The North African Journal of Scientific Publishing (NAJSP)
مجلة شمال إفريقيا للنشر العلمي (NAJSP)
E-ISSN: 2959-4820
Volume 4, Issue 4, 2026
Page No: 228-234
Website: https://najsp.com/index.php/home/index
SJIFactor 2024: 5.49 معامل التأثير العربي 0.69 :2025 (AIF) ISI 2024: 0.696

Chemical Screening of Ethanol Extract of Apium graveolens (Celery) and Its Volatile Oil Contents

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المسح الكيميائي لخام الايثانول لنبات الكرفس والتركيب الكيميائي للزيت

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Received: August 27, 2025	Accepted: November 05, 2026	Published: November 20, 2026

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Abstract:
This study was conducted at University of Kordofan during 2020 - 2026. It aimed to identify the chemical composition of Apium graveolens (celery) a member of the Umbelliferae (Apiaceae) family- due to its nutritional significance, particularly among the Chadian population, also utilize it as a remedy for various digestive system disorders. Plant samples were collected from the banks of the Chari River and the central vegetable market in N'Djamena, Republic of Chad. Crude extracts were obtained using organic solvents: hexane, chloroform, and ethanol successively following the Harvard method. Through chemical titration, the medically active constituents within the crude ethanol extract were identified at the Department of Plant Science laboratory, Faculty of Science, University of Kordofan. Additionally, the volatile oil was extracted using a Soxhlet apparatus at the Quality Control Center laboratory. The fatty acid composition of the oil was determined using High-Performance Liquid Chromatography (HPLC) at Zaria University in Nigeria. A phytochemical analysis of the ethanolic extract of raw celery revealed the presence of several active compounds: flavonoids, alkaloids, phenols, saponins, carboxylic acids, coumarins, tannins, and glycosides in significant quantities, alongside smaller amounts of steroids and terpenes. No ricins were detected. HPLC analysis of the volatile oil identified a limited number of fatty acids, specifically palmitic acid, oleic acid, and stearic acid, which aligns with previous research indicating that celery has a low fat and carbohydrate content, making it suitable for weight-loss purposes. The study concludes that celery contains chemically active compounds with biological properties, including antioxidant and antibacterial effects, as well as potential benefits for managing high blood cholesterol. Consequently, the study recommends that individuals struggling with obesity or high blood cholesterol incorporate celery into their diets.

Keywords: chemical screening, ethanol extract, volatile oil, Apium graveolens, High-performance liquid chromatography (HPLC).

الملخص:

