

ESSENTIAL OIL CHARACTERIZATION AND HEALING
ACTIVITY OF *A. GRAVEOLENS* VAR. *DULCE* (MILL.)
SEEDS

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Abstract

The present work aimed to study the phytochemical composition of the essential oil of *Apium graveolens* var. *dulce* (Mill.) growing in the region of Sidi Bel Abbés (Algeria, NW) as a condiment and a medicinal plant, and to evaluate its healing activity. CG/MS analysis of the essential oil obtained from the seeds led to the identification of 20 compounds representing 98.5% of the oil, with limonene (54%) and β -selinene (30.7%) as the dominant compounds. The essential oil showed exceptional healing power, with the burning surface shrinking to 100% after 15 days of application.

Key words: essential oil, *Apium graveolens* var. *dulce*, healing activity

Introduction. Celery (*Apium graveolens* var. *dulce* (Mill.)) is frequently used in Algeria and other Maghreb countries for its leaves, seeds, and stalks, which are traditionally used as anticoagulants [1,2]. Celery is also used in the treatment of gastrointestinal diseases as an antispasmodic [4]. Previous studies have confirmed its antimicrobial and fungal activities [3,4], and in vivo work on rats has confirmed its ability to lower blood sugar [5].

Essential oils, or plant essences, are oily, volatile, fragrant, and colourless or slightly tinted products obtained through steam distillation, expression, incision, or enfleurage of plant material [6].

Healing is a natural biological process that occurs after a soft part of the body is opened due to an external mechanical agent, aggression (direct trauma,

burn, injury, disease, surgical intervention, etc.), without the loss of substance. This healing of the wound can occur spontaneously; this is known as healing of the first intention. In some cases, however, human intervention is needed, referred to as secondary or second-line healing [6].

The aim of our work is to analyze the chemical composition of celery essential oil (*A. graveolens* var. *dulce* (Mill.)) and to assess its healing properties. Through our research, we intend to identify the active compounds present in the oil that may contribute to its healing properties and to evaluate their potential benefits.



Fig. 1. Stages of burning on the flank of the animal up to the application of the products

Materials and methods. Plant material. *A. graveolens* var. *dulce* (Mill.) seeds were grown in a typical greenhouse with two plots of 10 m in length and 3 m in width at the Technical Institute of Market Gardening and Industrial Crops of the province of Sidi Bel Abbes, Algeria. The soil was enriched with organic fertilizer and the seeds were planted at a depth of 1 cm and spaced 5 cm apart. The cultivation was carried out in September and the harvest was completed by the end of February 2016. The harvested seeds were dried and stored at room temperature until further use [7].

Extraction process. The extraction of essential oils from *A. graveolens* var. *dulce* (Mill.) seeds was performed using the hydrodistillation method in this research study. According to the standard protocol, 100 g of the seeds were placed in a Pyrex glass flask and mixed with 1000 ml of distilled water. The mixture was then heated until boiling, and the steam generated carried the essential oil. The steam was condensed using a vertically fixed condenser, which allowed for easy flow of the distillate. The entire extraction process took approximately 3 h. The distillate was collected in a separatory funnel and allowed to settle, resulting in the formation of two phases: an organic phase containing the essential oil and an aqueous phase. To preserve the quality of the essential oil, it was stored at a temperature of approximately 4 °C in a sterile glass bottle, hermetically sealed with aluminium foil to protect it from air and light.

Characterization of essential oil chemical compounds by Gas Chromatography-Mass Spectrometry (GC-MS). The essential oils were analyzed

using an Agilent Technologies GC Chromatograph, which was equipped with a GC 7890A Gas Chromatography System, an MS 5975C VL MSD Mass Spectrometer Detector, and a J&W Scientific HP-5MS Capillary Column. Data acquisition and processing were performed using MSD Chemstation software (Agilent) [5]. One microlitre of diluted essential oil (0.05 g in 1.5 mL of CH₂Cl₂) was injected. The experimental conditions were: Solvent delay: 2 min; Carrier gas: helium; Leakage rate: 1.2 ml/min; Split ratio: (60:1); Injector and detector temperatures: 250 °C; Oven temperature: 2 min at 80 °C, then 80 °C to 200 °C (5 °C/min), then 200 °C to 260 °C (20 °C/min), and finally holding the temperature for 5 min; Ionization voltage: 70 eV; Electron multiplier: 1 kV.

