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Identification of Cucurbitacin E Accumulated in Leaves and Fruits of the Iraqi *Citrullus colocynthis* During Two Seasons with Antioxidant Activity

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Abstract: The experiment was conducted by the Department of Pharmacognosy and Medicinal plants, College of Pharmacy, Basrah university during the agricultural season of 2023-2024, to investigate the content of cucurbitacin E in various portions of *Citrullus colosynthis* (leaves and fruits). The hot continuous method by reflux apparatus was used to extract the leaves and fruits of plant with different solvents, including chloroform and hexane. Cucurbitacin E is identified by thin-layer chromatography (TLC). The concentration of cucurbitacin was quantified once via high-performance liquid chromatography (HPLC). The antioxidant activity of the chloroform extract and cucurbitacin E was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method, with vitamin C serving as the standard. In summer, the chloroform extract of leaves exhibits a higher concentration than the other extracts (11. µg ml^{-1}). However, in winter, the chloroform extract of fruits has a greater concentration (20 µg ml^{-1}). The hexane extract is not advisable for two seasons. The findings indicated a non- significant difference between the antioxidant efficacy of chloroform extract and cucurbitacin E, as assessed by the DPPH radical scavenging activity, with IC50 values of 0.75, 1.52, and 1.61 for vitamin C, chloroform extract, and cucurbitacin E, respectively.

Keywords: antioxidant activity., *Citrullus colocynthis*, cucurbitacin E, fruits part, leaves part, seasons.

Introduction

The rising interest in traditional remedies, prompted by a greater awareness of the negative effects of synthetic drugs, has resulted in an uptick in ethnomedical research (Fallah-Huseini *et al*., 2023). Herbal medications address several ailments, leading to enhanced quality of life. This has led to a heightened demand for natural resources (Krishnaprabu, 2020). *Citrullus colocynthis* is known in Arabic as handhal, in English as

bitter apple or bitter cucumber, in German as koloquin, and in Indian as tumba (Cheng *et al*., 2023) The plant is a member of the Cucurbitaceae family, which encompasses 960 species over 125 genera, including both domestic and wild varieties (Chanda *et al*. 2020).

C. colocynthis is an angular, perennial, wild, herbaceous vine characterized by rough texture and lobular tendrils, capable of enduring various environmental conditions. The leaves possess elongated petioles, a green, hairy top surface, and a lighter-hued under surface. Monoecious flowers are characterized by five-lobed corollas and five-parted calyxes, appearing solitary, golden, and sub compounded. Each plant produces 15 to 30 globoid, smooth-surfaced, indehiscent fruits with a diameter ranging from 5 to 7.5 cm. The green and yellow stripes on the fruits transition to yellow as they ripen and develop. The little, brownish seeds possess a diameter of around 6 mm. (Kapoor *et al*., 2021). Cucurbitacin is one of the key chemicals in this family, and it extensively found in Cucurbitaceae family plants (Seong, 2015).

The plant is rich in nutrients that significantly enhance general health. C. colocynthis is a perennial plant exhibiting numerous biological properties, such as antioxidative, hypoglycemic, antibacterial, anti-cancerous, anti-inflammatory, analgesic, reproductive, protective, antimicrobial, antidiabetic, hypolipidemic, antineoplastic, profibrinolytic, anti-allergic, and pesticidal effects (Rao & Poonia, 2023). The biological effects in this family originated from active components, including cucurbitacin, glycosides, alkaloids, fatty acids, flavonoids, and essential oils. (Khan *et al*., 2023). Among these molecules, chemicals derived from secondary metabolism, notably phenolic compounds, are significant in oxidative stress resistance since they function as antioxidants (Chaves *et al*., 2020). Cucurbitacin is an efficacious antioxidant that may neutralize free radicals, including hydroxyl radicals, superoxide anions, and singlet oxygen. It can completely inhibit lipid peroxidation and oxidation (Rao & Poonia, 2023). Cucurbitacin types E and B are present in plants throughout development and generate additional naturally occurring cucurbitacin types, such as D and I, via secondary metabolism or other processes (Delgado-Tiburcio *et al*., 2022; Jing *et al*., 2020). However, cucurbitacin B can be reduced to create cucurbitacin E (Jing *et al*., 2020). Cucurbitacin-E (cuc. E) has wide interest due to its biological activities, and it a highly oxygenated triterpene (Abdelkhalek *et al*., 2017). Figure (1) shows the structure and the functional group of cucurbitacin E (Ramezani *et al*., 2021).

