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*Full Length Research Paper*

# Antiulcerogenic activity of *Apium graveolens* seeds oils isolated by supercritical $CO_2$

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**Isolation of volatile and fixed oils from *Apium graveolens* seeds have been obtained by supercritical fluid extraction (SFE) with carbon dioxide. The first extraction step (90 bar, 40°C) produced a volatile fraction (yield of 1.5% by weight of the charge) mainly formed by sedanolide, neocnidilide, β-selinene and 3-buthyl phtalide. The second extraction step (250 bar, 40°C) produced a fixed oil and the yield of this step was 3.4% by weight. The most represented fatty acids of fixed oil were petroselinic, linoleic, palmitic and oleic acid. Acute gastric ulcer was induced using HCl/ethanol. The volume, pH and total acidity of gastric secretion were determined by the pyloric ligature method. Treated groups received the essential oil of seeds isolated by supercritical $CO_2$ and hydrodistillation at doses (100, 200 and 300 mg/kg). The reference group (Omeprazole, 30 mg/kg) and control group (9% NaCl). Oral administration of essential oil of *A. graveolens* seeds inhibited HCl/ethanol ulcers by 59, 93 and 98%, respectively, to SFE essential oil and 72, 77 and 91%, respectively, to HD essential oil which was similar to that induced by omeprazole (71%). In the case of pylorus ligature, the essential oil reduced the volume of gastric juices and total acidity, and increased gastric pH.**

**Key words:** *Apium graveolens*, essential oil, fixed oil, fatty acids, antiulcerogenic activity, supercritical extraction, carbon dioxide.

## INTRODUCTION

The family Apiaceae is commonly known as the carrot family. It has approximately 2000 to 3000 species; out of these 174 grow in Mediterranean region. Celery has been cultivated for the last 3000 years, notably in pharaonic Egypt, and was known in China in the fifth century BC (Chevallier, 1998). Celery has been used as a food, and at various times both the whole plant and the seeds have been consumed as a medicine. The characteristic odor of celery essential oil is due to a series of phthalide derivaztives (Bjeldanes and Kim, 1977). Sedanolide, sedanonic anhydride, 3-n-butyl phthalide, and other minor phthalides are reported to be the major constituents of celery seed oil (Lund, 1978). Celery seed or celery seed extracts are used as flavoring agents for preparing herbal combinations sold as dietary supplements, and also in antirheumatic formulations. Celery seeds are also implicated in arthritic pain relief, for treating rheumatic conditions and gout (Chevallier, 1998; Bjeldanes and Kim, 1977; Satyavati and Raina, 1976). Other reports on the medicinal properties of celery seeds were related to asthma and bronchitis and, when used in combination with other herbs, to reduction of blood pressure (Chevallier, 1998; Satyavati and Raina, 1976). Nitrogenous compounds in celery seed essential oil have been reported to have effects on the central nervous system (Satyavati and Raina, 1976; Kulshrestha et al., 1967). *A. graveolens* extracts were assessed for their

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anti-inflammatory activity in intact rats by measuring the suppression of carrageen-induced paw edema (Al-Hindawi et al., 1989). However, any link between known constituents in the celery plant with the observed anti-inflammatory activity was not established.

In recent years supercritical fluid extraction (SFE) has received increased attention as an important alternative to conventional separation methods. Indeed, it has been demonstrated that SFE can produce superior quality products characterized by the absence of artifacts and by a better reproduction of the original flavor or fragrance. Supercritical fluids have adjustable extraction characteristics, due to their density, which can be controlled by changing the pressure or temperature. At lower pressures (near the critical point) volatile components, such as essential oils, are selectively extracted, while other components present in the vegetable matter such as waxes, resins and dyes have low solubility under these conditions. Globally, previous results (Reverchon, 1997; Marongiu et al., 2003; Marongiu et al., 2004) show that at extraction temperatures between 40 and 50°C and at extraction pressures lower than 100 bars, higher molecular-weight compounds are not co extracted with essential oils. A subsequent further run at higher pressure (250 bars) using the same exhausted matrix can be performed in order to obtain the fixed oil. Vegetable oils from seeds are traditionally produced by hexane extraction from ground seeds. The process is very efficient, but hexane elimination after extraction is a major problem. Three distillation units in series, operated under vacuum and other ancillary apparatus (deodorizers, degummers, etc.), have to be used. The possible thermal degradation of the oil and the incomplete hexane elimination are the main drawbacks of this process. Consequently, several authors have proposed the substitution of the traditional process by supercritical $CO_2$ (SC-$CO_2$) extraction of oil from seeds. Indeed, triglycerides forming seed oils are readily soluble in SC-$CO_2$ at 40°C and at pressures higher than about 250 bar. After extraction, the SC-$CO_2$ tryglicerides solution is sent to a separator working at sub-critical conditions. This operation reduces to near zero the solvent power of $CO_2$ and allows the recovery of oil. The complete elimination of gaseous $CO_2$ from oil is also obtained in the separator. The SFE of several seed oils has been successfully performed up to the pilot scale (Reverchon and De Marco, 2006).

