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كلية علوم الطبيعة والحياة



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BENKHEDDA Chaima

BOUGHANEM Serine

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Determination of phenolic content and evaluation of antioxidant activity in the aqueous extract of *Cachrys sicula* L.

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Jury

President	**BOUROUBA Romyla**	**MCA. UFA Sétif 1**
Promotor	**BOUDOUKHA Chahra**	**MCA. UFA Sétif 1**
Examinator	**AMOR Loubna**	**MCA. UFA Sétif 1**

Laboratory of Microbiology

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ملخص

تعتبر نبتة (*Cachrys sicula* L.) المنتمية الى العائلة الخيمية(Apiaceae) ،من النباتات العطرية المعمرة والمستوطنة في حوض البحر الأبيض المتوسط، والتي تكتسي أهمية طبية بالغة في الجزائر. تهدف هذه الدراسة إلى استكشاف التركيب الكيميائي وتقييم النشاط المضاد للأكسدة للأجزاء الهوائية للنبتة. أظهر الكشف اللوني الأولي وجوداً طفيفاً لعديد الفينول في المستخلص المائي المحضر بطريقة المغلى، مع غياب تام للفلافونويدات، والصابونينات، الكينونات، الانتراكينونات والتربينويدات. أجري القياس اللوني لعديد الفينولات باستخدام طريقة Folin-Ciocalteu، حيث أظهر المستخلص محتوى قدره 55.66 ± 4.53 ميكروغرام مكافئ حمض الغاليك /مغ من المستخلص. أما بالنسبة للفلافونويدات تم تقديرها باعتماد طريقة ثلاثي كلوريد الألومنيوم، فقد كشفت النتائج عن محتويات بلغت 2.55 ± 0.41 ميكروغرام مكافئ الكيرسيتين / مغ من المستخلص. تم تقدير الدباغ المكثفة بطريقة الفانيلين حيث أظهر 42.77 ± 6.69 ميكروغرام مكافئ الكاتشين / مغ من المستخلص. تم تقييم الأنشطة المضادة للأكسدة مخبرياً بواسطة ثلاث طرق مختلفة؛ حيث أظهر المستخلص قدرة على كنس الجذور الحرة بـ IC_{50} قدرها 69.43 ± 1.35ميكروغرام/مل في اختبارDPPH ، وفي اختبار السعة الكلية لمضادات الاكسدة (TAC) سجل المستخلص نسبة تثبيط بلغت 47.09±3.93% بالنسبة لمعيار الكيرسيتين و38.52± 3.21% بالنسبة لمعيار حمض الأسكوربيك. كما أظهر اختبار قوة الاختزال الحديدي (FRAP) نشاطاً معبراً عنه بـ EC_{50} قدرها 794.34±7.01 ميكروغرام/مل. تشير نتائج هذه الدراسة إلى أن نبتة *Cachrys sicula* L. لها تأثيرات بيولوجية مثيرة للاهتمام يمكن تثمينها في الصناعات الصيدلانية .

الكلمات المفتاحية :النشاط المضاد للأكسدة، العائلة الخيمية، المستخلص المائي، *Cachrys sicula* L.، DPPH, اختبار قوة الاختزال الحديدي(FRAP) ، السعة الكلية لمضادات الأكسدة(TAC) .

Abstract

Cachrys sicula L. (Apiaceae) is a perennial aromatic plant endemic to the Mediterranean basin, which holds significant medicinal importance in Algeria. The objective of this study is to explore the chemical composition and evaluate the antioxidant activity of the aerial parts of the plant. The phytochemical screening revealed a weak presence of polyphenols in the aqueous extract prepared by decoction, with a total absence of flavonoids, saponins, quinones, anthraquinones, and terpenes. Total polyphenols colorimetric determination was carried out using the Folin-Ciocalteu method, where the extract showed a content of 55.66 ± 4.53 µg GAE/mg of extract. As regards to flavonoids, it was estimated using the aluminum trichloride method ($AlCl_3$); the results revealed contents of 2.55 ± 0.41 µg QE/mg of extract. Condensed tannins were estimated by the vanillin method, showing 42.77 ± 6.69 µg CE/mg of extract. Antioxidant activities were evaluated *in vitro* by three different methods; the extract exhibited a radical scavenging activity with an IC_{50} of 69.43 ± 1.35 µg/mL in the DPPH assay. In the TAC assay, the extract recorded an inhibition rate of 47.09 ± 3.93% relative to the quercetin standard and 38.52 ± 3.21 % relative to the ascorbic acid standard. The FRAP assay also showed activity expressed as an EC_{50} of 794.34± 7.01µg/mL. The results of this study suggest that the plant. *Cachrys sicula* L. has interesting biological effects that can be valued in the pharmaceutical industries.

Keywords: Antioxidant activity, Apiaceae, Aqueous extract, *Cachrys sicula* L., DPPH, FRAP, TAC.