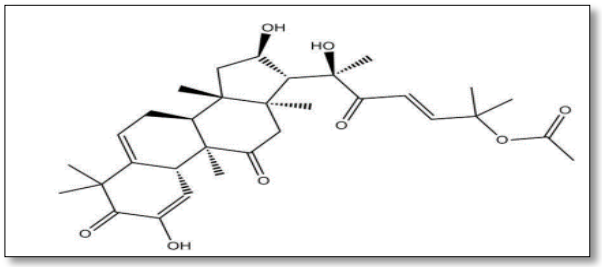


Fig. (1) chemical structure of cucurbitacin E

Examining the influence of climate on plants is essential since several climatic conditions which recognized in figure (2) affect the components and biological processes of plant growth (Hegazi *et al.*, 2024). It is widely recognized that assemblages of diverse plant species gathered throughout the year exhibited significant disparities in their chemical makeup, resulting in variations in their pharmacological properties (Al-Nablsi *et al*., 2022; Devendra *et al*., 2012).

The plant parts, developmental stage, and extraction and separation solvents all have a

direct impact on the quality, amount, and biological activity of these phytochemicals (Wakeel *et al*., 2019). Solvent type and polarity can affect the extract quality, quantity, extraction velocity, inhibitory compounds, toxicity, other biological activity, and biosafety.

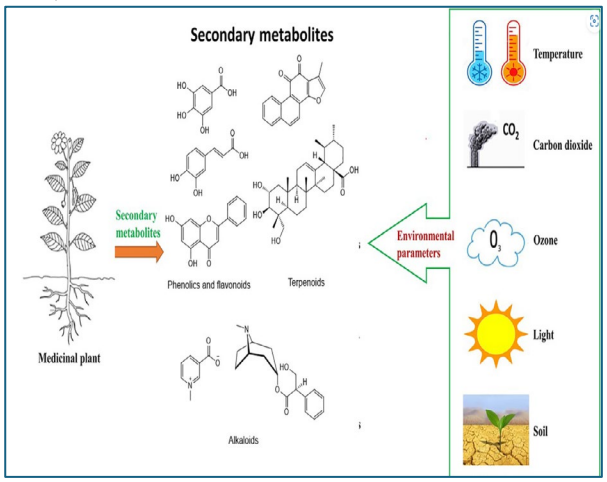


Fig. (2) climatic condition effect secondary metabolite (Pant *et al*., 2021)

The total secondary metabolites and their antioxidant capacity greatly depend on the solvent and plant part used for extraction (Zhang *et al*., 2019).

The use of suitable solvent in extraction of a plant is essential for the purification and isolation of active constituent that needs simple, rapid, economical, and efficient method to simultaneously isolate, and separate the compound for example 140ucurbittacin soluble in ethyl acetate, methanol, and chloroform but slightly soluble in water (Tosun & Baysar 2019; Panda *et al*., 2018).

Since cucurbitacin E is so important, the current study aims to analyze the variations in its concentration in the leaves and fruits of the C. colocynthis plant in relation to the various solvent systems used and the influence of the climate during the summer and winter seasons. The antioxidant capacities of the plant were examined in the experiment, and the concentrations were quantified using HPLC analytical methods.

Materials & Methods

Sample collection:

An extensive survey was undertaken in August 2023 in Basrah (Safwan at longitude ,47.7193r856 and latitude ,30.1097159) for *C. colosynthis* perennial, wild, herbaceous gather leaves and fruits were collected in two seasons, identified the plants based on their shape and fruit morphology according to Iraqi flora, table (1) recognized the region and time and weather at sampling.

Table (1) region and time of plant sampling

Plant part	Time of collection	Region	Temperature and humidity
Leaves collected in summer	August-2023	Basrah-Safwan	49 °C /45%
Fruit collected in summer	September -2023	Basrah-Safwan	47°C /42%
Fruits and Leaves in winter	February - 2024	Basrah-Safwan	12°C /19%

Chemical and materials

Chloroform and n hexane as a solvent for extraction, Triphenyl tetrazolium chloride for cucurbitacin test, thin silica gel plates, Methanol: water as 55:45 for thin layer chromatography, isopropanol and Methanol 50:50 for high performance liquid chromatography,2 ,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH), vitamin C, and cucurbitacin E as standard

Extraction method

Extraction was conducted by refluxing 10g of plant leaves with 200 ml of chloroform for 8 hours utilizing a continuous hot method (by using a reflux apparatus). Subsequently, a further 10 grams were extracted using 200 ml of hexane utilizing a reflux apparatus for 8 hours. The same method was carried out on fruits (Khan *et al*., 2023).

