

Chemical Composition and Anti-inflammatory Activity of *Apium graveolens* var. dulce Essential Oils from Senegal

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Abstract *Apium graveolens* var. dulce commonly known as celery, belongs to the *Apiaceae* family. It is used especially as a vegetable and medicinal plant. In Senegal, *A. graveolens* is used as a food condiment. The aim of this work is to study the chemical composition of *A. graveolens* stems (S), leaves (L) essential oils and antiinflammatory activity. GC/FID and GC/MS analyzes carried out on essential oils obtained by steam distillation showed that oils from both stems and leaves were characterized by the same major constituents α -pinene, the prominent compound of oils represented 69.3 and 68.4% for stems (S) and leaves (L), respectively. It is followed by limonene (9.5 and 9.8%), α -phellandrene (5.5 and 5.9%) and β -pinene (4.8 and 4.3%). These compounds represented 89.1% (S) and 88.4% (L) of the total essential oils content. Anti-inflammatory activity was measured by the inhibition of 5-lipoxygenase (5-LOX) by *A. graveolens* essential oil anti-inflammatory assays revealed an IC₅₀ of 29.5±2.0 µg/mL for *A. graveolens* oils and 23.7±0.5 µg/mL for quercetine used as a reference. This study showed that essential oils of *A. graveolens* was an important source of α -pinene who is probably responsible of its antiinflammatory properties.

Keywords: Apium graveolens, essential oils, α -pinene, linoleic acid, 5-lipoxygenase and anti-inflammatory

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1. Introduction

Inflammation is a positive and natural response of the immune system. It is triggered following tissue damage and penetration of pathogens into the body. It is characterized by leukocyte activation, increased vascular permeability, edema and pain [1]. Nowadays, lipoxygenases (LOX) and cyclooxygenases (COX1 and COX2) constitute the main source of production of pro-inflammatory mediators such as prostaglandins (lipid mediators neo formed following activation of mast cells), and cytokines (Tumor Necrosis Factor α (TNF α), Interleukins (IL1) and (IL6)), which represent the mediators released by mast cells and macrophases [2,3]. However, inhibition of these enzymes requires the use of nonsteroidal anti-

inflammatory (NSAI) drugs. These are often associated with certain side effects, including rheumatoid arthritis, bronchial asthma, inflammatory bowel disease, multiple sclerosis, type 1 diabetes, inflammatory skin disease and lupus [4,5]. In addition, lipoxygenases, in particular 5-LOX, are non-heme iron atom dioxygenases widely represented in the animal and plant kingdom [6], constitute one of the main routes of generation of leukotirenes (LT), main lipid mediators of inflammation [1]. In addition, due to the many diseases linked to the use of NSAI drugs to inhibit pro-inflammatory enzymes, the pharmaceutical industry is nowadays turning to the use of medicinal plants. For this purpose, when used properly, they can replace the synthetic drugs most commonly known for their unwanted side effects. This attractive approach to medicinal plants leads us to choose Apium graveolens because of its many healing properties. A.

graveolens is one of the most widely consumed vegetables of the Apiaceae family in the world. This family regroups about 400 genera and 3000 species [7]. In Senegal, A. graveolens var. dulce is the most widespread species. It usually grows in very humid areas and its leaves are used as condiment, because of Volatile Organic Compounds (VOCs) content and anti-inflammatoty properties. Studies have shown that celery stems contain mineral salts such as iron, phosphorus, calcium, magnesium and sodium as well as vitamins A, B1, B2 [8]. In addition, tests with celery seeds essential oils proven it to be very successful in the treatment of rheumatism [9]. Data from the literature shown a broad spectrum of celery use and valorization of their Essential Oils (EOs) in different applications, especially as stimulants, hypertensives and aphrodisiacs [10]; antifungal agents [11,12]; anti-ulcer and antiasthmatic drugs [13]; diuretics and rheumatism [14]; bactericides [15]; anti-oxidant agents [12,16]. Several studies reported the efficacy of celery essential oils against mosquitoes [17,18,19,20] and insecticidal properties [21,22,23]. The chemical composition of the essential oils of a species generally depends on the organs studied, the origin and the extraction method used. Among the studies carried out on the chemical composition of celery essential oils [15,24,25]. To our knowledge, there are no studies carried out on celery essential oils from Senegal. The aim of this work is to study the chemical composition of stems and leaves EOs from A. graveolens, and measuring its anti-inflammatory activity by inhibiting enzyme 5-LOX.