أُجريت هذه الدراسة بجامعة كردفان خلال الفترة من عام 2020م إلى عام 2026م، وهدفت إلى التعرف على التركيب الكيميائي لنبات الكرفس (Apium graveolens)، الذي ينتمي إلى الفصيلة الخيمية (Umbelliferae)، المعروفة علمياً باسم (Apiaceae)، وذلك نظراً لأهميته الغذائية، ولا سيما لدى السكان التشاديين، فضلاً عن استخدامه في علاج عدد من اضطرابات الجهاز الهضمي. جُمعت عينات النبات من ضفاف نهر شاري ومن سوق الخضروات المركزي بمدينة انجمينا، جمهورية تشاد. واستُخلصت المواد الخام باستخدام مجموعة من المذيبات العضوية، وهي: الهكسان والكلوروفورم والإيثانول، بصورة متتابعة، وفقاً لطريقة هارفارد (Harvard Method). كما جرى تحديد المركبات الفعالة ذات الأهمية الطبية الموجودة في المستخلص الإيثانولي الخام باستخدام المعايرة الكيميائية، وذلك في مختبر قسم علوم النبات بكلية العلوم، جامعة كردفان. بالإضافة إلى ذلك، استُخلص الزيت الطيار باستخدام جهاز سوكسليت (Soxhlet) في مختبر مركز ضبط الجودة. كما حُدّد التركيب الدهني للزيت باستخدام تقنية كروماتوغرافيا السائل عالية الأداء (HPLC) بجامعة زاريا في نيجيريا. أظهرت نتائج الفحص الكيميائي النباتي للمستخلص الإيثانولي للكرفس الطازج وجود عدد من المركبات الفعالة بكميات ملحوظة، شملت الفلافونويدات، والقلويدات، والفينولات، والصابونينات، والأحماض الكربوكسيلية، والكومارينات، والتانينات، والجليكوسيدات، إلى جانب وجود كميات قليلة من الستيرويدات والتربينات. كما لم يُكشف عن وجود مركبات الريسينات (Ricins). وأظهر تحليل الزيت الطيار باستخدام تقنية كروماتوغرافيا السائل عالية الأداء (HPLC) وجود عدد محدود من الأحماض الدهنية، تمثلت في حمض البالمتيك، وحمض الأوليك، وحمض الستياريك. وتتوافق هذه النتائج مع ما أشارت إليه الدراسات السابقة بشأن انخفاض محتوى الكرفس من الدهون والكربوهيدرات، الأمر الذي يجعله غذاءً مناسباً للأنظمة الغذائية الهادفة إلى إنقاص الوزن. وخلصت الدراسة إلى أن نبات الكرفس يحتوي على مركبات كيميائية فعالة تمتلك خصائص بيولوجية متعددة، من بينها التأثيرات المضادة للأكسدة والمضادة للبكتيريا، فضلاً عن إمكانية إسهامه في المساعدة على تنظيم مستويات الكوليسترول المرتفعة في الدم. وبناءً على ذلك، أوصت الدراسة بإدراج الكرفس ضمن النظام الغذائي للأشخاص الذين يعانون من السمنة أو ارتفاع مستويات الكوليسترول في الدم.

الكلمات المفتاحية: الفحص الكيميائي، المستخلص الإيثانولي، الزيت الطيار، نبات الكرفس (Apium graveolens)، كروماتوغرافيا السائل عالية الأداء (HPLC).

Introduction:

Celery (*Apium graveolens*) is an annual, aromatic, medicinal plant characterized by fleshy roots and broad, compound leaves with needle-like hairs along the veins and long petioles. The plant reaches a height of 60–80 cm and features a highly branched stem that terminates in umbels bearing regular, hermaphroditic, white flowers. Upon maturation, it produces brown-colored fruits with a strong, pungent aroma. Celery is a winter crop, though it can be cultivated year-round; it is considered an important crop in temperate regions, particularly in Europe, North America, and Asia. In Iraq, it is grown for nutritional purposes. It is notable for its diverse chemical composition, containing a variety of natural compounds, including essential oils, as well as other metabolites such as flavonoids, coumarins and their derivatives, and fatty acids. Its leaves are rich in minerals such as calcium, phosphorus, and iron as well as vitamins, including vitamins C, A, and B. It is low in calories, consisting of 94% water, and contains minimal carbohydrates and fats. It also contains various fatty acids, such as oleic, palmitic, stearic, and mevalonic acids, and possesses antioxidant properties, in addition to other medicinal benefits. Its volatile oil contains compounds like selinene and limonene, along with phthalide derivatives, which contribute to the plant's distinctive aroma.

Furthermore, it contains phenolic compounds such as chlorogenic acid and caffeic acid, while its flavonoids include substances like apigenin and luteolin. The chemical composition of celery varies depending on the specific plant part, environmental conditions, and the methods used for extraction and chemical analysis; this underscores the importance of conducting chemical analyses to identify the compounds present in plant samples and to quantify their concentrations (Salimi et al., 2022; Sowbhagya, 2014). It also has several other general uses. It is considered an excellent seasoning, added to many dishes to enhance their flavor; its leafy parts are also widely used in salads and pickles (Azazi, 2009). Its seeds are used as a remedy for asthma and bronchitis. Furthermore, celery is utilized in biological assays; studies have shown that celery extract when combined in equal proportions with the volatile oils of other plants is effective against bacteria such as *Bacillus subtilis* and *Proteus vulgaris*, as well as various fungi. Additionally, it has been shown to lower cholesterol and blood pressure in rats and mice. (Al-Anami & Ibrahim, 2011) In the field of plant tissue culture, several studies have been conducted, including the induction of callus in anise, parsley, and celery. Another study focused on the