The experiment included four samples

sample1 = fruits extracted by chloroform

sample2 = leaves extracted by chloroform.

sample3 =fruits extracted by hexane

sample4 =leaves extracted by hexane.

cucurbitacin test

Triphenyl tetrazolium chloride is a reagent used as an indicator of the presence of cucurbitacin (Kaushik *et al*., 2015). Preparation of the reagent by taking 1g of triphenyl tetrazolium chloride dissolved in the distilled water to make 100 ml (the PH of solution should be around 7byadding 0.1 molar NaOH) (Matter & Ayad 2022).

Thin layer chromatography qualitative analysis (TLC):

Thin silica gel plates measuring 20 × 20 cm and 0.25 mm thick were dried and subsequently spotted with chloroform and hexane extracts containing cucurbitacin E as a standard. Various solvent solutions were selected from the table (2) below for the detection of cucurbitacin substances and the analysis of the retardation factor (Rf) in the mobile phase (Kaushik *et al*., 2015).

Table (2): Mobile phase for TLC detection for cucurbitacin (Kaushik, 2015)**.**

Mobile phase	solvent ratio	Visualization
Toluene: ethyl acetate	40:60	Vanillin/orthophosphoric acid
Chloroform: ethanol	95:5	Vanillin phosphoric acid reagent
Methanol: water	55:45	UV 254 nm
EtOAc-C6H6	75:25	Vanillin-orthophosphate in EtOH
Ether: Hexane: methanol	70:30:5	UV 254
Chloroform: methanol	95:5	UV 254

***In Vitro* free radical scavenging activity evaluation of cucurbitacin E and chloroform extract's antioxidant properties using the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity:**

The antioxidant activity of cucurbitacin E (standard substance) and *C. colosynthis* extracted by chloroform was measured by their capacity to scavenge the radical DPPH in comparison with vitamin -C as standard (Sharma & Singh, 2012).

Solutions used in the test:

1- DPPH solution: A chemical balance was used to measure 7.89 mg of DPPH, which was then dissolved in 100 ml of 99.5% ethanol and left in the dark for two hours to create the DPPH solution (Xiao *et al*., 2020).

2- Vitamin-C Solution: To make a stock solution with a concentration of 2mg /ml 10mg of vitamin -c powder in 5ml water then titration was done to obtain 0.25, 0.5, 1.0 and 2 mg ml^{-1}

3- cucurbitacin E solution: the stock solution was made (2 mg ml^{-1}) to obtain the same concentration of vitamin C 0.25, 0.5, 1.0 and 2 mg ml^{-1}

4- plant extracted by chloroform: stock solution was made (2mg/ml) to obtain the same concentration of vitamin C 0.25, 0.5, 1.0 and 2 mg ml^{-1}

The mechanism of these *in vitro* studies depends on the ability of the substance to scavenge radicals was assessed by using the 2,2 diphenyl 1 picrylhydrazyl (DPPH) method DPPH, which is dependent on the colorimetric shift (from deep violet to bright yellow)

Statistical Analysis

The data were evaluated using the SPSS program by one-way ANOVAs. A p-value of less than or equal to 0.05 was considered statistically significant for plant extract, cucurbitacin E, and vitamin C as standard in different concentrations for antioxidant experiments

High performance liquid chromatography analysis (HPLC)

A Germany Sykama type HPLC was used to perform quantitative determination of the cucurbitacin E.

The gradient elution mobile phase was a mixture of 50:50 (methanol: isopropanol)

1- flow rate 1 ml min^{-1}.
2- U.V. detection at 237nm
3- temperature 30 º C.

So, the Sample injection was 10 µl, and the analysis was performed at a flow rate of 1 ml min^{-1} for 20 min; detection was conducted at 237 nm.

Preparing solution for standard with a concentration of 1 mg ml^{-1} of cucurbitacin E was prepared by dissolving around 1mg of it in a mixture of 0.5 mL methanol and isopropanol (Chanda *et al*., 2020)

Result & discussion

cucurbitacin Test

After the extraction of fruits and leaves, the preliminary phytochemical analysis indicated that the chloroform extract of *C. colosynthis* fruits and leaves yielded a positive result for tri triphenyl tetrazolium chloride, a reagent employed to identify cucurbitacin; the mechanism by which positive result obtained a red precipitate of formazan formation (Kaushik *et al*., 2015) due to reduction reaction as investigate in figure (3) After triphenyl-tetrazolium chloride (TTC) is reduced, an organic molecule known as triphenyl formazan (TPF) is produced (Shawali & Samy, 2015).

