBP inhibitor[10]. Another dangerous disease is diabetic mellitus [11] so making the identification of new active compounds a serious public health problem.

A Diabetes medications are quite efficient, but they can have significant side effects. On the other hand, medicinal herbs may serve as a different supply of mediators that lower blood sugar[12].Cucurbitacin performed an important role in regulating insulin-dependent glucose clearance and AMPK activation (AMP-activated protein kinase, a crucial regulator of energy homeostasis) promotes glucose transporter four (GLUt4)translocation to the plasma membrane, which in turn stimulates the uptake of this carbohydrate primarily in adipose tissue. This helps fight hyperglycaemia and improve glucose clearance without the need for insulin [13][14]. DNA and biological membranes can be damaged by radiation, which can also negatively impact living cells[15]Additionally, radiation is a well-known genotoxic agent in the biological system that can damage DNA and modify bases by generating fatty acid peroxidation inside the membrane's phospholipid structure. Study the effect of cucurbitacin E as DNA protection is crucial because reactive oxygen species can cause cell damage[16]

Citrullus colocynthis erratic occurrence can result in colitis with bloody diarrhea, severe cramping in the abdomen, vomiting, and hypotension because of toxicity[17]. Furthermore, it was found that cucurbitacin's toxicity was directly correlated with its chemical structure, particularly because of the double bond at position C-23 and the acetyl group at position C-25[18]so study hemolysis assay is important for this compound.

This study aimed to investigate the importance of cucurbitacin E as active metabolite from *C. colosynthis* as antibacterial and antidiabetic agent also, cucurbitacin E has antioxidant effect so study the effect of it as DNA damage protection was crucial issues Since cucurbitacin E is a cytotoxic chemical used to treat cancer, a toxicological review is necessary to guarantee patient safety[19].

2. Materials And Methods

1.2. Plant collection

The plant was collected in Basrah/Iraq (Safwan at longitude ,47.7193r856 and latitude ,30.1097159) and cleaned then left at room temperature to air-dry for five days. In the shade and then sealed in labeled plastic bags. After drying, the plant parts are ground with a mortar to assist the extraction of active chemicals. The powder of *C. colosynthis* (fruit and leaves) is then stored in a labeled plastic container tightly sealed.

2.2. Extraction and isolation of cucurbitacin E method

The extracts were prepared by weighing 10 g of each plant part (fruits and leaves) one by one, adding 200 ml of chloroform as a solvent in a 250 mL conical flask for each part of plant and then by using reflex apparatus extraction was done for 8 hours[20]. Isolation was done by using high performance liquid chromatography HPLC technique by using cucurbitacin E as standard. HPLC conditions for purification of cucurbitacin was[21]:

Mobile phase:	solvent A - acetonitrile	70%
	Solvent B-water	30

Column: 18 mm Pressure: less than 200 Flow rate: 1ml/min. Temperature: at 25 centigrade
Volume injection: 10 Ml for 20 min. UV detection: at 230 nm

2.3. Antibacterial assay invitro study

Antibacterial activity of cucurbitacin E was assessed qualitatively using the agar well diffusion method. The antibacterial activity assay employed a concentration detector with a concentration of 1 mg/ml. Bacterial isolates from the area were examined. Two gram-positive (*Bacillus subtilis and Staphylococcus aureus*) and two gram-negative (*Escherichia coli and Klebsiella pneumoniae*) bacteria are among the bacterial isolates, which were supplied by Basrah University's Pharmacy College's Microbiology Laboratory. Bacteria were cultured in Nutrient Broth (NB, Difco, MD, USA) at 37°C for 24 hours in order to reach the stationary growth phase with108 Colony Forming Unit (CFU) per ml was achieved by using McFarland 0.5. Pure Dimethyl sulfoxide (DMSO) served as the negative control, while gentamicin disk served as the positive controlEach plate (Mular Hinton agar) was inoculated with only one microorganism[22]. Three wells per plate were made using an 8 mm sterile cork borer. 100 µl of the positive, negative controls and cucurbitacin E solution,in each wells. After that, bacteria were cultured at 37°C for 18 hours. Every well's surrounding clear zone diameter was measured. Then, bacteria were incubated for 18 h at 37°C. The diameter of clear zones around each well was measured.