The relative retention index (RRI) was calculated using the Van der Dool and Kratz formula: $I_x = 100n + 100(t_x - t_n)/(t_{n+1} - t_n)$, where t_n and t_{n+1} are the retention times of the reference n-alkane hydrocarbons eluting immediately before and after the chemical compound "X"; t_x is the retention time of compound "X". The analysis of a mixture of n-alkanes C₅-C₂₅ (run at the same chromatographic conditions) was used to calculate RRI.

Determination of healing activity. This study aimed to compare the healing effect of a specific product with that of a control batch, corresponding to spontaneous physiological healing. In order to achieve this, twenty male and female Wistar rats, aged 2 to 3 months, with a weight between 180 g and 250 g, were randomly divided into four homogeneous groups. Each group was administered a dose of 40 mg/kg of thiopental via the intraperitoneal (IP) route and induction was obtained after 3 to 5 min. The hair removal step was then carried out, followed by the treatment of each batch with either a placebo or a product whose healing effect was to be tested. The product was applied to the wound, either in the form of *A. graveolens* oil at a rate of 50 µl each time, or as hebermin healing cream at a dosage of 0.5 g each time. The percentage reduction of the burnt surface, compared to the initial surface, was then measured according to the method described.

The following formula calculates the percentage change in the area burned, with respect to the area burned on the first day

$$S = (S_{J_1} - S_{J_n}) \times 100,$$

where S_{J_1} [cm²] is the area burned on the first day, S_{J_n} [cm²] is the area burned on the n -th day, and S is the percentage change in the area burned.

Statistical analysis. In this study, statistical analysis was performed using IBM SPSS (version 25). Experiments were repeated three times and results were expressed as the mean $\pm$ standard error of the mean (SEM). Significance was determined by unpaired two-tailed Student's t -tests, with p -values < 0.05 considered statistically significant.

Results and discussion. The yield of essential oil from the seeds of *A. graveolens* var. *dulce* (Mill.) is precisely 1.0017%, and has a light yellow colour, with

T a b l e 1

Chemical composition of the essential oil of *Apium graveolens* var. *dulce* (Mill.)

RT [min]	RRI	Compound	%
Monoterpenoids			56.0
3.460	925	α -Pinene	0.1
4.122	980	β -Pinene	0.3
4.254	985	Myrcene	0.9
5.032	1030	Limonene	54.0
7.365	1143	<i>trans</i> -Limonene oxide	0.2
9.317	1221	<i>trans</i> -Carveol	0.2
9.615	1230	<i>cis</i> -Carveol	0.1
9.97	1252	Carvone	0.2
Sesquiterpenoids			36.5
13.726	1398	β -Elemene	0.1
14.429	1432	β -Caryophyllene	0.3
15.265	1464	α -Caryophyllene	0.1
16.1	1485	β -Selinene	30.7
16.274	1497	α -Selinene	5.0
17.209	1531	γ -Selinene	0.1
18.342	1595	Caryophyllene oxide	0.2
Others			6.0
7.803	1155	Pentylbenzene	2.0
12.783	1340	1-Phenyl-1-Pentanone	0.1
19.864	1650	3-Butylphthalide	1.9
21.37	1672	<i>tert</i> -Butyl 4-(((trifluoromethyl)sulfonyl)oxy)-5,6-dihydropyridine-1(2H)-carboxylate	2.0
Total			98.5

RT – Retention time; RRI – Relative retention index, determined relative to a homologous series of n-alkanes (C₅-C₂₅) on HP-5MS column

a typical and highly aromatic smell of celery and a liquid appearance. This result is consistent with findings from a similar study, in which a yield of 0.9% was obtained from the same species [8]. A gas chromatography-mass spectrometry (GC/MS) analysis of the essential oil of *A. graveolens* var. *dulce* (Mill.) revealed 20 compounds (Table 1) which accounted for 98.5% of the essential oil.