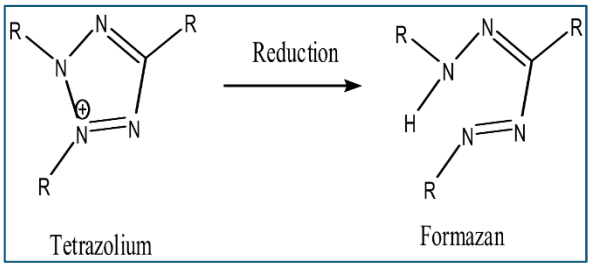


Fig. (3): Reduction of tetrazolium salt

Conversely, the hexane extract from these samples was ambiguous because the low concentration of cucurbitacin E was obtained in plants extracted by hexane (non-polar) This is the type of solvents employed for the extraction affects the yield and contents of secondary metabolites since different solvents have different polarity with different physicochemical characteristics (Khan *et al*., 2023). At normal temperatures, cucurbitacins are typically crystalline substances, the majority of them are highly soluble in petroleum ether, chloroform, benzene, ethyl acetate, methanol, and ethanol but very weakly soluble in water (Tosun & Baysar, 2019). This observation was conducted across two seasons,

as below in Table (3). Ared precipitate of formazan indicates the presence of Cucurbitacin.

Table (3): Result of tri triphenyl tetrazolium chloride reagent

Plant part	Chloroform extract	Hexane extract
Leaves	Strong (+)	(-)/Weakly (+) after24hr
Fruits	Strong (+)	(-)

Thin layer chromatography (TLC) qualitative analysis:

The visualization of cucurbitacin E spots on silica gel was accomplished by spraying samples with a spraying stander of cucurbitacin E to help the location and characterization of the compound spots on chromatogram at wavelength 254 nm. The Rf value of cucurbitacin E using different eluents appeared by using mobile phase methanol: water as 45:55 which gives good spot with the stander which is recognized in figure (4) and table (4)

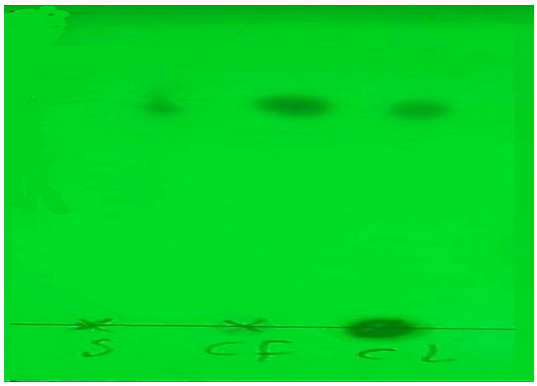


Fig. (4) TLC chromatography S standard of cucurbitacin E, CL leaves extracted by chloroform, CF fruit extracted by chloroform.

The presence of one spot with the same Rf value as compared with cucurbitacin E standard for chloroform extract (sample 1) indicate good purification for cucurbitacin E by using chloroform as solvent these results because the solubility of most cucurbitacins in chloroform make it is good solvent for extraction of cucurbitacin E and the value of RF value agree with (Ponsankar *et al*., 2020).

Table (4): Cucurbitacin E standard and chloroform extract of Citrullus leaves in T.L.C. Solvent system.

Solvent system	Rf Value of standard	Rf Value of sample 1
Methanol: water at 55:45	0.63	0.63

***In Vitro* free radical scavenging activity**

A stable nitrogen-centered free radical known as DPPH is commonly employed to evaluate the radical-scavenging ability of compounds or plant extracts. Colorimetric measurements indicated that the violet hue of the DPPH radical diminished to a yellow tint in the Diphenyl picrylhydrazine radical upon the stable DPPH radical accepting an electron from the antioxidant compound (Gulcin & Alwasel, 2023). The scavenging action of the plant extract by chloroform and cucurbitacin E is seen in (Figure 5 All extracts and cucurbitacin E exhibited variable levels of scavenging activity about vitamin C at concentrations ranging from 2 to 0.25 mg ml^{-1}, with IC50 values for vitamin C, chloroform extract, and cucurbitacin E determined to be 0.75, 1.52, and 1.61 mg ml^{-1}. Upon comparison of the IC50 values for the chloroform extract of *Citrullus colocynthis* and cucurbitacin E, it is noteworthy that the results were similar. This may be attributed to two factors: the extract's activity exhibited for cucurbitacin E and the extraction method's specificity for the purification of cucurbitacin E. This observation aligns with the findings of E. Tosun *et al*. (2019) which indicate that chloroform is an optimal solvent for the isolation and purification of cucurbitacin