Although there are many products used for the treatment of gastric ulcers, most of these drugs produce several adverse reactions (Ariphisi et al., 1986). Plant extracts are among the most attractive sources for developing new drugs and have been shown to produce promising results in the treatment of gastric ulcers (Hiruma-Lima et al., 2000a, b; Hiruma-Lima et al., 2001). In traditional medicine for example, several plants have been used to treat gastrointestinal disorders, including gastric ulcers (Toma et al., 2002).

The objective of this work was to explore the potential application of supercritical $CO_2$ to the extraction of volatile and fixed oils from *Apium graveolens* berries growing in Tunisia and antiulcer effect of fixed and essential oil from this plant.

## MATERIALS AND METHODS

### Chemicals

Triolein, trilinolein, fatty acids, fatty acid methyl esters and desferal (deferoxamine mesylate salt), were purchased from Sigma-Aldrich (Milan, Italy). All solvents used, of the highest available purity, were also purchased from Sigma-Aldrich. Methanolic HCl (3 N) was purchased from Supelco (Bellefonte, PA). cis, trans-13-Hydroperoxyoctadecadienoic acid (c,t-13-HPODE) and cis,trans-9-hydroperoxyoctadecadienoic acid (c,t-9-HPODE) were obtained from Cascade (Cascade Biochem. Ltd., London, U.K.).

All the other chemicals used in this study were of analytical grade. $CO_2$ (purity 99%) was supplied by Air Liquid Italia, Cagliari, Italy.

### Plant material

Seeds of *A. graveolens* were collected in winter 2009 from Monastir, Tunisia. Before using the vegetable matter was ground with a Malavasi mill (Bologna, Italy) and the particles sizes were in the range (250-425) μm.

### Hydrodistillation

Hydrodistillation was performed for four hours in a circulatory Clevenger-type apparatus up to exhaustion of the oil contained in the matrix, according to the procedure described in the European Pharmacopoeia (Council of Europe, 1997).

### Apparatus for supercritical fluid extraction

Supercritical $CO_2$ extractions were performed in a laboratory apparatus, equipped with a 320 $cm^3$ extraction vessel and two separator vessels of 300 and 200 $cm^3$ respectively connected in series. Extraction was carried out in a semi batch mode: batch charging of vegetable matter and continuous flow solvent. The volatile oil was obtained working at 90 bar and 40°C in the extraction vessel, at 90 bar and –10°C in the first separator and at 20 bar and 15°C in the second one. The extraction of the fixed oil was run on the same samples of seeds previously treated at 90 bar; the fixed oil was obtained working at 250 bar and 40°C in the extraction vessel and by using only one separator (at 20 bar and 15°C ) to recover the extract.

### GC and GC-MS analysis of essential oil

Analysis of the volatile extracts was carried out by gas chromatography (GC) and by gas chromatography-mass spectrometry (GC-MS).

Analytical GC was carried out in a gas chromatograph (Agilent, Model 7890A, Palo Alto, CA), equipped with a flame ionization detector (FID), an auto sampler (Agilent, Model 7683B), Agilent HP5 fused silica column (5% phenyl-methylpolysiloxane), 30 m × .25 mm i.d., film thickness 0.25 μm, and a Agilent ChemStation software system. Oven temperature was settled at 60°C, raising at

3°C/min to 250°C and then held 20 min at 250°C; injector temperature: 250°C; carrier gas: helium at 1.0 ml/min; splitting ratio 1:10; detectors temperature: 300°C.