2.4. Minimum Inhibitory (MIC) Assay

Cucurbitacin E was tested against these pathogens on a plate with MH agar seeded with five concentrations (1,0. 5,0 .0.25 ,0.125, and 0.1 mg/mL). Well diffusion agar was used to determine the least inhibitory concentration (MIC). For 18 to 24 hours, agar was incubated at 37°C. The antibacterial activity was then assessed by measuring the no-growth zone around each well. The MIC values were the lowest concentrations of the extract that showed a clear zone. DMSO was used as a solvent as a negative control.

2.5. Antidiabetic activities: In Vitro glucose uptake assay in yeast cells

After obtaining commercial baker's yeast, it was centrifuged three times at 3000g for five minutes with distilled water until a clear supernatant fluid appeared. A 10% (v/v) suspension was then made in distilled water. One milliliter of glucose solution10 mM (0.05g in 30 ml) was mixed with various quantities of plant extracts and cucurbitacin E (1, 0.5 ,0.25, and 0.125 mg/ml), and the mixture was incubated at 37 °C for 10 minutes. The reaction started with 100 µl of yeast suspension, vortexed, and then incubated for further 60 minutes at 37 °C. After 60 minutes, the tubes were centrifuged (2500 g, 5 minutes) to determine the supernatant's glucose content. Metformin was used as standard drug in same concentration as *C. colosynthis* and cucurbitacin E which used. The percentage increase in glucose absorption by yeast cells was estimated using the following formula:

% increase in glucose uptake = {(Ac - At)/Ac} multiplied by 100.

Where Ac is shown the absorbance of the control and At indicated the absorbance of the test sample. All experiments were carried out in triplicate[23]. A linear regression analysis was conducted to establish the inhibition of half concentration of glucose (IC50) value of the specimen by plotting the absorbing capacity of cucurbitacin E or the standard specimen against the percentage increase in glucose absorption[24].

2.6. DNA damage protection test:

The FAVORGEN DNA extraction kit was used to extract human genomic deoxyribonucleic acid (DNA) from human white blood cells (WBCs). Every extraction procedure was carried out precisely in accordance with the manufacturer's guidelines.

This method was determined by utilizing human genomic DNA subjected to photolysis using UV light in the presence of hydrogen peroxide (H_2O_2). Agarose gel electrophoresis was subsequently employed to evaluate the range of DNA damage [25].different concentration of cucurbitacin E prepared (1 ,0.5, and 025) mg /ml. the procedure was:

1- The experiment mixture consisted of 5µl specimens of human genomic DNA with a concentration of 20 ng/µl mixed together with each concentration of cucurbitacin. E in polyethylene microcentrifuge tubes.

2- Tube contains all contents but does not contain cucurbitacin E which is called irradiated control (IC)

3- added 5 µl of H2O2 (3%) to all tubes (including IC)

4- Then all tubes were put onto a UV transilluminator that emitted light with a wavelength of 300 nm and left for a duration of 10 minutes at ambient temperature

5- A non-irradiated control (negative control) tube was identified as the five tube and contained 1 µl of human genomic DNA. Ultimately, electrophoresis was performed on all the specimens.

6- Electrophoresis procedure[26]: for preparation of Agarose Gel done by volume of approximately 25 ml of 1x tris-borate-EDTA (TBE) buffer was put into a beaker. Subsequently, 0.25 g of agarose were made into the TBE buffer solution. The solution was further heated using a hot plate until all the agarose particles were dissolved. The mixture was subsequently cooled to a temperature from 50–60 °C

7- precise volume of 0.2 µl of ethidium bromide was cautiously introduced into the cooled solution

8- Loading and running Genomic DNA in Agarose Gel, these done by 9 µl of DNA was combined with 3 µl of bromophenol blue loading dye and placed into the wells of the 1% agarose gel.

9- 60 voltages electrophoresed to the gel until the bromophenol blue tracking dye reached the end of the gel.