propagation of *Thapsia garganica*, a medicinal plant. Secondary metabolites have been extracted from celery and from the callus of dill. Other research has utilized somatic embryos to produce synthetic seeds for carrot, celery, and anise. (Dagher, 2009) Volatile oils, like many other natural compounds, have diverse medical applications. They can help lower blood sugar levels; protect the skin from dryness, superficial burns, and redness, and act as carminatives (expelling intestinal gas) and appetite stimulants. They are incorporated into various pharmaceutical products used to treat a wide range of human and animal diseases, and they also serve as remedies for certain insect stings.

Furthermore, these oils are used to address sleep disorders, in the production of fragrances, perfumes, cosmetics, and decorative items, as well as in the manufacturing of confectionery and cleaning products, serving as natural agents that impart specific scents and flavors. Volatile oils are composed of terpenes, which are classified into monoterpenes and sesquiterpenes, with isoprene serving as the fundamental building block (El-Shahat, 1992; Bruneton, 1999). These oils play a significant role for the plant itself; they help attract insects to flowers to facilitate cross-pollination and act as natural agents that repel or eliminate fungal and bacterial pathogens. Furthermore, they accelerate the healing of wounds and prevent the leakage of cellular sap (Al-Husseini et al., 1990; Hijawi, 2004; Iburg, 2006). Volatile oils are utilized in the pharmaceutical, food, and cosmetic industries. They are also a subject of significant research interest, as certain components have demonstrated biological activities, such as antibacterial and antioxidant properties. The acquisition of high-quality oils depends on the plant species, its chemical composition, the specific plant part used, and the extraction method employed (Kant and Kumar, 2022; Katekar et al., 2023)

Methods:

1. **Preparation of the Plant Sample:** Celery plants were obtained from the banks of the Chari River via the central vegetable market. The plant material was cleaned with water and separated into its constituent parts (roots, stems, and leaves). The parts were air-dried in the shade. Once dried, the plant parts were ground into a powder. The ground plant material was stored in individual plastic bags.
2. **Crude Extraction of the Plant Sample Celery (*Apium graveolens*):** 50 grams of the powdered plant material were weighed and mixed with 150 mL of petroleum ether (40–60°C). The mixture was shaken thoroughly in a glass container and left to stand for three days. It was then filtered, and the filtrate was left exposed to the air to evaporate. Subsequently, 150 mL of chloroform was added to the same plant material in the original container and left for another three days; the mixture was then filtered, and the filtrate was left to dry. Finally, 150 mL of 70% methanol was added to the same plant material in the same glass container and left for an additional three days, after which it was filtered and the filtrate was air-dried. The filtrates obtained from the various solvents (petroleum ether, chloroform, and methanol) were collected and dried. The weights of the crude extracts obtained after filtration were recorded, and the extracts were stored in small colored glass containers.
3. **Extraction of oil from celery (*Apium graveolens*)**: Three empty flasks for a Soxhlet extractor were weighed, and anti-bumping granules (crushed glass) were added to them. Hexane was used as an organic solvent to extract oil from celery plant tissues, following the AOAC (2019) method. The weighed samples were placed in holders and completely covered with cotton to prevent any loss of the sample material. The holders were connected to condensers, through which a continuous flow of water was maintained. 300 ml of hexane was added to the samples. The flasks were heated electrically to initiate the extraction process, which was conducted over a fixed duration of 8 hours, after which the solvent was condensed and removed. The experiment was repeated three times. The extracted oil samples were transferred into small dark-colored containers and stored in a refrigerator until further use. The extraction yield was calculated using the following formula: Extraction Yield = [(Weight of flask with oil – Weight of empty flask) / Weight of sample] × 100