GC-MS analyses were carried out in a gas chromatograph (Agilent, Model 6890N, Palo Alto, CA) equipped with a split-splitless injector, an autosampler Agilent model 7683 and an Agilent HP5 fused silica

column; 5% phenyl-methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm. GC conditions used were: programmed heating from 60 to 250°C at 3°C/min followed by 20 min under isothermal conditions. The injector was maintained at 250°C. Helium was the carrier gas at 1.0 ml/min; the sample (1 μl) was injected in the split mode (1:10). The GC was fitted with a quadrupole mass spectrometer, MS, Agilent model 5973 detector. MS conditions were as follows: ionization energy 70 eV, electronic impact ion source temperature 200°C, quadrupole temperature 150°C, scan rate 3.2 scan/s, mass range 30 to 480 u. Software adopted to handle mass spectra and chromatograms was a ChemStation. NIST 02 and LIBR (TP) Mass Spectra Libraries were used as references (NIST, 2002; Adams, 2007). Compounds were identified by matching their mass spectra and retention indices with those reported in the literature. Moreover, whenever possible, identification has been confirmed by injection of pure compounds. Percentage of individual components was calculated based on GC peak areas without FID response factor correction.

## Fixed oil

### Preparation of fatty acids

Separation of fatty acids was obtained by mild saponification (Rosa et al., 2011) as follows: 3 mg of the fixed oil were dissolved in 5 ml of EtOH and 100 µL of Desferal solution (25 mg/mL of $H_2O$), 1 mL of a water solution of ascorbic acid (25% w/v), and 0.5 mL of 10 N KOH were added. The mixtures were left in the dark at room temperature for 14 h. After addition of 10 mL of n-hexane and 7 ml of $H_2O$, samples were centrifuged for 1 h at 900 g. The hexane phase was collected, and after addition of further 10 mL of n-hexane to the mixtures, samples were acidified with 37% HCl to pH 3-4 and then centrifuged for 1 h at 900 g. The hexane phase (saponifiable fraction) with free fatty acids and conjugated diene fatty acids hydroperoxides (HP) was collected and the solvent was evaporated. A portion of the dried residue was dissolved in 500 µl of $CH_3CN$ with 0.14% $CH_3COOH$ (v/v) and aliquots of the samples were injected into the HPLC system.

An aliquot of dried fatty acids was methylated with 1 ml of methanolic HCl (3 N) (Christie, 1993) for 30 min at room temperature. After addition of 4 ml of n-hexane and 2 ml of $H_2O$, samples were centrifuged for 20 min at 900 g. The hexane phase with fatty acid methyl esters was collected, the solvent was evaporated, the residue was dissolved in 250 µl of n-hexane and aliquots of the samples were injected into the GC system. The recovery of fatty acids during saponification was calculated by using an external standard mixture prepared dissolving 1 mg of triolein and trilinolein in 5 mL of EtOH and processed as samples. All solvents evaporation was performed under vacuum.

### HPLC analyses

Analyses of unsaturated fatty acids, and oxidative products were carried out with an Agilent Technologies 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a diode array detector. Analyses of unsaturated fatty acids and HP, detected at 200 and 234 nm, respectively, were carried out with a XDB-$C_{18}$ Eclipse (150 × 4.6 mm, 3.5 µm particle size) (Agilent Technologies) equipped with a Zorbax XDB-$C_{18}$ Eclipse (12.5 × 4.6 mm, 5 µm particle size) guard column (Agilent Technologies), with a mobile phase of $CH_3CN/H_2O/CH_3COOH$ (75/25/0.12, v/v/v), at a flow rate of 2.3 ml/min (Rosa et al., 2011). The temperature of the column was maintained at 37°C. The identification of fatty acids and HP was made using standard compounds and the second derivative, as well as conventional UV spectra, generated with the Agilent Chemstation A.10.02 software. Calibration curves of all of the compounds were constructed using standards and were found to be linear with correlation coefficients > 0.995.