10- by digital camera obtain the result of the DNA bands were observed and analyzed under ultraviolet (UV) transillumination.

2 .7. Hemolysis assay for human red blood cells

1- human red blood cells has been done according to study in 2014 [27]with some modification such as EDTA was employed as anticoagulant instead of sodium citrate, and 1% Sodium lauryl sulphate(SDS) as a positive control instead of Triton X-100.

2- 2 mL of blood was combined with 38 mL of Ringer solution.

3- The assay examined various product concentrations (2, 1, 0.5 ,0.25, and 0.125) that were added to each test tube, with the volume being reached at 2 ml by the combination of blood and Ringer solution.

4- They were incubated for one to two hours at 37°C.

5- The samples underwent centrifugation at a speed of 5000 revolutions per minute for a duration of 12 minutes, in contrast to a negative control consisting of blood mixed with Ringer solution, and a positive control consisting of blood mixed with SDS.

2.8. Statistical Analysis

The SPSS software was used to evaluate data using one-way ANOVAs. A p-value of less than or equal to 0.05 was considered statistically significant.

3. Results And Discussion

3.1. High performed liquid chromatography (HPLC)

Cucurbitacin E standard beak which appeared at 3.10 min recognized in figure (٣) and recognized in table 1while figure (٤) and table 2 for extract by chloroform

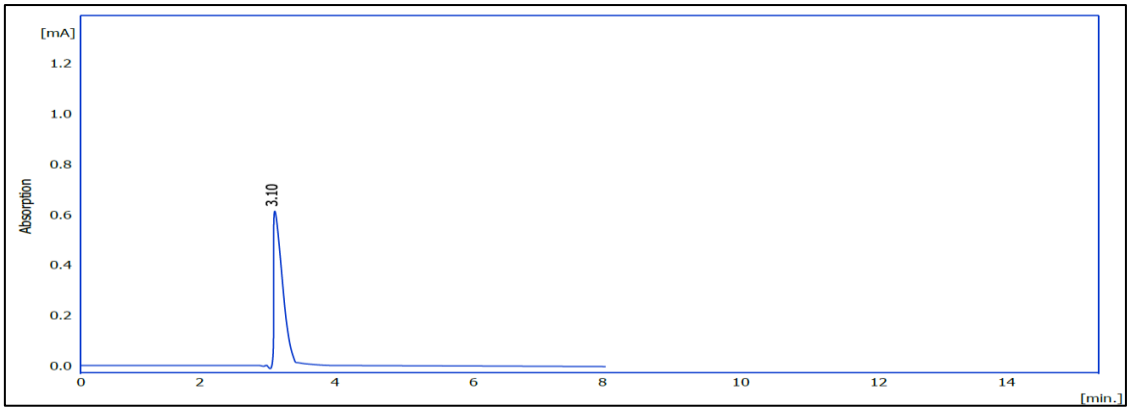


Fig. 3. cucurbitacin E standard 1mg/0.5ml methanol and isopropanol

Table 1. Retention time and peak area of HPLC analysis for standa

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	3.10	2148.02	620.34	100.00
Total	3.10	2148.02	620.34	100.00

