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Comparative Some Phytochemical Components of Both Aqueous and Ethanol Extracts of Libyan Citrullus Colocynthis

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مقارنة بعض المكونات الكيميائية النباتية للمستخلصين المائي والإيثانولي لنبات الحنظل الليبي *Citrullus colocynthis*

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Abstract:
This study's objectives were to perform atomic absorption spectroscopy (AAS) and a variety of phytochemical assays on plant extracts in the lab. Aqueous and alcoholic extracts are made by soaking plant fruit powder in distilled water and ethanol for 72 hours, then filtering the resulting extracts. Fruit samples of the Citrullus colocynthis plant were gathered from the Dawoon region of Libya and prepared for study. They were first cleaned with tap water, then distilled water, dried in a drying oven, ground, sifted, and stored in tight, opaque cans. In order to detect the presence of various phytochemicals, a variety of chemicals have been prepared for the aqueous and alcoholic extract of the fruits of the C. colocynthis plant. A qualitative analysis using standard methods has revealed the presence of proteins and amino acids, alkaloids, flavonoids, phenols, tannins, carbohydrates, cardiac glycosides, alkaloids, flavonoids, and steroids. After weighing and burning the fruits using conventional techniques, the finely crushed plant material is used to make ash. Atomic absorption spectroscopy (AAS) is used to identify some minerals and estimate the amount of heavy elements present in the ashe of plant specimens (fruits). They are minerals magnesium. Zinc, nickel, lead and cobalt (Mg, Zn, Ni, Pb, Co) respectively. Alkaloids, phenols, tannins, flavonoids, proteins and amino acids, glycosides, saponins, carbohydrates, and steroids were all found in abundance in the in vitro phytochemical examination for qualitative assessment, with the exception of Phlobatannins, which are absent from fruits, and atomic absorption analysis confirmed the abundant presence of Mg, and the presence of Coin trace amounts, while heavy metals such as Pb, Ni and Zn were below the limit of detection.

Keywords: Citrullus colocynthis plant, Phytochemical, Medicinal Plants, phytochemical screening.

الملخص:
هدفت هذه الدراسة إلى إجراء التحليل الطيفي للامتصاص الذري (AAS) بالإضافة الى اجراء مجموعة واسعة من الاختبارات الكيميائية النباتية على مستخلصات النبات مخبرياً. تم تحضير المستخلصين المائي والكحولي عن طريق نقع مسحوق ثمار النبات في الماء المقطر والإيثانول لمدة 72 ساعة، تلي ذلك ترشيح المستخلصات الناتجة، جُمعت عينات ثمار نبات الحنظل من منطقة الداوون في ليبيا وأُعدت للدراسة؛ حيث نُظفت أولاً بماء الصنبور ثم بالماء المقطر، ثُم جُففت في فرن التجفيف، وطُحنت، ونخلت، وحُفظت في علب معتمة ومحكمة الغلق .وللكشف عن وجود المركبات الكيميائية النباتية المختلفة، جُهزت محاليل كيميائية متعددة للمستخلصين المائي والكحولي لثمارالنبات. وقد كشف التحليل النوعي باستخدام الطرق القياسية عن وجود: البروتينات والأحماض الأمينية، القلويات، الفلافونويد، الفينولات، التانينات، الكربوهيدرات، الجليكوسيدات القلبية، والستيرويدات. وبعد وزن وحرق الثمار باستخدام التقنيات التقليدية، استُخدمت المادة النباتية المسحوقة لتحضير الرماد. واستُخدم التحليل الطيفي للامتصاص الذري (AAS) للتعرف على بعض المعادن وتقدير كمية العناصر الثقيلة الموجودة في رماد الثمار، وهي على التوالي: المغنيسيوم، والزنك، والنيكل، والرصاص، والكوبالت (Mg, Zn, Ni, Pb, Co). أظهر الفحص الكيميائي النباتي للتقييم النوعي وفرة كل من: القلويات، الفينولات، التانينات، الفلافونويد، البروتينات والأحماض الأمينية، الجليكوسيدات، الصابونين، الكربوهيدرات، والستيرويدات؛ باستثناء الفلوباتانينات التي غابت تماماً عن الثمار. وأكد تحليل الامتصاص الذري الوفرة العالية لعنصر المغنيسيوم(Mg) ، ووجود الكوبالت (Co) بكميات ضئيلة، بينما كانت المعادن الثقيلة مثل الرصاص(Pb) ، والنيكل(Ni) ، والزنك (Zn) دون حد الاكتشاف.
الكلمات المفتاحية: نبات الحنظل (Citrullus colocynthis)، المواد الكيميائية النباتية، النباتات الطبية، الفحص الكيميائي النبات.