### GC analysis

Fatty acid methyl esters were measured on a gas chromatograph Hewlett-Packard HP-6890 (Hewlett-Packard, Palo Alto, USA) with a flame ionisation detector and equipped with a cyanopropyl methyl-polysiloxane HP-23 FAME column (30 m × 0.32 mm × 0.25 µm) (Hewlett-Packard). Nitrogen was used as carrier gas at a flow rate of 2 ml/min. The oven temperature was set at 175°C; the injector temperature was set at 250°C; and the detector temperature was set at 300°C. The fatty acid methyl esters were identified by comparing the retention times to those of standard compounds. The composition of individual fatty acid was calculated as a percentage of the total amount of fatty acids (g%), using the Hewlett-Packard A.05.02 software.

## Antiulcerogenic activity

### Animals

Wistar rats of both sexes, weighing 150 to 200 g, provided from Siphat pharmaceutical company (Tunis, Tunisia) were used in this study. The animals were maintained under standard environmental conditions and had free access to standard rodent feed and water. Housing conditions and *in vivo* experiments were approved according to the guidelines established by the European Union on Animal Care (CCE Council 86/609).

### Gastric lesions induced by HCl/ethanol

The anti-ulcerogenic activity was evaluated by the HCl/EtOH method which causes injury to the gastric mucosa (Hara and Okabe, 1985). A total of 80 rats fasted for 24 h (with free access to water) were divided into 8 groups (10 rats/group). Sex treated groups received orally the essential oil (HD and SFE) extracts of *A. graveolens* dissolved in 1% Tween-80 aqueous solution at the concentration (100, 200 and 300 mg/kg), vehicle (1% Tween-80 aqueous solution). The reference group was given a solution of omeprazole (Mopral®, 30 mg/kg) and the control group received a 9% NaCl solution (5 mL/kg). After 30 min, all groups were orally treated with 1 ml of HCl/EtOH (40:60, v/v) solution for gastric ulcer induction. The animals were killed 1 h after the ulcerogenic agent administration; their stomachs were excised and opened along the great curvature, washed and stretched on cork plates. The surface was examined for the presence of lesions and the extent of the lesions was measured. The summative length of the lesions along the stomach was recorded as lesion index (mm).

### Gastric secretion parameters

Gastric secretion volume, pH and HCl concentration were measured according to the method of Shay et al. (1945). All groups of rats were fasted 24 h, with free access to water. Immediately

**Table 1.** Retention indices, $I_R$, and chromatographic area percentages of constituents of *Apium graveolens* seeds oil obtained by SFE and by HD.

| $I_R$ | Compound | SFE | HD | Identification[a] |
|---|---|---|---|---|
| 938 | α-pinene | - | 0.4 | MS, $I_R$, Inj |
| 976 | sabinene | - | 0.1 | MS, $I_R$, Inj |
| 980 | β-pinene | 0.1 | 3.3 | MS, $I_R$, Inj |
| 992 | myrcene | 0.1 | 2.6 | MS, $I_R$, Inj |
| 1031 | limonene | 3.4 | 39.4 | MS, $I_R$, Inj |
| 1161 | pentyl cycloexa-1,3-diene | 0.8 | 4.0 | MS, $I_R$ |
| 1256 | piperitone | - | 0.3 | MS, $I_R$ |
| 1299 | trans-pinocarvyl acetate | - | 0.3 | MS, $I_R$ |
| 1419 | β-caryophyllene | 0.3 | 0.7 | MS, $I_R$, Inj |
| 1471 | 7-epi-1,2-dehydro-sesquicineole | - | 0.3 | MS, $I_R$ |
| 1486 | β-selinene | 8.2 | 15.4 | MS, $I_R$ |
| 1488 | n.i. | - | 0.2 | |
| 1494 | α-selinene | 1.4 | 2.5 | MS, $I_R$ |
| 1522 | myristicin | 0.4 | 1.3 | MS, $I_R$ |
| 1527 | kessane | 0.1 | 0.3 | MS, $I_R$ |
| 1581 | caryophyllene oxide | 0.1 | 0.3 | MS, $I_R$, Inj |
| 1599 | 6-methoxy elemicin | 0.1 | 0.1 | MS, $I_R$ |
| 1625 | dill apiole | 0.3 | 0.7 | MS, $I_R$ |
| 1635 | 3-butylhexahydro phtalide | 0.1 | 0.2 | MS, $I_R$ |
| 1651 | 3-butyl phtalide | 6.2 | 4.8 | MS, $I_R$ |
| 1677 | n.i. | 0.2 | 0.5 | |
| 1682 | apiole | 1.0 | 1.5 | MS, $I_R$ |
| 1685 | α-bisabolol | 0.2 | 0.3 | MS, $I_R$ |
| 1720 | sedanolide | 60.4 | 9.0 | MS, $I_R$ |
| 1726 | neocnidilide | 15.8 | 11.0 | MS, $I_R$ |
| 1733 | *Z*-ligustilide | 0.5 | 0.5 | MS, $I_R$ |
| Monoterpene hydrocarbons | | 4.5 | 49.7 | |
| Oxygen containing monoterpenes | | 83.0 | 26.1 | |
| Sesquiterpene hydrocarbons | | 9.9 | 18.6 | |
| Oxygen containing sesquiterpenes | | 0.3 | 1.2 | |
| Phenylpropanoids | | 1.8 | 3.7 | |
| Total identified | | 99.7 | 99.3 | |