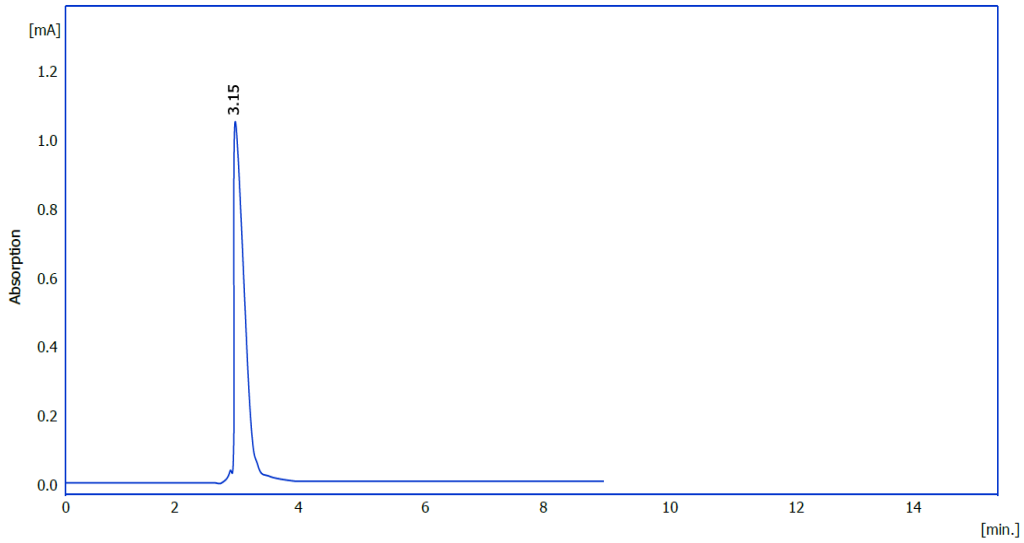


Fig.4. for cucurbitacin E which isolated from extract

Table 2. retention time and area under the curve for cucurbitacin E which isolated from extract

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]
1	3.15	7125.99	1050.14	100.00
total	3.15	7125.99	1050.14	100.00

3.2. Antibacterial activity and minimum inhibitory concentration

The antibacterial activity of cucurbitacin E was evaluated qualitatively using the agar well diffusion method. The antibacterial assay's concentration detector was calibrated to read 1 mg/ml. The inhibitory zones for microorganisms were found in table 1. The bacterial inhibitory zones are displayed in table 3 in contrast to the standard gentamicin in figure (5).

Table 3. inhibition zones for bacteria

Bacterial isolates	Inhibition zone (mm) for cucurbitacin E	Inhibition zone (mm) for gentamicin
Staphylococcus aureus	26	27
Klebsiella pneumoniae	5	20
Escherichia coli	Non	12
Bacillus subtilis	10	15

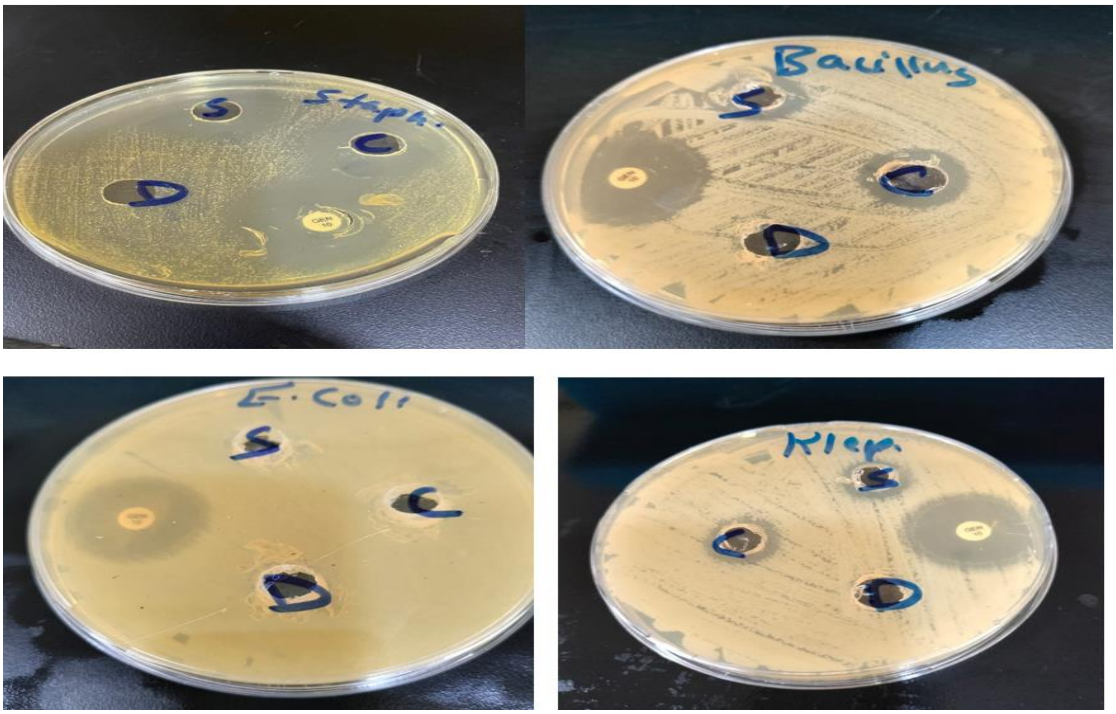


Fig. 5. inhibition zone of cucurbitacin E for *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Klebsiella pneumoniae* respectively*:* S is cucurbitacin E, C is plant extract, D DMSO, gentamicin disk as positive control