(Tosun & Baysar 2019). The scavenging activity of the extracts was assessed in the following order: vitamin C > chloroform extract > cucurbitacin E, with vitamin C concentrations ranging from 2 to 0.25 mg ml^{-1}as the standard. The IC50 value was determined to be 0.75 mg ml^{-1}. The standard and all samples exhibited suppression of DPPH in a dose-dependent manner. Cucurbits produce cucurbitacin to defend against illnesses and insect pests. They are produced in response to environmental stressors, such as ultraviolet radiation. They may serve as antioxidant molecules to mitigate cell damage induced by reactive oxygen species (ROS), including free radicals like O2, H2O2, OH^{-}, and 1O2. Moreover, the accumulation of cucurbitacin has been associated with increased drought tolerance (Mkhize *et al.*, 2023). Statistically a non- significant difference among vitamin C, cucurbitacin E, and chloroform extract, as the P value was 0.145.

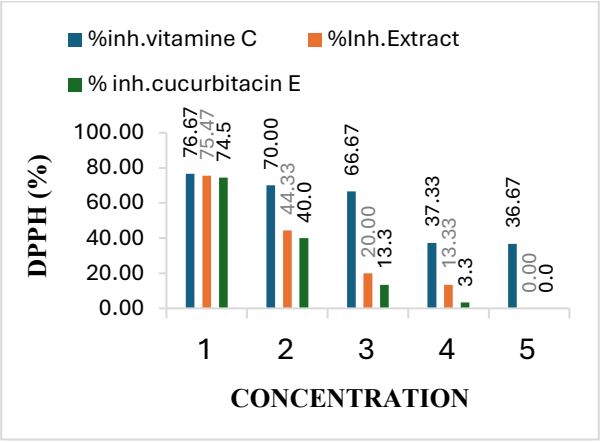


Fig. (5): DPPH radical scavenging activity of chloroform extract for *Citrullus colocynthis*, cucurbitacin E and vitamin C at different concentrations from (2mg to 0.25mg).

High-performance liquid chromatography (HPLC)result

The analysis of cucurbitacin E was conducted in Figure (6) and Table (5) using the cucurbitacin E standard peak, which was observed at 2.69 minutes. During the summer season, the chloroform leaf extract exhibited a pronounced peak and a greater area under the curve compared to the fruit, while the hexane extract displayed the lowest area under the curve for both fruits and leaves.

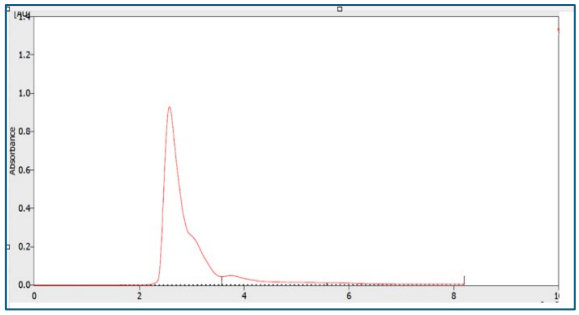


Fig. (6) for cucurbitacin E standard 1mg in 0.5ml methanol and isopropanol

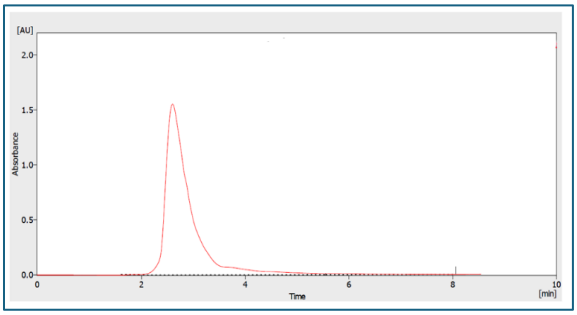


Fig. (7) leaves extract by chloroform (sample2)

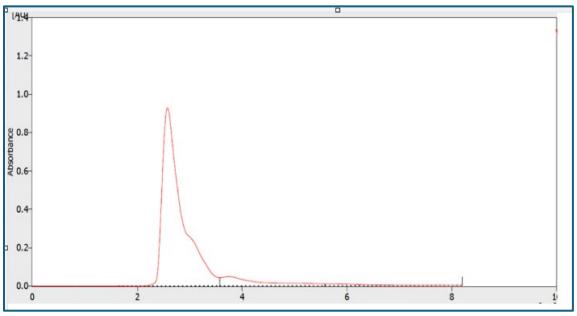


Fig. (8) fruits extracted by chloroform (sample1)