Introduction:

Phytochemicals and natural substances such as tannins, terpenes, alkaloids, saponins, phenolic compounds, and flavonoids are abundant in medicinal plants. Researchers can uncover the constituents of plant extracts and pinpoint the predominant ones by doing phytochemical screening, which facilitates the hunt for bioactive substances with prospective applications as dietary supplements. Many scientists agreed that plants are important. A greater understanding of the significance of medicinal plants has emerged in recent years, and the plant kingdom provides a rich supply of possible therapeutic medications. There is growing interest in medicinal plants because they are easily accessible, affordable, safe, highly effective, and have few side effects. However, many people, especially those in rural areas, cannot afford the high cost of pharmaceutical drugs. Despite the lack of information regarding the safety and toxicity of plants, they have been widely used to cure and alleviate health issues in such circumstances (Ekor, 2014). Interestingly, there have been several reported incidents of herbal treatment toxicity in recent years, notwithstanding their effectiveness (Deng, 1994, July); O'Hara et al., 1998).

Nevertheless, despite their still incomplete knowledge of biological components, herbal medications are nevertheless frequently given because of their efficacy, negligible side effects, and affordability (Kumar et al., 2009).

Table (1): The Taxonomy of *C. colocynthis*: (Scientific classification)

Kingdom	**Plantae**
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Rosids
Order	Cucurbitales
Family	Cucurbitaceae
Genus	Citrullus
Species	C. colocynthis
Binomial name	Citrullus colocynthis (L.) Schrad.
Traditional Arabic and Indigenous Libyan names	Handal



Figure (1): Unripe and ripe fruit of *C. colocynthis* in field.

Materials and Methods:

Selection of fruit material:

The majority of the tribal people and villagers commonly employed *Citrullus colocynthis* to treat common illnesses. We selected the material as an experimental instrument based on that context. The bitter fruit of *Citrullus colocynthis Linn (C. colocynthis)* was carefully collected from the Dawoon area of Tarhuna, Libya. After that, the *C. colocynthis* fruit samples were moved to Elmergib University's Faculty of Science. The Department of Chemistry is located in town of Alkhoms. There, the samples underwent a laborious process of sorting and thorough cleaning. Before being handled with distilled water, they were first gently cleaned with tap water. The samples were then spread out in a protected location and carefully dried, making sure they were not exposed to any direct sunlight. To guarantee a uniform, efficient drying process, the samples were often agitated during the drying period. In the end, the drying process was successfully finished in an electric oven that kept the temperature at 40°C for three days.

Fruit material extraction process:

Using a maceration extraction process, the bitterness of the *C. colocynthis* plant was extracted both aqueous and alcoholic. The fruits weighed 6.02g, and a crudely powdered sample of the plant material was carefully gathered. After that, the sample was carefully put into the conical flask to make sure it fit perfectly inside the 500 ml capacity. Next, 100 milliliters of appropriate solvents (either 75% ethanol (Et-OH) or distilled water (aqus)).

The sample was then put on an orbital shaker that was set for 72 hours at 150 rpm.A vacuum rotary evaporator was used to filter and dry the material following the extraction time. It should be noted that the ethanol extract had a pleasing oily green color, but the aqueous extract looked brown.

Phytochemical Screening:

The test samples were subjected to phytochemical examination using established procedures (Shri me et al., 2011); (He FJ et al., 2007).

1. **Alkaloids Test:** a) Dragendroff 's test. b) Mayer's test.
2. **Test for Flavonoids:** a) Alkaline Reagent test. b) Lead Acetate test.
3. **Test for Tannins:** a) Ferric Chloride Solution (1%) test.
4. **Test for Phenols:** a) Ferric Chloride Solution (10%) test**.**
5. **Test for Steroids:** a) Salkowski test.
6. **Test for Cardiac glycosides:** Keller-Killiani test
7. **Test of Protein and Amino Acid:** a) Xanthoprotein test.
8. **Test of Carbohydrates:** a) Molisch's test b) Benedict's test.
9. **Test for Saponins:** a) foma test.

10. **Test for Phlobatannins:** a) HCl test

***Note*:** The results of the previous investigations are shown in Table 1.

Analysis by Atomic Absorption Spectroscopy (AAS):

The presence of some metals and heavy elements in plants has been detected through an analysis by AAS, and the results are displayed in Table 2. The sample of powdered plant was wet digested by adding 5 mL of 65% HNO_3 and then gently boiling the mixture for 30 to 45 minutes. After cooling, 2.5 mL of 70% $HClO_4$ was added, and the mixture was gently boiled until dense white fumes appeared. The mixture was then allowed to cool, and 10 mL of deionized water was added, followed by additional boiling until the fumes were completely released (Hseu, 2004).

