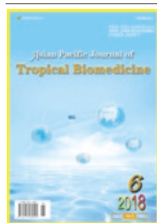

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Coriandrum sativum grown under organic or chemical fertilizer effectively prevents DNA damage: Preliminary phytochemical screening, flavonoid content, ESI (−) FT−ICR MS, *in vitro* antioxidant and *in vivo* (mice bone marrow) antimutagenic activity against cyclophosphamide

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ABSTRACT

Objective: To evaluate the influence of fertilization and phenological stages on secondary metabolites production and chemoprotective effects of *Coriandrum sativum* (*C. sativum*) L. **Methods:** The plants were grown under organic or chemical fertilizer, collected at vegetative and flowering development stages and their hydroalcoholic extracts were analyzed by phytochemicals methods, mass spectrometry, antioxidant and antimutagenic assays. **Results:** All extracts exhibited metabolites such as coumarins, flavonoids and steroids, and mass spectrometry showed similar molecular peaks among the extracts evaluated, suggesting the presence of palmitic and α-linolenic acids. Vegetative *C. sativum* extract grown under chemical fertilizer showed better antioxidant activity, according to the DPPH assay. Vegetative *C. sativum* extracts grown under organic and chemical fertilizer were able to effectively reduce micronucleous frequency in the simultaneous and pre-treatment protocols, especially reaching 55.90% of damage reduction in the pre-treatment protocol. **Conclusions:** These findings suggest that chemical fertilization promotes an increase in the content of flavonoids in *C. sativum* and, consequently, leads to better antioxidant and antimutagenic activities, as well as reinforces the potential uses of this culinary plant in health promotion and disease prevention.

1. Introduction

Plants have been traditionally used as food and in the treatment and prevention of various diseases. The variability of effects that these plants exhibit is related to their capacity to produce many components, especially components of secondary metabolism[1]. Secondary metabolites are not directly related to the growth and

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development of the plant, being synthesized with different purposes, such as attractive agents, intra and interspecific interactions and defense against predators and parasites[2]. These substances may aid in the prevention and treatment of several diseases such as cancer, inflammation, cardiovascular and neurodegenerative diseases[3]. Moreover, endogenous factors, such as plant species and stage of development, and exogenous factors, such as the absence or excess of production factors, can interfer in the production of secondary metabolites[4,5].

Apiaceae family consists of several species which are widely used as condiments and in prevention of disease due to their antioxidant and/or pharmacological and antiproliferative properties[6,7].

Coriandrum sativum (*C. sativum*) L. or coriander, belonging to the Apiaceae family, is known for its aromatic, culinary and medicinal properties and can be used as a natural medicine and also in the cosmetic industry[8]. *C. sativum* presents bioactive compounds that are accounted for a wide range of pharmacological activities such as anti-inflammatory, anxiolytic, antimicrobial, diuretic, cognition improvement, antidiabetic, antiseptic, antioxidant, anticancer and neuroprotective activities[9,10]. These biological activities showed by *C. sativum* have been related to the presence of many phytochemicals such as camphor, geraniol, α–pinene, γ–terpinene, geranyl acetate, limonene and linalool, which are abundantly found in seeds and recognized for their abilities to modulate key pathogenesis pathways of diseases[9,10].

The formation of free radicals by oxygen is associated to the development of cancers and heart disease. On the other hand, good antioxidant substances may act as antimutagenic and antigenotoxic agents and be able to reduce the action of chemical or physical agents that induce damage to genetic material[11]. The phytochemicals of natural products can present therapeutic applications, since they can act as antioxidants and prevent genomic damage. Therefore, it is indispensable for the therapeutic validation of medicinal plants to characterize their chemical composition and possible biological activities *in vitro* and *in vivo*.

Some researchs showed that most carcinogens are mutagens and they are strongly correlated with mutagenesis and carcinogenesis[12]. The ability to control cell response against mutagens through diet provides a new way for prevention and control of cancer. The micronucleus assay *in vivo* is commonly used to investigate mutagenic effects and is recommended by regulatory agencies around the world as part of safety assessment of products[13]. In addition, the micronucleus test in rodents can also indicate the ability of medicinal plant compounds to prevent or avoid mutagenic damage by antimutagenicity assays.

In this context, this study aimed to evaluate the influence of fertilization and phenological stages on secondary metabolites production and chemoprotective effects of the crude hydroalcoholic extracts (CHEs) of *C. sativum* aerial parts.