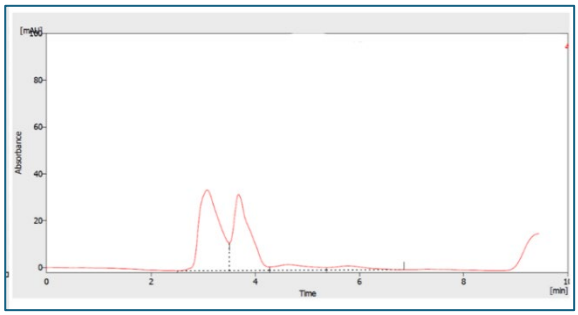


Fig. (9) fruits extracted by hexane (sample3)

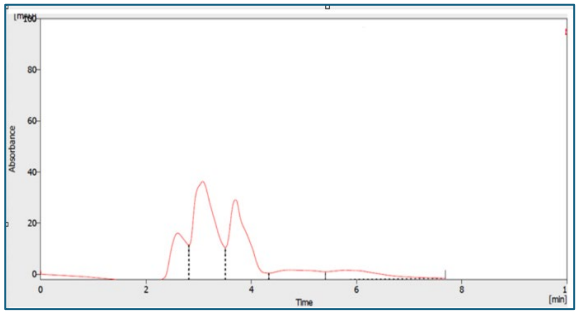


Fig. (10) extracted by hexane (Sample 4)

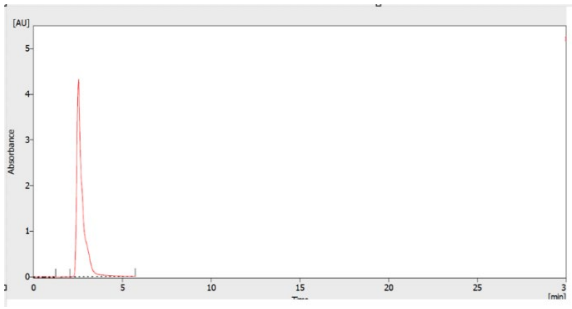
Table (5) and Figure (11) provide a summary of the findings, showing that the mount of cucurbitacin E in leaves chloroform extract is higher than in other kinds in the summer.

Table (5): summarizes the result of cucurbitacin concentration in summer.

Sample	Retention time Minutes	Retention time of standard Minutes	Concentration Of cucurbitacin µg ml^{-1}
1	2.573	2.693	5.333
2	2.6	2.693	11.06
3	3.077	2.693	0.207
4	2.6	2.693	0.0856

Table (5) and Figure (11) provide a summary of the findings, showing that the amount of cucurbitacin E in leaves chloroform extract is higher than in other kinds in the summer.

In winter, the chloroform extract of fruits exhibited the most pronounced peak with the greatest area under the curve. In contrast, the chloroform extract of leaves showed a lesser peak. Conversely, the hexane extracts of both



fruits and leaves were minimal in quantity

Fig. (12): fruits extracted in winter by chloroform (sample1)

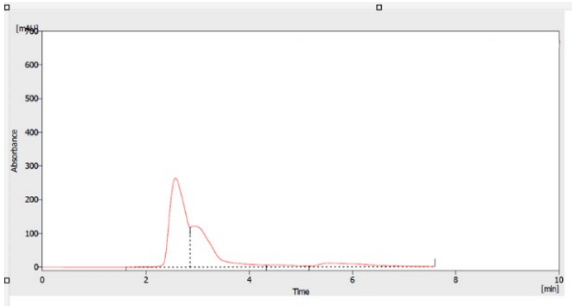


Fig. (13): leaves extracted in winter by chloroform (sample 2)

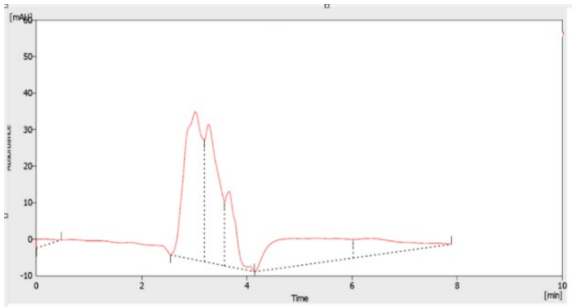


Fig. (14) sample (3)

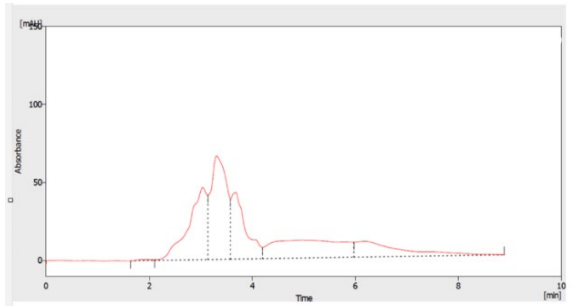


Fig. (15): sample (4)

The results are summarized in (Table 6 and Figure 16), indicating that the concentration of cucurbitacin E, calculated based on the area under the curve, is greater in the chloroform extract of fruits compared to other varieties during winter.