The minimum inhibitory concentration (MIC) is the compounds at what concentration that is no discernible growth (turbidity)[28]. The result of MIC for cucurbitacin E as antibacterial was summarized in table (4) for five different concentrations were used:

Table 4. MIC value for bacterial isolates

Bacterial isolates	MIC mg/ml
Staphylococcus aureus	0.1
Klebsiella pneumoniae	0.25
Bacillus subtilis	0.15

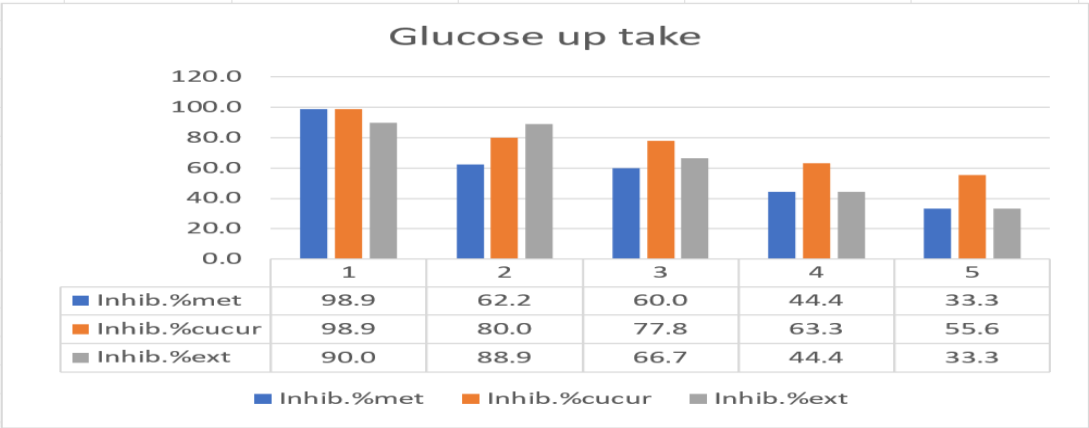
The mechanism in which cucurbitacin acts as antibacterial comes from the fact that cucurbitacin has the ability to bind with protein nucleophilic amino acid to form complex like the way that β-lactams work against bacteria is via inactivating penicillin binding protein (PBPs) by covalent binding. Given those details, we can also propose that cucurbitacin may function as a PBP inhibitor[10]

cucurbitacin E had great impact on Gram positive bacteria than Gram negative these agree with [29]these owing to cell membrane permeability or other genetic factor may be the cause of the observed variances in the inhibitory zones.

Although antibacterial activity of terpenes remains challenging due to their poor solubility, terpenes show a strong activity especially against Gram-positive bacteria the mechanism how they work was Terpenes' lipophilic characteristics, which enable them to penetrate microbial cell walls, are intimately linked to their antimicrobial actions[30]

The structural variations in these bacterial types of cell walls could be the cause of this. Gram-negative bacteria have an extra layer of membrane surrounding their cells, giving them a hydrophilic membrane that acts as a barrier to permeability in contrast to a variety of chemicals, including natural compounds. Additionally, efflux pumps which move various substances, including toxins and antibiotics—from the periplasm to the exterior of the cell support the innate resistance of Gram-negative bacteria[31]

3.3. antidiabetic activities: In Vitro glucose uptake assay in yeast cells



	1	2	3	4	5
Inhib.%met	98.9	62.2	60.0	44.4	33.3
Inhib.%cucur	98.9	80.0	77.8	63.3	55.6
Inhib.%ext	90.0	88.9	66.7	44.4	33.3