Results and Discussion:

Preliminary phytochemical screening:

1. Phytochemical Results:

Table (1): Displays the results of the preliminary phytochemical analysis of aqueous and ethanol extracts of *C. colocynthis* fruits*:*

Components'	Fruits	
	Aqus	EtOH
Alkaloids	+++	++
Flavonoids	++++	+++
Tannins	++++	++++
Phenols	++++	+++
Steroids	++++	++++
Cardiac glycosides	++++	++++
Proteins and amino acids	++++	+++
Carbohydrates	+++	+++
Saponnins	+++	__
Phlobatannins	__	__

The results of the initial detection of metabolic products in the aqueous and alcoholic extracts of the *C. colocynthis* plant are displayed in Table 2. The results of the aqueous and alcoholic extracts of the fruits (alkaloids, flavonoids, tannins, phenols, steroid (terpenoids), Cardiac glycosides, proteins and amino acids, and carbohydrates) ranged from very good to excellent, while saponnins were abundant in aqueous extracts but absent from alcoholic extracts in the plant because they are polar compounds in water while their solubility in alcohol is low. Phlobatannins, on the other hand, were absent from both aqueous and alcoholic extracts in fruit of the plant and they may be present in other parts of the plant.

2. Results from Determination of some Metals and Determining Heavy Elements Content in Ash of The Plant:

Table (3): Results of showed the obtained from determination of some metals and determining heavy elements content in ash of the plant analysis by AAS (Atomic Absorption Spectroscopy):

Some minerals and Heavy Element Content	Conc. ppm				
	Mg	Zn	Ni	Pb	Co
Fruits	13.91	-*	-*	-*	0.02

Key: -* =Under the limit of detection, Conc. ppm= concentration parts per million.

The results of an analysis using AAS (Atomic Absorption Spectroscopy) to detect some minerals and heavy elements in the fruits of the *C. colocynthis* plant are displayed in Table 3. The table shows small abundance of the element Cobalt (Co) was below the sensitivity of detection, while the three heavy elements Lead (Pb), Zinc (Zn), and Nickel (Ni) had results below the limit of detection, while Magnesium (Mg) metal was found in abundance. The absence of lead indicated from a non-contaminated environment. And the biological importance of these elements is that:

- **Magnesium:** As magnesium has an important role in the human body and its deficiency leads to the clinical manifestations of hypomagnesemia may affect every system including neuromuscular, cardiovascular, renal, and gastrointestinal systems (Jahnen-Dechent & Ketteler, 2012) ;(Martin et al., 2009).
- **Zinc:** Where zinc has multiple functions, including: A sufficient zinc intake can help boost immunity and prevent respiratory infections of all kinds, as well as other common illnesses (Skalny et al., 2020); (Stefanidou et al., 2006). Zinc has anti-inflammatory and antimicrobial properties, which can help prevent or reduce the occurrence of acne, dermatitis and other skin-related conditions (Zou et al., 2023). Additionally, zinc is involved in the synthesis of collagen, which is essential for maintaining healthy skin (Molenda & Kolmas, 2023). An increasing body of research suggests that hyperglycemia and zinc metabolism are linked, and also that zinc supplementation could contribute to the improvement of glycemic control in diabetic patients (Wang et al., 2019).
- **Nickel:** Whereas accumulation of nickel and nickel compounds in the body through chronic exposure may be responsible for a variety of adverse effects on the health of human beings, such as lung fibrosis, kidney and cardiovascular diseases and cancer of the respiratory tract (McGregor et al., 2000); (Seilkop & Oller,2003).
- **Lead:** Whereas Lead the interesting point to include is that lead has been found to have no physiological role in the body (Woolf et al., 2007); (Rubin & Strayer, 2008). As lead disrupts the maintenance of the cell membrane, red blood cells with a damaged membrane become more fragile, which results in anemia (White et al, 2007).
- **Cobalt:** Where Cobalt has multiple functions, including: Cobalt is a necessary component of vitamin B12 (hydroxocobalamin) and a fundamental coenzyme of cell mitosis. Moreover, cobalt is very important for forming amino acids and some proteins to create myelin sheath in nerve cells (Ortega et al.,2009); (Battaglia et al., 2009). Cobalt also plays a role in creating neurotransmitters, which are indispensable for correct functioning of the organism (Battaglia et al., 2009). The salts of cobalt stimulate the synthesis of erythropoietin, which is the most important function in the activation of different stages of erythropoiesis, which, in turn, is connected with the formation of erythrocytes in bone marrow (Simonsen et al., 2012); (Simonsen et al., 2011).

Conclusion:

Citrullus colocynthis (L.) Schrad whole fruit extracts contain phytochemicals that show promise as a source of principles that could lead to new medications. Therefore, more research is recommended to determine their antimicrobial, antispasmodic, and anti-helminthic properties. Additionally, it will be fascinating to study the phytochemicals' separation using other solvents, purification, and characterization.

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