[a]Identification has been realized by comparing mass spectra (MS), retention indices ($I_R$) and by injection of authentic compound (Inj).

after pylorus ligature, solution of NaCl 9% (5 mg/kg), *A. graveolens* essential oil and omeprazole (30 mg/kg) were administered intraduodenally. After 4 h, the animals were killed by cervical dislocation, the abdomens were opened, the stomachs were removed and the gastric content was collected to determine the total amount of gastric juice (ml) and pH values were measured by a pH meter. Total acid in the gastric secretion was determined by titration to pH 7.0 with 0.1 N NaOH.

### Statistical analysis

The statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's t-test for multiple comparisons. The significance of difference was accepted at $p < 0.05$.

## RESULTS AND DISCUSSION

A comparison of the composition of the volatile oil obtained by supercritical $CO_2$ extraction (SFE) at 90 bar and of the essential obtained by hydrodistillation (HD) is reported in Table 1 from which main facts can be inferred: the identified components are the same in all the extracts but the quantity is different; substantially high amounts of oxygenated monoterpenes are predominant in SFE volatile oil (83.0 vs. 26.1%) while the hydro

**Table 2.** Composition (%) of fatty acids of *A. graveolens* seed fixed oil by GC.

| Fatty acid | Classification | g/100 g |
|---|---|---|
| Lauric acid | 12:0 | 0.08 ± 0.01 |
| Miristic acid | 14:0 | 0.22 ± 0.01 |
| Palmitic acid | 16:0 | 7.02 ± 0.01 |
| Palmitoleic acid | 16:1 *n-7* | 0.21 ± 0.01 |
| Stearic acid | 18:0 | 1.95 ± 0.04 |
| Cis-vaccenic acid | 18:1 *n-7* | 0.44 ± 0.01 |
| Oleic acid | 18:1 *n-9* | 0.52 ± 0.02 |
| Petroselinic acid | 18:1 *n-12* | 57.85 ± 0.02 |
| Linoleic acid | 18:2 *n-6* | 17.14 ± 0.02 |
| α-linolenic acid | 18:2 *n-3* | 0.42 ± 0.04 |
| Arachid acid | 20:0 | 4.67 ± 0.30 |
| Gondonic acid | 20:1 *n-9* | 0.26 ± 0.03 |
| SFA | | 13.93 ± 0.26 |
| MUFA | | 65.28 ± 0.01 |
| PUFA | | 17.56 ± 0.01 |

SFA, satured fatty acids,; MUFA, monosatured fatty acids; PUFA, polysatured fatty acids; means and standard deviation of 4 samples.