Fig. 6. *Invitro* glucose uptake assay in yeast cells. A comparative percentages of glucose uptake exhibited by inhibition % of metformin, cucurbitacin E and chloroform extract of *C. colosynthis*

This *invitro* glucose uptake study was investigated in yeast cells. The results showed that there was a direct link between the dose of the plant extracted by chloroform and cucurbitacin E with the amount of glucose absorbed by yeast cells for each plant extract, cucurbitacin E and metformin which was used as stander. The IC50 values found for cucurbitacin E, extract, and metformin were 0.108, 0.313, and 0.377mg/ml, respectively.

statistically there was no significant difference because p-value was 0.592 which more than 0.05 and yeast transports glucose via facilitated diffusion. Due to its importance, researchers are actively concentrating on yeast's sugar transport mechanism. Yeast has the ability to use one or more sugars as its principal source of carbon and energy. Furthermore, sugar is converted into ethanol[32]. The mechanism was that Cucurbitacin increase glucose absorption by boosting glucose transporter four (GLUT4) protein activity in skeletal muscle via PI3K/Akt and antioxidant defense in plasma. It also preserves homeostasis[14].the result agree with [33][34][13].

3.4. DNA damage protection

Analysis of DNA pictures indicated the protective effect of cucurbitacin E. The study measured both the percentage of DNA protection and the concentration-dependent effect of cucurbitacin E after UV exposure and 3% H2O2 therapy. These findings were compared to two control groups: a negative control (non-irradiated, untreated DNA) and a positive control (UV-irradiated, 3% H2O2-treated DNA) the result showed in fig. ٧ and table (5)

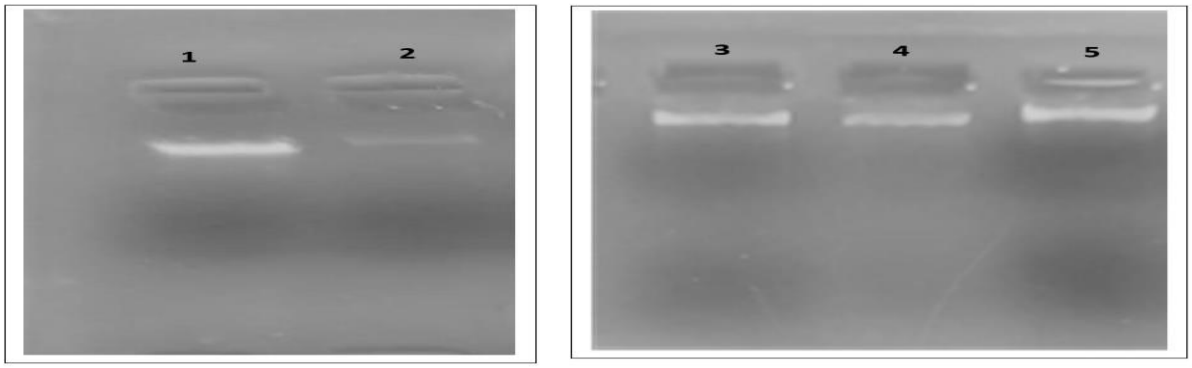


Fig . 7. Electrophoresis of genomic DNA on agarose gel at concentration 1% demonstrates the ability of cucurbitacin E as DNA protection

Table 5. Exhibit the protection percentage of cucurbitacin E and the concentration of DNA at various cucurbitacin E concentrations (0.25 ,0.5, and 1mg)

Band No.	Protection %	DNA concentration ng/µl
1	100	10 ng
2	ND*	ND*
3	87.52 concentration 1mg/ml	8.75
4	44.92concentration 0.25 mg /ml	4.49
5	72.21concentration 0.5 mg/ml	7.22

***ND= Not detected**

The result showed in table (5) that cucurbitacin E which belongs to triterpenoid groups has DNA protection activity in three concentrations (1 ,0.5 and 0.25) mg /ml in dose dependent manner to demonstrate the effect in this assay after ionizing radiation which agree with [35]. Ionizing radiations have the ability to harm DNA and cellular membranes and can have a detrimental effect on live cells[15]. also in the biological system, Radiation is a widely recognized genotoxic agent that can cause base modification and oxidative DNA damage by causing fatty acid peroxidation inside the phospholipid structure of the membrane, UVB-induced reactive oxygen species (ROS) harm cell membranes[16].