2. Materials and methods

2.1. Cultivation and obtaining *C. sativum* extracts

The cultivation of *C. sativum* was carried out under field conditions in the municipality of Venda Nova do Imigrante/ES (GPS 20º23’30.6” S 41º07’04.1” W) during the period from December 2014 until January 2015. Considering the type of fertilizer used, two treatment groups were established: one with soil supplemented with bovine manure without the presence of herbicides (organic fertilizer) and the other with soil supplemented with NPK (4-14-8) at a concentration of 150 g/m (chemical fertilizer). Each site was the size of 2 m × 6 m, the seedlings were transferred to the beds using the spacing between seedlings of 25 cm × 10 cm and were irrigated daily. The soil composition analysis was conducted by the Agronomic Analysis Laboratory and Consulting LTDA- FULLIN (Linhares/ES), methodology as described by Da Silva *et al*.[14].

The plants were collected in the vegetative stage and flowering for 45 and 55 d, respectively, after the transfer of seedlings to the beds. The aerial part of the plant was dried in oven at 40 ℃ for 72 h. Subsequently, the dried plant material was triturated and subjected to maceration in hydroalcoholic solution at 70% v/v for 72 h. Each sample was filtered and rotary evaporator was used to remove solvents under low pressure to obtain the CHEs of *C. sativum*.

2.2. Preliminary phytochemical

The Phytochemical prospecting was carried out according to the method of Costa[15] in order to identify secondary metabolites groups such as alkaloids, flavonoids, saponin triterpenoids, steroids, tannins, coumarins and anthraquinone present in CHEs from vegetative and flowering stages grown under conditions of chemical and organic fertilizers.

2.3. Spectrometric quantification of flavonoids

The flavonoid concentration was determined by spectrometric quantitation according to the methodology proposed by ANVISA[16].

2.3.1. Standard curve to flavonoids

An amount of 25 mg of rutin were dissolved in methanol, the volume was completed to 50 mL in order to yield final concentration of 0.5 mg/L. This solution was withdrawn 4 aliquots (0.25, 0.5, 1.0 and 1.5 mL), which were placed in a 25 mL volumetric flasks. Each of these volume was made up to 2 mL with methanol and then was

added in each volumetric flask: 0.6 mL of glacial acetic acid; 10 mL of pyridine and water solution (20:80); 2.5 mL of aluminum chloride solution in 6.5% methanol and then distilled water was added to the mixture until the volume of 25 mL was complete. After 30 min, the absorbance was taken in a spectrophotometer at 420 nm. For the calibration of the spectrophotometer all reagents listed above (except rutin and aluminum chloride) were used as "white" solution. As the absorbance of the solvents used in photometric tests may influence the colorimetric test results, it was necessary to prepare the solution "white", a solution containing all the solvents used in this test, to perform the calibration of the spectrophotometer and to measure its influence on the obtained obsorbancies. Analyses were performed in triplicate.

2.3.2. Quantification of flavonoids using CHEs

A total of 50 mg of CHEs were weighed, diluted in 15 mL of ethanol and, after this addition, methanol was used to complete the volume to 50 mL. This solution was removed with a 250 μL aliquot, which was poured into a volumetric flask. The volume was completed to 8 mL with methanol, and were added with: 0.6 mL of glacial acetic acid; 10 mL of pyridine and water solution (20:80); 2.5 mL of aluminum chloride in 6.5% methanol. Then the volume was completed to 25 mL with distilled water. Readings were performed in a spectrophotometer at 420 nm, using as solution "white" all reagents listed above (except rutin and aluminum chloride) for calibration.

2.4. Mass spectrometry

The *C. sativum* hydroalcoholic extracts were solubilized in 1 mL of methanol. Ten μL of the diluted extract were produced again in 1 mL of acetonitrile solution and water basified with NH_4OH. The resulting solution was analyzed by ESI (-) FT-ICR MS.

The solutions were analyzed by direct infusion at a flow rate of 5 μL/min for the electrospray source in negative ion mode of acquisition [ESI (-)], and acquired on a region *m/z* 200 to 1 000. The [ESI (-)] source conditions were following: nebulizer gas pressure of 1.0 bar, a capillary voltage of 3.2 kV and the capillary temperature 250 ℃. The ion accumulation time was 5.10-4 s, each spectrum has been acquired by accumulation of 32 scans with a time domain 4 Mega-point[17].

All FT-ICR MS spectra were externally calibrated using a solution NaTFA (*m/z* 200 until 1 200). A resolving power of 500 000, *m/z* of 428 and a mass less accuracy than 1 ppm provided unambiguous molecular formulas monocarregados molecular ions. FT-ICR MS spectra were acquired and processed using the Data Analysis software (Bruker Daltonics, Bremen, Germany). The degree of introduction for each molecule was determined from the value of double bond equivalent (DBE)[18] by equation 1:

$$DBE = c - h/2 + n/2 + 1 \quad (1)$$

Where c, h, and n correspond to the numbers of carbon, hydrogen and nitrogen, respectively, in the given minimum formula from FT-ICR MS data.

Hydrogen deficiency provides a measure of aromaticity and, in addition, DBE is a more direct index used to define the number of rings plus double bonds. DBE indicates the presence of double and/or triple bonds, as well as cyclic systems in its structure.