Chemical Screening of *Apium graveolens* (Celery):

This analysis was performed in the Plant Laboratory at the Faculty of Science, University of Kordofan. 3 grams of the methanolic extract of celery were weighed and mixed with 30 ml of methanol. The mixture was shaken thoroughly until the extract was completely dissolved. Subsequently, tests were conducted to detect the presence of Flavonoids, Alkaloids, Phenols, Terpenes, Glycosides, Steroids, Coumarins, Ricins, Tannins and Carboxylic Acids:

4. **Detection of Fatty Acids Components of celery (*Apium graveolens*) plant oil**: 0.2 µL sample of the celery oil dissolved in hexane (10% solution) was injected into an HPLC (High-Performance Liquid Chromatography) system to determination fatty acid contents.

Results:

Table (1): Crude extracts of the plant sample *Apium graveolens* (celery)

Sample weight (g)	| Solvent (mL)	Extract weight (g)
50	Petroleum ether 150	0.8
50	Chloroform 150	4
50	| Ethanol 150	4.8

Table (2): percentages oil % of tissues

Tissue Type	Net Weight of Extracted Oil (g)	Percentage of OIL %
Roots	8.3475	1.669 %
Stems	8.3625	1.6725 %
Leaves	8.45	1.69 %

Table (3): Phytiochemical screening of the ethanol extract of *Apium graveolens* (celery)

Metabolite	Concentration
Flavonoids	++ +
Alkaloids	+++
Phenols	+++
Glycosides	++
Coumarins	++
Tannins	+++
Carboxylic Acids	+++
Terpenes	+
Steroids	+
Ricins	---

+++ High Concentration, ++ modrate Concentration, + low Concentration, --- Absent

HPLC analysis of the volatile oil:

Resulted a limited number of fatty acids as palmitic acid, oleic acid, and stearic acid which aligns with previous research indicating that celery has a low fat and carbohydrate content

Discussion:

Table (1) presents the yields obtained from the entire plant tissue of celery (*Apium graveolens*) using various organic solvents of differing polarities. The extract yields by ethanol had highest amount than the extract yields by chloroform but the Petroleum ether very fewer compared the other solvents.

The oil content percentages were determined by calculating the averages for the different plant parts. The oil content was highest in the leaves; this is attributed to a greater abundance of glandular hairs and oil-secreting ducts in the leaves compared to the stem. In the roots, glandular hairs are entirely absent, and any oil obtained is derived solely from secretary ducts an anatomical fact.

Displays the results of the chemical screening of the ethanol extract of *Apium graveolens*. The analysis revealed the presence of the following active chemical compounds: flavonoids, alkaloids, phenols, saponins, carboxylic acids, coumarins, tannins, and glycosides (in significant quantities), as well as steroids and terpenes (in smaller amounts). Conversely, ricins not detected. The relative abundance (high or low) of these compounds was determined based on the intensity of the color produced or the concentration of precipitates formed during titration. The absence of a compound in the crude extract was concluded when no such indicators neither color changes nor precipitates were observed, as was the case with ricins illustrates the compositional content of the oil extracted from the celery plant (*Apium graveolens*), as determined via High-Performance Liquid Chromatography (HPLC).

Three fatty acids were identified: palmitic acid, oleic acid, and stearic acid. It can be concluded that celery oil has low fatty acid content. This aligns with previous studies suggesting that celery helps reduce blood cholesterol levels due to its low-fat content; therefore, it may be useful for weight loss and lowering blood cholesterol. Our study supports these previously reported findings. A study was conducted to investigate the effect of an alcoholic extract of celery leaves on the concentration of certain biochemical parameters in chicken through the concentrations of cholesterol and low-density lipoprotein (LDL) in the group treated with the alcoholic celery leaf extract. (Dagher, 2019).