2. Material and Methods

2.1. Plant Material and Essential Oils Extraction

A. graveolens plants were collected inside of the Institute of Food Technology of Dakar (14° 45' N, 17° 20' W), Senegal in august 2019. Identification of taxonomic plants was carried out in the herbarium of Fundamental Institute of Black Africa of Cheikh Anta DIOP University of Dakar where a voucher specimen (N° HM₁) was deposited. The collected plants were washed with water to remove foreign matter and then dried for 5 days. EOs were extracted by submitting separately both stems (S) and leaves (L) from plants to steam distillation for 2 hours using a Clevenger apparatus. Essential oils obtained were stored in amber vials at 4 °C until analysis. All extractions were repeated 3 times.

2.2. Gas Chromatographic Methods

EOs were analyzed by gas chromatography using an Optima-5-accent type, 5% phenylmethylsiloxanecapillary column: 30 m x 0.25 mm i.d., 0.25 μ m film thickness (Macherey-Nagel, Düren-Germany). The oven temperature was programmed as follows: isotherm at 40°C for 5 min, after the temperature increase with a gradual ramp of 8°C/min up to of 280°C where it is finally maintained for 5 min. The carrier gas was helium at a constant rate set at 1.5 mL/min. Detector temperature was held at 290°C and the injector works at 280°C in splitless mode. The volume of sample injected by analysis was 1 μ L (10mg of

EO/40mLin *n*-hexane).The air and hydrogen flows were 350 and 35 mL/min, respectively.

GC/FID: A Trace Ultra GC (Thermo Electron Corporation, Interscience Louvain-La-Neuve, Belgium) coupled with a flame ionization detector was used for EO quantification. The percentage of each constituent represented the ratio of peak area on the total of GC peak areas.

GC/MS: A mass spectrometer from Agilent5973 Network Mass Selective Detector Quadrupole was associated to a gas chromatograph, Agilent Technologies 6890N (G1530N), USA. The relative abundance of the peaks of the spectra was between 50 and 550 m/z, with ionization energy of 70 eV. EOs constituents were identified by matching their mass spectra and retention indices with those from the computerized libraries (Wiley 275L and Pal 600K) and those given in the literature [26,27]. Identification was completed basing on the comparison of GC data with those of standards: α -pinene (268070), β -pinene (402753), α -phellandrene (W285609) and limonene (183164) from SIGMA ALDRICH (Boornem, Belgium).

3.2. Anti-inflammatory Assays

The anti-inflammatory activity of EOs from A. graveolens leaves was measured by in vitro inhibition of 5-LOX, enzyme whose is responsible of the inflammatory reaction. This method was inspired by those of [28,29,30], with some modifications. The inhibition of 5-lipoxygenase was measured at 234 nm using a spectrometer (Ultrospec 7000). Five oils concentrations: 12.5; 25; 50; 100 and 250 µg/ml were prepared with methanol. Quercetin, was used as a reference and methanol represented the control. To measure anti-inflammatory activity of the samples, $100 \ \mu L$ of each concentration of EOs or Quercetine were mixed with 2.4 ml of the borate buffer solution (0.2 M and pH =9.3), 100 µL of LOX (1343 U/mL) and 100 µL of linoleic acid (0.01 M). Finally, the inhibition of the active sites of the enzyme by the EOs or Quercetin prevents the reaction between the enzyme and the substrate, which results in a decrease in absorbance. The anti-inflammatory activity (percentage of inhibition of the enzyme, 5-LOX) of quercetin and the oil was evaluated after 3 minutes by the following equation (1):

% Inhibition =
$$\left(\frac{A_0 - A_i}{A_0}\right) 100$$
 (1)

 A_0 = Absorbance without HE or Quercetine A_i = Absorbance with HE or Quercetine.