Our results are similar to those obtained by other researchers, in particular the work of SIMO et al. [9] from the Yaoundé region, which characterizes the chemical composition of the essential oil extracted from the leaves of *A. graveolens* by the same analysis technique and has identified that the main component is limonene 50.7%. In parallel, EL MOSTAPHI et al. [8] worked on the seeds of *A. graveolens* from the Marrakech region to point out that the majority component is limonene

T a b l e 2

Mean area (cm²) and % shrinkage of initial burn area over time

Treatments	D ₀	D ₃	D ₆	D ₉
Untreated	5.86 ± 0.3 —%	4.95 ± 0.3 —%	4.27 ± 0.23 —%	3.6 ± 0.32 —%
Reference (Hebermin)	5.95 ± 0.3 0%	2.99 ± 0.35 39.57%	2.10 ± 0.29 50.78%	1.42 ± 0.37 60.38%
Essential oil	5.69 ± 0.3 2.93%	3.84 ± 0.37 22.82%	2.74 ± 0.05 35.81%	1.99 ± 0.2 44.72%
Treatments	D ₁₂	D ₁₅	D ₁₈	D ₂₁
Untreated	3.26 ± 0.27 —%	2.99 ± 0.2 —%	2.68 ± 0.2 —%	0.79 ± 0.28 —%
Reference (Hebermin)	0.005 ± 0.0 99.83%	0.0 ± 0.0 100%	0.0 ± 0.0 100%	0.0 ± 0.0 100%
Essential oil	0.18 ± 0.02 94.22%	0.05 ± 0.0 98.25%	0.0 ± 0.0 100%	0.0 ± 0.0 100%

Values expressed as mean ± deviation.

SEM. (Standard error on the mean) with $n = 5$ (D: Days)

with 79.15%. Additionally, MARONGIU et al. [10] worked on the essential oil of *A. graveolens* L. from Italy and found the presence of α -selinene at a percentage of 5.0%, and β -selinene at a percentage of 3.73%. These results differ significantly from our results, as the amount of β -selinene in their study was almost ten times lower than in our study. We also highlighted the presence of 3-butylphthalide which represents a rate of 1.9%, a compound that was not identified by the other researchers [5,10,11].

The results of our study revealed that during the initial three days of healing, the group treated with the essential oil of *A. graveolens* var. *dulce* (Mill.) exhibited a wound surface shrinkage percentage of 2.93%, which was not significantly different from the reference group treated with hebermin (0%) [12]. However, from days 6 to 12, the healing percentage of the essential oil-treated group significantly increased to 94.22%, while the hebermin-treated group achieved a percentage of 99.83% ($p < 0.001$) [13]. During the final phase of healing, which extended from day 15 to 20, the batch treated with hebermin marked a shrinkage rate of 100%, as did the batch treated with essential oil of *A. graveolens* var. *dulce* (Mill.), with a shrinkage rate of the burnt surface estimated at 100% [14]. The morphological evaluation of the wounds showed that both batches treated with hebermin and the essential oil, respectively, had a successful healing outcome [14]. According to pathophysiological studies, this period corresponds to the proliferative phase of the scarring process, which is characterized by the formation of granulation tissue.

Conclusion. This study has demonstrated the potential of *A. graveolens* var. *dulce* (Mill.) essential oil to promote wound healing. The essential oil yielded 1.0017% with a light yellow colour, a typical and highly aromatic smell of celery and a liquid appearance. GC/MS analysis revealed 20 compounds which accounted for 98.5% of the essential oil, most notably limonene and β -selinene. Furthermore, the application of the essential oil to rats with skin lesions resulted in regrowth of hair and a successful healing outcome. These findings suggest that *A. graveolens* var. *dulce* (Mill.) essential oil can be used as a natural and non-invasive alternative for wound healing.

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