Studies had demonstrated the efficacy of celery extract as an antibacterial agent against *Bacillus subtilis* and *Proteus vulgaris*. Furthermore, when mixed in equal proportions with volatile oils from other plants, celery seed extract has shown potential in combating fungal diseases, as well as in reducing cholesterol levels and blood pressure in rats and mice. Our previous study indicates that the oil

extracted from various parts of the celery plant including the leaves, stems, and roots exhibits significant inhibitory potency against bacterial growth, particularly against bacteria associated with food poisoning; this aligns was indicated by previous studies. Additionally, three fatty acids were identified in the volatile oil using HPLC analysis and this confirm that had previously been detected by (Salimi et al 2022) through GC-MS analysis.

ملف البيانات C:\Chem32\1\Data\I DRI SS_ADAM MOUSSA_FFA_1 2025-12-12 15-52-47\004-0401.D اسم
العينة: أوراق

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جهاز الاستحواذ: HPLC		موقع:	4
تاريخ الحقن: 5:43:1 12/12/2025	مساءً 6:		1

Inj Volume : 10.000 µl

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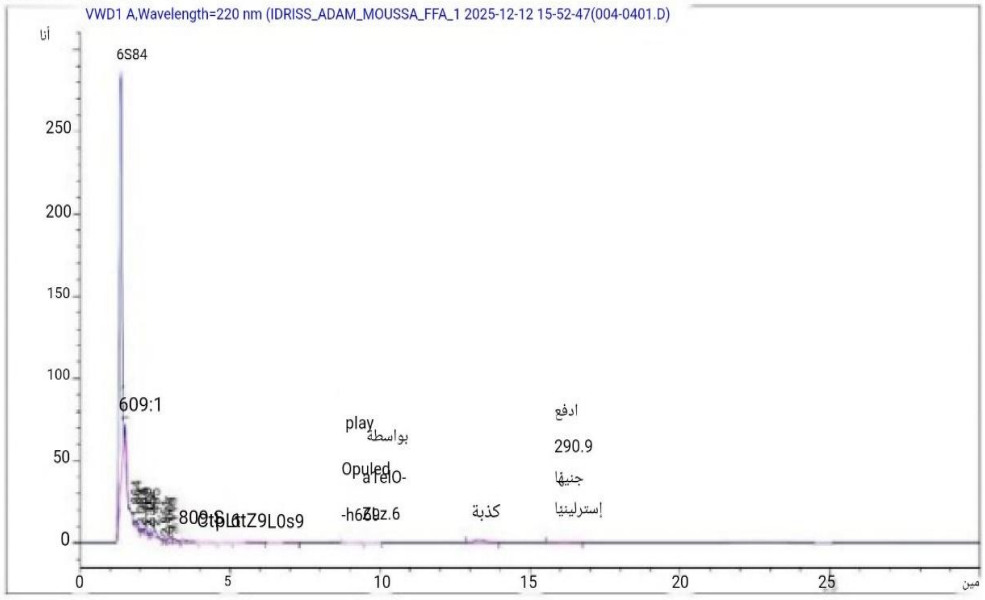
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آخر تحديث ١٦/١٢/٢٠٢٥ الساعة ٣:٤٧:٣٧ مساءً بواسطة النظام

معلومات عن الطريقة قياس كمية الأحماض الدهنية بواسطة مختبر أبحاث الطب بجامعة زاريا (MUSRL A.B.U Zaria) على جهاز ZORBAX Eclips XDB-C18 أسيتونيتريل في الماء + 0.085% حمض الفوسفوريك/ميثانول، 12:53:35 عند 35 درجة مئوية. فهرنهايت H2O/95% ،مم، 5 ميكرومتر 150 × 4.6 مل/دقيقة 1=R/