Acknowledgements

Summary

List of abbreviations

List of figures

List of tables

1. Introduction: 6

2. Materials and Methods 6

2.1. Chemical reagents 6

2.2. Plant material collection 6

2.3. Extraction 7

2.4. Extraction yield 7

2.5. Phytochemical screening 7

2.5.1. Polyphenol Detection 8

2.5.2. Flavonoid Detection 8

2.5.3. saponin Detection 8

2.5.4. Quinone Detection 8

2.5.5. Anthraquinone Detection 8

2.5.6. Terpenoid Detection 9

2.6. Total Phenolic Content 9

2.7. Total Flavonoid Content 10

2.8. Condensed Tannin Content 10

2.9. Evaluation of the antioxidant activity 11
2.9.1. DPPH radical scavenging activity assay 11
2.9.2. Total antioxidant capacity 12
2.9.3. Ferric reducing antioxidant power 13
3. Results and discussion 14
3.1. Extraction yield 14
3.2. Phytochemical screening 14
3.3. Total Phenolic and Flavonoid Contents 16
3.4. Total condensed Tannin Content 18
3.5. Evaluation of the antioxidant activity of the plant 18
3.5.1. DPPH Radical Scavenging Activity Assay 19
3.5.2. Total antioxidant capacity 21
3.5.3. Ferric reducing antioxidant power 22
4. Conclusion 24
5. References 25

1. Introduction:

Since the dawn of civilization, nature has formed the basis of medicine for humanity (Yuan et al., 2016), medicinal plants represent a fundamental source for drug discovery, as traditional healing systems have long relied on them and many modern pharmaceuticals are derived from their bioactive compounds (Fabricant & Farnsworth, 2001). Natural products remain a key source for the discovery of new medicines, particularly in light of global challenges such as drug resistance and the side effects of synthetic compounds, which underscore the need for ongoing research into bioactive compounds derived from nature (Newman & Cragg, 2020). The renewed interest in natural products is aimed at studying bioactive compounds extracted from plants as safer and more sustainable alternatives for promoting human health (Atanasov et al., 2015).

Given the global significance of medicinal plants, we find that Algeria thanks to its vast territory and diverse climates, is rich in vegetation, including a large number of medicinal and aromatic plants. This natural heritage constitutes an important resource in the field of phytotherapy; it is home to thousands of plant species, many of which are endemic (Belhouala and Benarba, 2021). Among this rich flora, *Cachrys sicula* L. (*C. sicula* L) belonging to the Apiaceae family, stands out as a plant of significant medicinal importance (Palá-Paúl et al., 2002).

Cachrys sicula L. (synonym: *Hippomarathrum libanotis* Koch subsp. *Pterochlaenum*) is a perennial aromatic plant native to the Western and Central Mediterranean regions; southern Europe, Asia and north Africa (Larit et al., 2025). Its flowering season extends from April to June, during which its biological characteristics are most evident (Castroviejo & Jury, 2003). It grows primarily in subtropical regions which is also adapted and thriving in arid areas, along hedgerows and in agricultural areas (Larit et al., 2025).

Botanically, the plant is characterized by its yellowish-green foliage, growing to a height of between 30 and 100 cm, with a large taproot, its stems are erect and ribbed. The basal leaves are large divided into 4–5 pinnate segments with short petioles and their terminal segments are filiform 1.5–10.5 mm long, the stem leaves are similar and shorter segments. The upper

corymbose inflorescence has 7–15 thick unequal rays, with 5–7 (in the variety crispatum) complete bracts divided into 1–2 pinnate segments; the smaller corymbose inflorescences have an involucre of 5–7 sharp undivided bracts, with fewer than 4–9 rays. The flowers are yellow, with entire petals and have very prominent calyx teeth on the fruit (2–3.5 mm); the styles (3–4.5 mm) are widely spaced larger than the style base and flat-convex. The fruits are ovoid, pome-like or subcylindrical. The fruits have 5 very prominent ribs are winged tuberculate or covered with woolly hairs (figure 1) (Castroviejo & Jury, 2003).



Figure 1: Aerial part of *Cachrys sicula* L. (Anonymous).

A long-standing historical significance within traditional therapeutic practices is possessed by *C. sicula* L. having been extensively utilized since antiquity in Mediterranean folk medicine. This prolonged and intensive historical reliance is attributed to its unique pharmacological properties, establishing the species as an integral component of the region's ethnobotanical heritage. Specifically, in Turkish folk medicine as a tonic and antiflatulent, for treatment of intestinal worms and wounds especially hemorrhoids and to stop external bleeding. Roots of *Cachrys* species are used in Turkey as an aphrodisiac (Doković et al., 2004). Moreover,

its oils and fruits exhibit therapeutic effects similar to *Ammi majus* in treating vitiligo (Ena et al., 1989).