3. Results and Discussion

3.1. Results

3.1.1. Essential Oils Composition

The oils extracted from both stems and leaves were colorless. The yields obtained were of $0.4\pm0.1\%$ and $1.1\pm0.2\%$ (*w/w*) for stems and leaves, respectively. Twenty-two and twenty-three compounds were identified in the stems and leaves oils, respectively. Oils were

dominated by monoterpenic hydrocarbons that represented 95.5% (S) and 94.9% (L). Sesquiterpenic hydrocarbons constituted 1.9 and 3.1%, oxygenated sesquiterpenes 0.8 and 1.0% and oxygenated monoterpenes 0.7 and 0.2% in the stems and leaves oils, respectively (Table 1). Four (4) major compounds were identified in stems and leaves oils, representing 89.1% (S) and 88.4% (L) of the total EOs content. They were α -pinene (69.3 and 68.4%), limonene (9.5 and 9.8%), α -phellandrene (5.5 and 5.9%) and β -pinene (4.8 and 4.3%) in the stems and leaves oils, respectively. Other compounds, which percentages varied between 1.0 and 2.2%, were identified in the oils. Among them, we may cite sabinene (1.3 and 1.3%), myrcene (2.1 and 1.8%), allo-ocimene (0.7 and 1.1%) and in the stems and leaves oils, respectively. (E)- β -Ocimene and γ -terpinene, have been revealed in traces in leaves oils but they were present in the stems oils at 0.1% and 0.2% respectively. There are also five compounds

in trace form in the stems but present in the leaves, these are (*E*)- β -caryophyllene, β -selinene, α -amorphene, bicyclogermacrene and δ -cadinene. Unidentified compounds represent 1.9% of *A. graveolens* EOs. They were distributed as follows 1.1 and 0.8% in the stems and leaves oils, respectively.

3.1.2. Anti-inflammatory Activity

The inflammatory reaction between enzyme 5-LOX and linoleic acid (LA) was studied. First, activity between 5-LOX and LA is measured, which is manifested by an increase of absorbance. Then, to inhibit this inflammatory reaction, *A. graveolens* EOs from Senegal was used. The results of this study showed an inhibition of the active sites of 5-LOX after incubation of it with *A. graveolens* EOs or Quercetin (control), which led to a decrease in absorbance (Figure 1). The inhibition of the enzyme is also proportional to the concentration of EOs and quercetin used (Figure 2).

Table 1. Chemical composition of the EOs from stems and leaves of Apium graveolens var. dulce

| Compounds | Retention indice | % of Compounds | | T1 |
|----------------------------|------------------|----------------|-------------|-------------------------------------|
| | | Stems | Leaves | Identification methods ^a |
| α-Pinene | 937 | 69.3±1,9 | 68.4±3.1 | RI, MS, inj. |
| Camphene | 954 | 0.3±0.0 | 0.2±0.2 | RI, MS |
| Sabinene | 976 | 1.3±0.1 | 1.3±0.1 | RI, MS |
| β-Pinene | 982 | 4.8±0.2 | 4.3±0.5 | RI, MS, inj. |
| Myrcene | 989 | 2.1±0.2 | 1.8 ± 0.1 | RI, MS |
| α-Phellandrene | 1009 | 5.5±4.9 | 5.9±5.4 | RI, MS, inj. |
| α-Terpinene | 1020 | 2.1±2.2 | 2.0±2.2 | RI, MS |
| Limonene | 1033 | 9.5±0.5 | 9.8±0.6 | RI, MS, inj. |
| (<i>E</i>)-β-Ocimene | 1046 | 0.1±0.0 | tr | RI, MS |
| γ-Terpinene | 1061 | 0.2±0.1 | tr | RI, MS |
| allo-Ocimene | 1128 | 0.7±0.5 | 1.1 ± 0.8 | RI, MS |
| Terpinen-4-ol | 1187 | 0.3±0.3 | 0.1±0.1 | RI, MS |
| α-Terpineol | 1200 | 0.4±0.2 | 0.1±0.2 | RI, MS |
| δ-Elemene | 1343 | 0.2±0.0 | 0.2±0.2 | RI, MS |
| α-Ylangene | 1375 | 0.3±0.2 | 0.3±0.2 | RI, MS |
| Not identified | 1434 | 0.4±0.1 | 0.5±0.1 | RI, MS |
| (E)-β-Caryophyllene | 1437 | tr | 1.0±0.0 | RI, MS |
| γ-Elemene | 1446 | 0.2±0.0 | 0.3±0.1 | RI, MS |
| Aromadendrene | 1460 | 0.2±0.0 | 0.1±0.0 | RI, MS |
| α-Humulene | 1468 | tr | 0.1±0.0 | RI, MS |
| β-Selinene | 1473 | tr | 0.1±0.0 | RI, MS |
| α-Amorphene | 1487 | 0.3±0.1 | 0.1±0.0 | RI, MS |
| Germacrene D | 1496 | 0.2±0.1 | 0.3±0.2 | RI, MS |
| Bicyclogermacrene | 1509 | tr | 0.2±0.1 | RI, MS |
| Not identified | 1526 | 0.3±0.1 | 0.2±0.3 | RI, MS |
| Shyobunol | 1529 | 0.7±0.7 | 0.8±0.6 | RI, MS |
| δ-Cadinene | 1533 | tr | 0.3±0.3 | RI, MS |
| Spathulenol | 1594 | 0.1±0.0 | 0.2±0.1 | RI, MS |
| Not identified | 1644 | 0.4±0.5 | 0.1±0.1 | RI, MS |
| Monoterpene hydrocarbons | | 95.5 | 94.9 | |
| Oxygenated monoterpenes | | 0.7 | 0.2 | |
| Sesquiterpene hydrocarbons | | 1.9 | 3.1 | |
| Oxygenated sequiterpenes | | 0.8 | 1.0 | |
| Total | | 98.9 | 99.2 | |
| Not identified | | 1.1 | 0.8 | |
| Oil yield (%) | | 0.4±0.1 | 1.1±0.2 | |