**Table 3.** Unsaturated fatty acids (UFA) composition (mg/g weight) of *Apium graveolens* seed fixed oil by HPLC.

| UFAs | Value | *sd* |
|---|---|---|
| 18:1 *n-9* | 41.34 | 1.67 |
| 18:1 *n-12* | 367.01 | 3.86 |
| 18:2 *n-6* | 116.59 | 1.55 |
| 18:3 *n-3* | 3.22 | 0.03 |

Data are mean values (4 samples) with standard deviations (*sd*).

distillated oil is rich in monoterpenes hydrocarbon (49.7 vs. 4.5%). The SFE volatile oil is mainly formed by sedanolide (60.4%), neocnidilide (15.8%), β-selinene (8.2%) and 3-buthyl phtalide (6.2%). The main components in HD oil are: limonene (39.4%), β-selinene (15.4%), neocnidilide (11.0%), sedanolide (9.0%) and 3-buthyl phtalide (4.8%).

On the exhausted matrix a further extraction at higher pressure (250 bar) and 40°C ($\rho_{CO2}$ = 0.886 g $cm^{-3}$) was performed for the extraction of fixed oil, with a yield of 3.4%.

Quali-quantitative information on the individual fatty acids that compose the lipid classes of *A. graveolens* seeds fixed oil was obtained by GC analysis. The composition of fatty acids present in the saponifiable matter of oil is shown in Table 2 and expressed as percentage of total fatty acids.

Fixed oil showed a concentration of approximately 14% of saturated fatty acids (mainly 16:0, 18:0 and 20:0), 65% of monounsaturated (mainly 18:1 *n-12* and 18:1 *n-9*, 57.8 and 6.5%, respectively), and 17.6% of polyunsaturated (mainly 18:2 *n-6* and 18:3 n-3). Petroselinic acid (18:1 *n-12*), the unusual positional isomer of oleic acid (18:1 *n-9*), has been found to be the major component of the triacylglycerols of fixed oil from *A. graveolens* seeds, as previously reported (Ngo-Duy et al., 2009).

By HPLC, the unsaturated fatty acid content in the fixed oil was detected as follows: 367.01 mg/g of 18:1 *n-12*, 41.34 mg/g of 18:1 *n*-9, 116.59 mg/g of 18:2 *n-6*, and a minor amount of 18:3 *n-3* as reported in Table 3.

Administration of the essential oil of *A. graveolens* inhibited the formation of ulcerative lesions induced by absolute ethanol. The percentage of ulcer inhibition was 59, 93 and 98% in animals pretreated with SFE essential oil at doses of 100, 200 and 300 mg/Kg, respectively, and ulcer inhibition was 72, 77 and 91% in animals pretreated with SFE essential oil at doses 100, 200 and 300 mg/Kg, respectively compared to the control group (32.6 mm). The animals that received Omeprazole (30 mg/Kg) presented an inhibition of the lesions corresponding to (71%) (Table 4).

In order to rule the possibility that the protective effect

**Table 4.** Effect oral administration of *Apium graveolens* seeds essential and fixed oil on gastric ulcer induced by HCl/EtOH in rats.

| Group (n = 6) | Dose mg/kg) | Ulcer index (mm) | Inhibition (%) |
|---|---|---|---|
| Control | - | 32.60 ± 6.8 | - |
| | 100 | 24.10 ± 2.69 | 59 |
| SFE essential oil | 200 | 3.00 ± 1.05 ** | 93 |
| | 300 | 0.50 ± 0.34 ** | 98 |
| | 100 | 13.90± 3.04 ** | 72 |
| HD essential oil | 200 | 12.10 ± 1.20** | 77 |
| | 300 | 4.54 ± 1.03** | 91 |
| SFE fixed oil | 300 | 34.10 ± 6.86 | 21 |
| Omeprazole | 30 | 12.50 ± 3.56 ** | 71 |

Data are expressed as mean ± S.E.M. (n=6), *p<0.05; **p<0.01. The statistical analysis was performed using one-way analysis of variance (ANOVA) followed by t Student test.