In Algerian, *Cachrys sicula* L. have demonstrated significant anticholinesterase activity in its essential oils, suggesting its potential role in managing neurodegenerative disorders such as Alzheimer's disease (Smaili et al., 2021). Additionally, it is used to treat human melanoma by inhibiting tumor cell growth and inducing apoptosis (Marrelli et al., 2021). Alongside its therapeutic uses, this species has been traditionally integrated into human and animal diets, it is used as a condiment in food products and traditionally added to tarhana soup (Matejić et al., 2012), transfers a pleasant smell to milk and derivatives and is mainly used as fodder on arid pastures (Doković et al., 2004) as well as assayed for their insecticidal activity (Smaili et al., 2021), producing crops and as renewable sources for the production of fine chemicals (Musolino et al., 2023).

Manunta, suggested that the plant *C. ferulacea* is botanically identical to the plant on coins of the old Greek colony Cyrene (Kyrene), reflecting the prominent status that the *Cachrys* species held in ancient civilizations (Doković et al., 2004). However, it is important to note that contact with this plant can cause skin irritation and blistering, especially when the skin is humid and exposed to UV light, due to the presence of certain coumarins such as psoralens (Grande et al., 1986).

The traditional uses highlight the pharmacological importance of *C. sicula* where its therapeutic effects are closely linked to its secondary metabolite composition, in particular coumarins mainly furanocoumarins, stand out among the main phytochemical compounds, widely found both as a heteroside and free form in the seeds, roots, and leaves of Apiaceae families. Furthermore, the phytochemical profile showed the ubiquitous presence of the terpene such as estragole which represent the primary constituents of the plant's essential oils (Musolino et al., 2023).

In addition to these major compounds, the plant contains minor amounts of phenolic constituents (Musolino et al., 2023), which are defined as natural metabolites arising biogenetically from either the shikimate/phenylpropanoid pathway (Vuolo et al., 2019). They

are secondary metabolites, ubiquitous in plants and plant-derived foods and beverages, exhibiting a wide diversity of structures (Cheynier, 2012), which are made of an aromatic ring containing one or more hydroxyl substituents (Tsao, 2010) such as polyphenols.

Polyphenols were defined as water-soluble phenolic compounds having molecular weights between 500 and 3,000 Da (Cheynier, 2012). Its basic monomer is the phenolic ring, which is generally classified as phenolic acids and phenolic alcohols. According to this ring, polyphenols can be classified into several classes, the main ones being phenolic acids, flavonoids, stilbenes, phenolic alcohols, and lignans (Abbas et al., 2017). Based on its phytochemical similarity to *C. pungens*, this phenolic fraction in *C. sicula* includes the flavonoid catechin, the glycosides naringin and quercitrin, as well as low concentrations of cinnamic acids (caffeic and ferulic acid) and gallic acid (Musolino et al., 2023).

The presence of these diverse phytochemical constituents, particularly the synergy between coumarins and phenolic compounds, provides *C. sicula* with a broad spectrum of biological properties. This chemical diversity plays an important role in enhancing the plant's biological efficacy, which is reflected in the multiple biological activities observed, such as antioxidant, antimicrobial, anti-inflammatory (Musolino et al., 2023), anticholinesterase (Smaili et al., 2021) and anticancer effects (Marrelli et al., 2021).

Specifically, the abundance of the phenolic antioxidants emphasizes the remarkable ability of *C. sicula* to combat oxidative stress. Antioxidants are chemical substances, that even at low concentrations significantly delay or prevent the oxidation of vital substrates. Their primary mechanism involves donating electrons or hydrogen atoms to unstable free radicals, effectively neutralizing them (Lobo et al., 2010). Oxidative stress is a pathological condition, resulting from the disparity between the production of free radical and the body's capability to counteract them, which induces apoptotic signaling that is consequent to reactive oxygen species (ROS) increases and/or antioxidants decreases, disruption of intracellular redox homeostasis and irreversible oxidative modifications of lipid, protein, or DNA (Deoxyribonucleic Acid) (Circu & Aw, 2010). The imbalance can damage cells and contribute to many diseases, including cancer, neurological disorders, atherosclerosis, hypertension, ischemia/perfusion, and diabetes (Birben et al., 2012).

To date, most available literature on the *Cachrys* genus has focused almost exclusively on its essential oils. Consequently, there is a significant lack of data regarding the antioxidant activities of its extracts. However, this scarcity of research does not imply a lack of such properties, but rather highlights an unexplored area that warrants deeper investigation.

In this context, the present work contributes to the valorization of the Algerian flora, specifically focusing on the species *C. sicula* L. This study aims to provide a comprehensive analysis of this species, focusing on the aqueous extract prepared via decoction. It involves the quantification of major bioactive constituents, particularly polyphenols, flavonoids and condensed tannins, while simultaneously evaluating the antioxidant activity of the extract through in vitro assays (using DPPH, FRAP, and TAC tests).