^aRI: Retention indices, MS: Mass Spectrometry, Inj.: Injection of pure compound, tr = trace ([<] 0.05%).

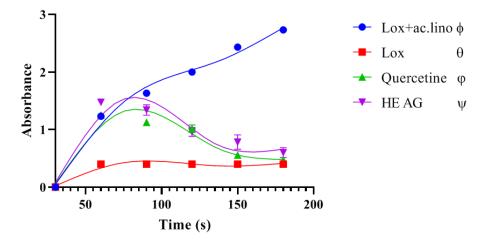


Figure 1. Evolution of absorbance as a function of time: ϕ Inflammatory reaction between 5-Lox and linoleic acid; θ 5-Lox activity; ψ Inhibition of active sites of 5-LOX enzyme by *A. graveolens* EOs. ϕ Inhibition of active sites of 5-LOX enzyme by quercetin. The absorbance is even lower than when the active sites of the enzyme are inhibited

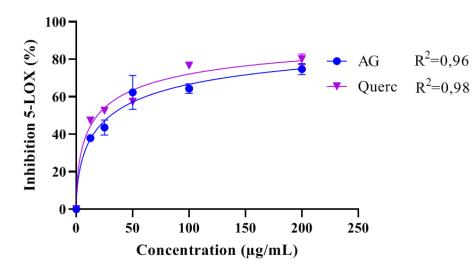


Figure 2. Evolution of the inhibitory activity of 5-LOX by A. graveolens essential oil and quercetin

3.2. Discussion

3.2.1. Essential Oils Composition

Depending on the part of the plant studied and the extraction method used, the yield of EOs can vary qualitatively and quantitatively. EOs yields of stems (0.4 \pm 0.1%) and leaves $(1.1 \pm 0.2\%)$ of A. graveolens from Senegal obtained by steam distillation showed a very significant difference. Thus, within the same species, the yield can vary depending on the organ studied. The extraction of EOs from the stems of A. graveolens from Senegal gave a lower yield than [24] from Tunisia, which obtained 0.7%. On the other hand, the yield extraction of A. graveolens leaves from Senegal is higher than the same work of [24] which obtained 0.8% and that of [25] from Tunisia (0.25%). On the other hand, it remains lower in the works reported by [31] from Lithuania, which obtained 1.7%. This difference in yield depends on several factors, including harvest period, duration of extraction, organ studied and vegetative stage of species [32,33,34]. In the literature, several studies on chemical composition of A. graveolens EOs showed different constituents. a-Pinene, limonene, α -phellandrene and β -pinene were the main constituents identified in the EOs from stems and leaves oils of A. graveolens from Senegal. These results corroborate those reported by [35] from Cuba and [24] from Tunisie. However, the majors compounds obtained A. graveolens oils from Senegal differ from those reported by [36] from Nigeria, [16] from South Korea and [25] from Tunisia. In addition, α-phellandrene content obtained in the oils is higher than the rate reported by [8] from India. Moreover, β -selinene, a characteristic compound of celery oils, is present in trace in stems oils and at 0.1% in leaves oils. The difference noted in the chemical composition of oils from Senegal and those reported in the literature could be explained by the fact that chemical composition of EOs can vary according to the site and period of harvest, organ studied, soil salinity and duration of extraction [34,37,38]. However, several authors have reported that limonene and β -selinene are the main constituents of A graveolens. Among them, we can cite [8], which obtained 72.1% of limonene and 12.1% of β -selinene, [36] identified 40.5% limonene and 16.3% β -selinene. This work disagrees with our own where limonene and β -selinene levels were relatively low (9.8-0.1% in leaves and 9.5%-(tr) in stems), respectively. On the other hand, our study corroborates the work of [25] from Tunisia. The observed compositional difference between A. graveolens found in Senegal and the rest of the world could be due to climactic and environmental conditions, chemotypes, nutritional status