**Table 5.** Effect of *Apium graveolens* essential and fixed oil administered intraduodenally, on gastric secretion parameters in Wistar rats subjected to pylorus-ligature.

| Treatment | Dose (mg/Kg) | Gastric juice (mL) | pH (units) | Total acidity Mequiv.[H+]./mL/4h |
|---|---|---|---|---|
| Control | - | 3.14 ± 0.52 | 1.58 ± 0.14 | 121.45 ± 12.79 |
| | 100 | 0.67 ± 0.20* | 3.25 ± 0.25** | 45.20 ± 0.67* |
| EO (SFE) | 200 | 0.89 ± 0.06* | 2.28 ± 0.18** | 55.67 ± 4.6* |
| | 300 | 0.55 ± 0.1** | 2.00 ± 0.26** | 50.40± 4.05* |
| | 100 | 0.92 ± 0.15* | 2.36 ± 0.15* | 61.49 ± 4.06* |
| EO (Hydro) | 200 | 0.87 ± 0.07* | 2.27 ± 0.19* | 56.27 ± 4.53* |
| | 300 | 0.78 ± 0.05* | 2.36 ± 0.20* | 56.90 ± 3.67* |
| Fixed oil | 300 | 0.98 ± 0.12 | 2.21 ± 0.24 | 62.00 ± 2.23* |
| Omeprazole | 30 | 0.76 ± 0.16* | 3.49 ± 0.39* | 48.40 ± 7.86* |

Data are represented as mean ± S.E.M. (n = 8); *p < 0.05; **p < 0.01 and ns: not significant. The statistical analysis was performed using one-way analysis of variance (ANOVA) followed by t Student test for multiple comparison. (EO (SFE): Essential oil extracted by SFE; EO (Hydro): Essential oil extracted by hydro distillation).

of essential oil mucosa depend on local action, direct contact of the oil with the mucosa forming a protective barrier to the action of ethanol. Animals treated with the fixed oil *A. graveolens* showed no anti-ulcer activity (300 mg/kg, 21%).

After 4 h of pylorus ligature it was observed that the essential oil of *Apium graveolens*, when administered intraduodenally, decreased the gastric volume secreted when compared the control group (3.8 ± 0.2 ml) at all doses. As for the pH of gastric juices, the doses of 100, 200 and 300 mg/kg of SFE essential oil from *A. graveolens* decreased pH by 3.25, 2.28 and 2.00 respectively, rats treated with HD essential oil get a pH of gastric juice by 2.36, 2.27 and 2.36 respectively, when compared to the control and reduced the total acidity ($H^+$ concentration) of acid secretion by 1.58 and Omeprazole modified these three parameters, reducing the volume and gastric acidity, while increasing the pH (3.49) of gastric juices (Table 5). This study investigated the antiulcerogenic activity of the essential oil dried seeds of *A. graveolens* on gastric lesions induced by different necrotizind agents and as well as acid secretion parameters.

This is the first report establishing the antiulcerogenic activity of the essential oil of this species. The results obtained demonstrate that the essential oil of dried seeds of *Apuim graveolens* had gastroprotective effects on both models tested. Necrotizing agents such as ethanol and HCl-induce the formation of gastric mucosal injury, an effect that involves depression of gastric defense mechanisms and gastric blood flow stasis (Andreo et al., 2006). In addition, ethanol releases free radicals,

increasing lipid peroxidation, decreasing production of gastric mucus and inhibiting prostaglandins (Abdel-Salam, 2001). In model of HCl/ethanol-induced ulcer, *A. graveolens* displayed a significant gastroprotective dose related affect at all doses administered orally, which demonstrates that the antiulcer activity important.

These results indicate that the essential oil exhibits probably acts to increase the protective elements of the gastric mucosa, such as mucus and bicarbonate, since it inhibited the formation of ulcerative lesions in this model. In the pyloric ligature method, the digestive effect of accumulated gastric juices and interference in gastric blood flow are responsible for inducing ulceration (Patel et al., 2000). In this model, treatment with the essential oil of *A. graveolens* changed all gastric secretion parameters, reduced the volume of gastric juices and total acidity, and increased gastric pH when compared with the control group. The results suggest that the pharmacological mechanism of the oil may be related to antisecretory activity, via the receptor system or mediators of the parietal cell.

## Conclusion

The activities shown in this study contribute to chemical and pharmacological properties of some of the species studied such as *A. graveolens*. The results indicate that the essential oil of *A. graveolens* seeds has an antiulcerogenic activity, as evidenced by its significant inhibition of the formation ulcers in both models. This effect could be related to an increase in gastric mucosal defensive factors. Further pharmacological studies are conducted to provide more accurate elucidation of the mechanism of action involved in this activity.

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