Table (6): concentration of cucurbitacin E in winter.

Sample	Retention time Min.	Retention time of standard MIN.	Concentration Of cucurbitacin µg ml^{-1}
1	2.55	2.693	20
2	2.57	2.693	1.1
3	3.02	2.693	0.212
4	3.03	2.693	0.252

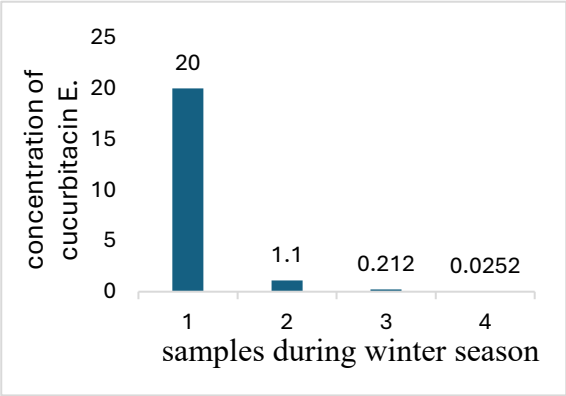


Fig. (16): chart table for samples percentage in winter.

Numerous studies have demonstrated that different parts of the plant possess varying levels of cucurbitacin. Regarding the parts of plants, fruits and roots have higher levels of cucurbitacin compared to leaves and stems (Kim *et al.,* 2018). Stems have a significant role in the circulation of cucurbitacin, but only fruits are associated with storage, despite this, the leaves' cucurbitacin content was low these agree with (Devendra *et al*., 2012).

The HPLC analysis revealed that the highest concentration of cucurbitacin E was present in the chloroform fruit extract (20 µg ml^{-1}) in winter and in the chloroform leaf extract (11.0620 µg ml^{-1}) in summer.

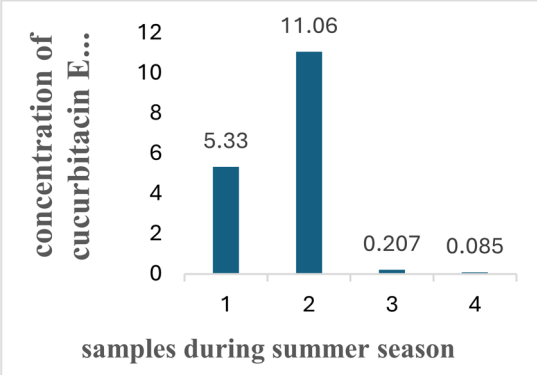


Fig. (11) chart table for samples percentage in summer

The cucurbitacin concentration in the fruit was determined to be fourty times higher than that in the leaves since storage is primarily linked with the fruit, within the same family as *C. colosynthis*, *Ecballium elaterium* (L.) A. Rich. is a minor crop used in the study (Jafargholizadeh *et al*., 2016).

Additionally, multiple extraction procedures utilizing different solvents were examined to identify the most efficient ways for optimizing cucurbitacin recovery from the different plant parts (Patel *et al.*, 2020). cucurbitacin E is a moderate polar substance that is extracted by using solvents such as methanol. The solubility of most cucurbitacin is notable in moderate polar solvents like dissolve such as chloroform, leading to their high concentration in extracts using chloroform as a solvent (Kaushik *et al*., 2015; Patel *et al*., 2020).

Secondary metabolites of plants (SMS) are significantly impacted by several environmental factors, including soil fertility, moisture content, light, warmth, and salt (Khalaf, 2024). Even when other parameters remain constant, modifications to one of these components can affect the amount of S.M.s

present in the majority of plants. (Yang *et al*., 2018**;** Maja *et al*., 2022).

Temperature fluctuations affect plant development and the metabolic pathways governing signaling, physiological regulation, and defense systems. Significant climatic parameters, like temperature, can profoundly influence the composition of S.M.s by disrupting photosynthesis and reducing their vulnerability to stressors Seasonal fluctuations significantly influence the majority of the plant's chemical contents.

The elevated temperature and increased transpiration rates during summer resulted in a reduction in water content in *C. colocynthis* (Salama *et al*., 2017) so the content of cucurbitacin E in winter was more than in the summer season all these factors were summarized in figure (17).