of the plants, time of harvest, and other factors, which can influence EOs composition. Similarity of constituents in stalks and leaves of celery allows us to conclude that the two parts of the plant studied have the same nutritional and medicinal properties [13]. Therefore, for this sample of celery studied, it is preferable for the consumers to use it as much as stems as leaves.

3.2.2. Anti-inflammatory Activity

EOs composition of the stems and leaves is almost the same. The major compound identified in A. graveolens EOs from Senegal (a-pinene) is known for their antibacterial and antifungal activities. They are also used for the treatment of simple respiratory conditions such as colds, coughs and asthma [14]. This is why the antiinflammatory activity was only tested with oils of the leaves. The results of this study showed that the inhibition of 5-LOX is proportional to the concentration of EOs used. Consequently, the active sites of the enzyme are blocked and the reaction between the enzyme and the substrate is almost nonexistent: oils owned anti-inflammatory activity. The IC₅₀ value of HE from A graveolens (IC₅₀ = 29.5 ± 2.0 μ g/mL) compared to that of quercetin (IC₅₀ = 23.7±0.5 μ g/mL) showed a promising effect of inhibiting 5-LOX. The latter plays an important role in the pathophysiology of several inflammatory diseases [39]. This 5-LOX inhibitory activity is due on the one hand to the significant presence of α -pinene level (68.4%) found in EOs of A graveolens, and on the other hand to the presence of limonene (9.8%). These two monoterpenic compounds represented 78.2% of total chemical composition of A. graveolens EOs, and were known for their antiinflammatory properties [28,40,41,42,43]. They could act in perfect synergy to inhibit on the one hand the 5-LOX activation protein; and on the other hand by chelating the iron atom, or even, by competing with linoleic acid for occupy the active site of the enzyme of the manner of quercetin, known for its 5-LOX inhibitory power [30,44,45]. The results obtained in this study demonstrated potential inhibitory effect of EOs from A. graveolens on 5-LOX. This is important in the search of alternative sources for the treatment of inflammatory diseases involving this enzyme.

4. Conclusion

The present study is the first conducted on EOs of *A. graveolens* var. dulce from Senegal. The results showed that both stems and leaves oils were characterized by four monoterpenes hydrocarbons distributed as follows: α -pinene (69.3 and 68.5%), limonene (9.5 and 9.8%), α -phellandrene (5.5 and 5.9%) and β -pinene (4.8 and 4.3%) in the stems and leaves oils, respectively. Results of this study also showed a promising effect of inhibiting 5-LOX. In other words, *A. graveolens* EOs has an anti-inflammatory activity (IC₅₀ = 29.5±2.0 µg/mL) compared to that of quercetin (IC₅₀ = 23.7±0.5 µg/mL). This is important in the search for alternative sources for the treatment of inflammatory conditions involving this enzyme.

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Statement of Competing Interests

The authors have no competing interests.

List of Abbreviations

COX 1: Cyclooxygenase 1 COX 2: Cyclooxygenase 2 EOs: Essential Oils IL 1: Interleukin 1 IL 6: Interleukin 6 LA: Linoleic acid 5-LOX: 5-Lipoxygenase LT: Leukotirene NSAI: Nonsteroidal Anti-Inflammatory TNFα: Tumor Necrosis Factor α VOCs: Volatile Organic Compounds

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