معلومات إضافية: تم دمج الذروة (الذروات) يدويًا



تقرير المعايير الخارجية

مرتبة حسب	إشارة
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مُثبِّت متعدد	1.0000
من الحزن	1.0000

لا تستخدم عامل الضرب والتخفيف مع المعايير الداخلية

Si gnal 1: VWD1 A, Wavel ength=220 nm

وقت إعادة التشغيل [أسفي]	يكتب	منطقة [mAU*s]	المقاطعة/المنطقة	كمية [ملغم/مل]	اسم المجموعة
8.994	بي بي	12.34670	1.28294e-2	1.58400e-1	حمض البالميتيك
9.712	مم	2.15084	0.00000	0.00000	مرحباً، لديك بطاقة.

اسم العينة: C:\Chem32\1\Data\I DRI SS_ADAM_MOUSSA_FFA 1 2025-12-12 15-52-47\004-0401.D ملف البيانات
أوراق

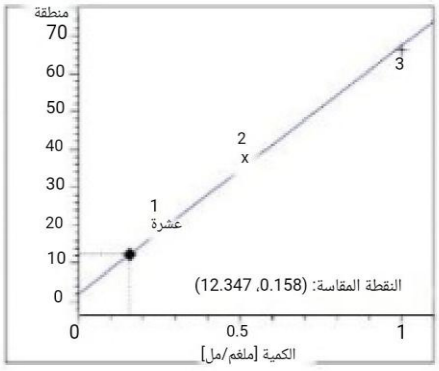
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الإجمالي: 1.69939e-1

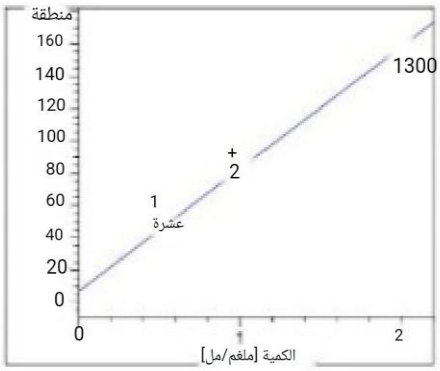
1- تحذيرات أو أخطاء:

(Ol ei c Ai cd) ،تحذير: يتم تعيين النتائج السلبية إلى الصفر (نقطة تقاطع منحنى الحساب)

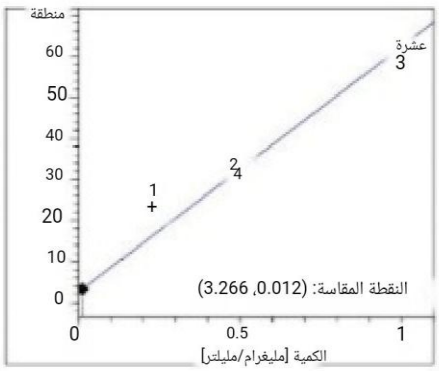
منحنيات المعايرة



حمض البالميتيك عند درجة حرارة التجربة: 8.954
طول الموجة = 220 نانومتر، VWD1 A
علاقة: 0.99772
الانحراف المعياري المزدوج للسكن: 2.31088
الصيغة أ: ص = م س + ب
م: 65.37951
ب: 1.99056
س: الكمية [ملغم/مل]
و: المنطقة



Oleic Ai cd at exp. RT: 9.607
طول الموجة = 220 نانومتر، VWD1 A
علاقة: 0.99473
الانحراف المعياري المزدوج للسكن: 8.18220
الصيغة أ: ص = م س + ب
م: 75.93360
ب: 6.78207
س: الكمية [ملغم/مل]
و: المنطقة



حمض الستياريك عند درجة حرارة التجربة: 16.059
طول الموجة = 220 نانومتر، VWD1 A
علاقة: 0.99251
الانحراف المعياري المزدوج للسكن: 3.85018
الصيغة أ: ص = م س + ب
م: 59.83439
ب: 2.57559
س: الكمية [ملغم/مل]
و: المنطقة

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