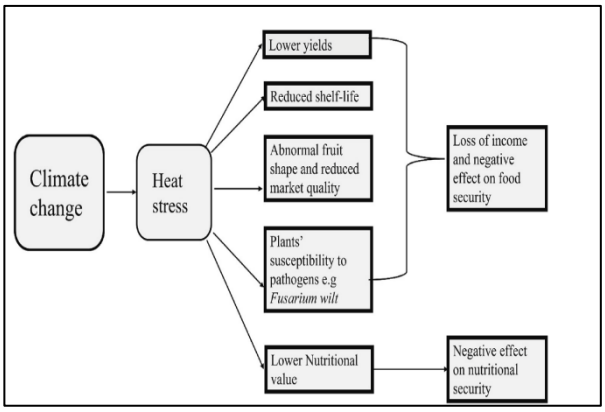


Fig. (17): An overview of the effect of climate change on Cucurbitaceae plants (Olarewaju *et al*., 2023)**.**

In Basrah, summer temperatures might reach 52 ºC, while winter temperatures may drop to 8 ºC, these significant fluctuations in weather impact secondary metabolites. Excessive quantities may result in diminished efficacy, causing researchers to misjudge the use of plants as conventional pharmaceuticals. Perennial herbs should be assessed for the ideal harvesting season depending on their commercial and/or medicinal properties (Soni *et al*., 2015).

Conclusion

Climate change has negatively affected food supply and abundance, which depends significantly on favorable weather conditions. Different plant parts that have been extracted using various solvents with varying polarity indexes have varying amounts of secondary metabolites .This article examines how plant secondary metabolite (cucurbitacin E)content respond to environmental challenges through various biochemical mechanisms by comparing the amount of cucurbitacin E in different tissue of *C.colosynthis* plant and the type of solvent that is used for extraction and it's antioxidant activity due to cucurbitacin E is of great interest has wide range of biological activities, finally the result was fruits parts which extracted by chloroform in winter season had a highly content of cucurbitacin E followed by leaves part extracted by chloroform in summer season and cucurbitacin E, C.colosynthis extract both have antioxidant activity.

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H.E.M.: Resources, Methodology, Investigation, Formal Analysis, Writing original draft

U.M.N. Supervision Visualization, investigation, review and editing

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Conflicts of interest

The authors declare no conflict of interest

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تحديد تراكم الكوكربيتاسين نوع (E) في أوراق وثمار نبات الحنظل العراقي *Citrullus colocynthis* خلال موسمين مع دراسته كمضاد للأكسدة

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المستخلص :تم إجراء التجربة من قبل قسم العقاقير والنباتات الطبية، كلية الصيدلة، جامعة البصرة خلال الموسم الزراعي 2023-2024، للتحقيق في محتوى الكوكوربيتياسين E في أجزاء مختلفة من نبات الحنظل تم استخدام الطريقة الساخنة المستمرة بواسطة جهاز التقطير لاستخراج أوراق وثمار النبات باستخدام مذيبات مختلفة، بما في ذلك الكلوروفورم والهكسان. يتم تحديد كوكيربيتاسين E بواسطة الكروماتوغرافيا الطبقية الرقيقة. تم قياس تركيز الكوكوربيتاسين مرة واحدة باستخدام الكروماتوغرافيا السائلة عالية الأداء. تم تقييم النشاط المضاد للأكسدة لمستخلص الكلوروفورم والكوكيربيتاسين E باستخدام طريقة التقاط الجذور الحرة مع استخدام فيتامين سي كمعيار. في الصيف، يظهر مستخلص الكلوروفورم من الأوراق تركيزًا أعلى من المستخلصات الأخرى (11. ميكروغرام مل-1). ومع ذلك، في الشتاء، يحتوي مستخلص الكلوروفورم من الفواكه على تركيز أكبر (20 ميكروغرام مل-1). استخراج الهكسان غير مستحسن لموسمين. أشارت النتائج إلى عدم وجود فرق ذو دلالة إحصائية بين فعالية مضادات الأكسدة لمستخلص الكلوروفورم والكوكيربيتاسين E كما تم تقييمه من خلال نشاط امتصاص الجذور الحرة، بقيم IC50 تبلغ 0.75 و1.52 و1.61 لفيتامين سي ومستخلص الكلوروفورم، والكوكوربيتاكين E على التوالي.

الكلمات المفتاحية: النشاط المضاد للأكسدة، نبات الحنظل، الكوكيربيتاسين نوع ي، جزء الفاكهة، جزء الأوراق، الفصول