3.5. Hemolysis assay for human red blood cells

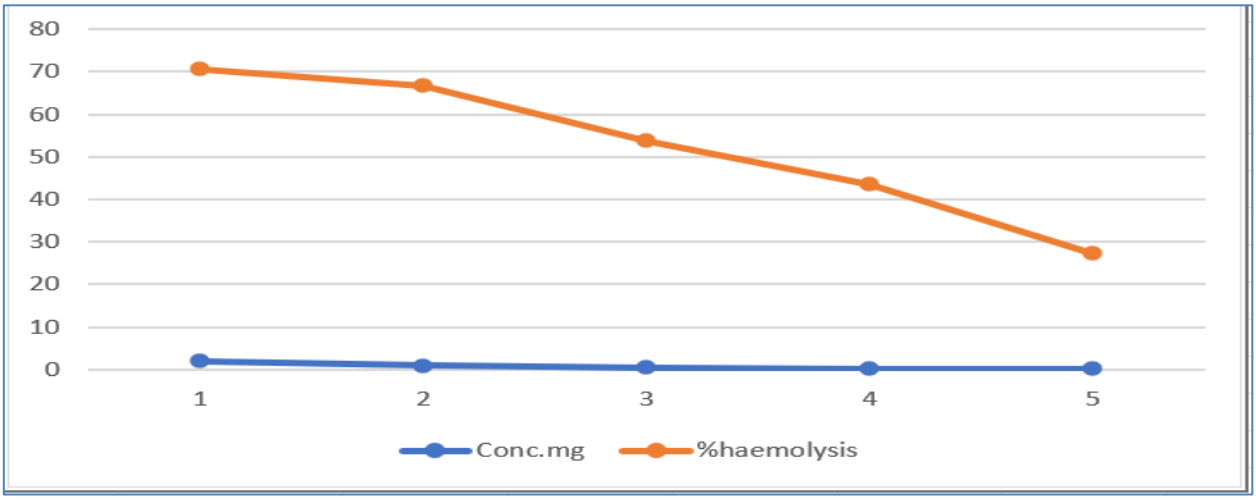


Fig.8. Hemolysis result of cucurbitacin E in number from(1 to 5) mean different concentration from (2mg, 1mg, 0.5 mg, 025 mg. and 0125 mg).

Red blood cells (erythrocytes) are ruptured or destroyed in the process known as hemolysis. Chemical substances, poisons, or mechanical stress are all possible causes. Cucurbitacin E is known as cytotoxic substance used in cancer treatment so the need of toxicological evaluation to ensure patient safety[19]. Understanding the percentage that cuc, E cause hemolysis and interact with red blood cells is essential for assessing its potential hemolytic effects. The result from Table 6 and figure (^) illustrates that there is an increase in hemolysis when increasing the concentration in comparison to negative control (0% lysis) and positive control by H2O2 which cause distraction (100% lysis).

Table 6. the heamolysis % of cucurbitacin E according to the concentration

Concentration for cucurbitacin E	% Hemolysis for cucurbitacin E
Negative control	0
Positive control SDS	100
2 mg/ml	68.69806094
1 mg/ml	65.78947368
0.5 mg /ml	53.32409972
0.25 mg /ml	43.35180055
0.125 mg /ml	27.1468144

Cucurbitacin has therapeutic promise but may cause toxicity in non-cancerous cells at high doses[36] . in other hand study the toxicity *in vivo* is important *in vivo* hemolysis assay for mice to cucurbitacin isolated from *Apodanthera congestiflora* which belong to Cucurbitaceae family the result was the complete blood count of the animals' peripheral blood revealed no changes in hematological parameters, supporting the data obtained in vitro in the hemolysis test[37].Also agree with same study for *C. colosynthis* extract were examined for cytotoxicity in vivo, and the findings showed that even at 400 mg/kg, the liver cells had mild morphological alterations, such as ballooning, lipid droplets, and a tiny buildup of extracellular matrix. [7]

4. Conclusion

According to this study, the good source of cucurbitacin E is *citrullus colosynthis*. Study of biological properties like antibacterial, antidiabetic cucurbitacin E and DNA protection effect shows that this plant possesses glucose reuptake also is a good source of antibacterial for gram positive bacteria more than gram negative bacteria and cucurbitacin E which belongs to triterpenoid groups has DNA protection activity in dose dependent manner although the use of its in high dose may cause toxicity in non-cancerous cells.

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Declaration of competing interest

The authors declare that they have no competing financial interest or personal relationship that could have appeared to influence the work reported in this paper.

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دراسة تأثيرات الحماية للحمض النووي و المضادة للبكتيرياو والمضادة للسكري لمركب الكوكيربيتاسين المستخرج من نباتالحنظل .العراقي مع دراسة سميته في المختبر

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معلومات البحث

الاستلام 3 شباط 2025
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القبول 15 اذار 2025
النشر 30 حزيران 2025

الكلمات المفتاحية

كوكوربيتاسينE ، مضاد للبكتيريا، مضاد للسكري، حماية من تلف الحمض النووى، تحلل كريات الدم

الملخص

لطالما تم استخدام العلاجات النباتية لأغراض طبية، وقد زاد استخدامها جنبًا إلى جنب مع الحضارة البشرية. نبات الحنظل هو عضو في عائلة القرعيات ويحتوي على العديد من المستقلبات الثانوية، أحدها هو الكوكوربيتاسين .E كشفت الدراسات عن أهمية الكوكوربيتياسين E بسبب خصائصه العلاجية المتنوعة مثل النشاط المضاد للبكتيريا والنشاط البيولوجي ضد البكتيريا، ودراسة النشاط المضاد للسكري والتحقيق في تأثير حماية الحمض النووي للكوكوربيتياسين E مع دراسة تأثيره على كريات الدم الحمراء البشرية لهذه النبتة ومستقلبها) الكوكوربيتياسين E لإظهار نشاطه في هذه الدراسة. تم استخراج ثمار الحنظل بطريقة التسخين المستمر باستخدام الكلوروفورم كمذيب، ثم تم الكشف عن الكوكوربيتاسين E باستخدام الكروماتوغرافيا السائلة عالية الأداء(HPLC) ، وطريقة الانتشار في الأطباق للخصائص المضادة للبكتيريا، وطريقة إعادة امتصاص الجلوكوز للنشاط المضاد للسكري، وتم استخدام الرحلان الكهربائي للهلام الأجاروزي لتقييم مدى تلف الحمض النووي، كما تم استخدام طريقة بينتو وآخرون (2012) لاختبار النشاط المسبب لانحلال الدم. تم التحقيق في نشاط حماية تلف الحمض النووي لكوكوربيتاكين E باستخدام الحمض النووي البشري. تُظهر النتائج النشاط المضاد للبكتيريا القيم للكوكوربيتاكين E الذي تم اكتشافه باستخدام العزلات البكتيرية، وكانت قيم MIC للكوكوربيتاكين E للعزلات البكتيرية (0.1، 0.25، 0.15) ملغ/مل لكل من) المكورات العنقودية الذهبية، كليبسيلا الرئويةو الباسيلس على التوالي. فيما يتعلق بتأثير مضاد السكري، تم دراسته باستخدام اختبار امتصاص الجلوكوز في المختبر حيث تم استخدام الميتفورمين كمعيار، وكانت النتائج أن قيم IC50 التي تم العثور عليها للكوكربيتاسينE ، المستخلص، والميتفورمين كانت 0.108، 0.313، و0.377 ملغ/مل على التوالي، وبعد الاستخراج الناجح، حدد اختبار حماية تلف الحمض النووي التركيزات المثلى (1، 0.5، 0.25) ملغ/مل التي أعطت حماية بنسبة 87.52%، 72.21%، و44.92% على التوالي، وتسبب في انحلال الدم لخلايا الدم الحمراء بشكل يعتمد على الجرعة.

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