2.5. Antioxidant activity

The antioxidant activity was evaluated by the test of scavenging activity of stable free radical 2,2-diphenyl-1-picryl-hidrazila (DPPH) in microplate[19], expressed as percentage, compared to the control ascorbic acid. The antioxidant substances in extracts react with DPPH radical which is stable, and convert it into 2,2-diphenyl-1-picryl hydrazine. The degree of discoloration indicates the potential antioxidant of extract.

An aliquot of 200 μL of methanol solution of DPPH 120 mg/L was added to 100 μL of ethanolic extract of *C. sativum*. After 30 min in the dark, there was the absorbance read at 517 nm in a spectrophotometer for microplate (Epoch Microplate Spectrophotometer - BioTek®). Concentrations extract were used: 1 000.00, 500.00, 250.00, 125.00, 62.50, 31.25 and 15.62 μg/mL, in triplicate. Trolox solution was used as control and "white" corresponding to the DPPH assay. Methyl alcohol was used for calibration of the spectrophotometer.

The calculations were performed with the aid of the following formula[20]:

$$\%\text{Discoloration of DPPH} = \frac{\text{Abs. control} - (\text{Abs. sample} - \text{Abs. sample blank})}{\text{Abs. control}} \times 100$$

Where Abs. control is white absorbance of DPPH test at 517 nm; Abs. sample is sample absorbance at 517 nm; Abs. sample blank is blank sample absorbance at 517 nm.

2.6. Micronucleus assay

2.6.1. Animals

All experiments were conducted in accordance with standards established by the Ethics Committee on Animal Use of the Federal University of Espirito Santo (CEUA / UFES – Certificate number – 019 / 2015).

A total of 96 albino male mice Swiss strain (Mus musculus) were used, aged 6 to 8 weeks and approximately 40 g of body weight (bw). Mice were randomly separated into 16 experimental groups, 6 animals per group (n = 6).

The mice were placed in polypropylene cages with metal bars and wood shavings. They passed an acclimatization period of 7 days in vivarium pass before the start of the experiments, with free access to

standard commercial feed and water, and they were kept under light-dark cycles of 12 hours.

The antimutagenicity tests were carried out from the analysis of the results of phytochemical and antioxidant activity. Following previous analyzes, two experimental groups were established: organic and chemical fertilization at vegetative stage.

2.6.2. Micronucleus test in rat bone marrow cells

The micronucleus test in bone marrow was performed according to the methodology described by Krishna and Hayashi[21]. The evaluation of the antimutagenic effect of extracts of *C. sativum* against damage induced by cyclophosphamide was performed according to the protocols of simultaneous treatment and pre-treatment[22].

In the simultaneous treatment, all three concentrations tested extract (50, 100, 200 mg/kg bw) were administered a single dose by gavage and then the mice were given cyclophosphamide intraperitoneally (100 mg/kg bw). After 24 hours of the administration of extracts and cyclophosphamide, euthanasia was performed.

In the pre-treatment protocol, mice were treated via gavage, once daily, always at the same time for seven consecutive days with *C. sativum* extracts at the three concentrations tested (50, 100 and 200 mg/kg bw). On the 8th day of treatment, animals received mutagen cyclophosphamide i.p. (100 mg/kg bw) and 24 h after this application, the animals were sacrificed by cervical dislocation.

For each experimental group were used six Swiss mice ($n = 6$), which were administered proportional volumes of extract the body weight of each animal. As a negative control, in the experimental group, the mice received a single dose of saline 0.9% NaCl (via gavage), which were euthanized 24 h after this application. The animals of the experimental group received cyclophosphamide as positive control, with a single dose, at a concentration of 100 mg/kg bw and 24 hours after application the animals were sacrificed.

Subsequent to euthanasia, the bone marrow of the femur from each animal was sampled, injecting 0.5 mL fetal bovine serum to a syringe and centrifugated for 10 min at 1 000 rpm. The supernatant was discarded and the material was subjected again to centrifugation for 10 min at 1 000 rpm. By smear method is sewed 2 slides per animal, fixed in methanol for 10 min and stained with eosin-methylene blue Leishman.

The slides were examined under an optical microscope (Nikon Eclipse E200) with an increase of 1 000 times, and recorded in 2 000 polychromatic erythrocytes (PCE) per animal, 1 000 PCE per slide, considering the micronucleated polychromatic erythrocytes (PCEMNs). Cytotoxicity analysis was determined by the relationship between PCE and normochromatic erythrocytes (NCEs) for the reason PCE / PCE + NCE in a total of 200 cells counted per slide.

The percentage reduction in damage (decrease of frequency of micronucleated cells) at different concentrations and protocols were calculated according to Serpeloni *et al.*[23], using the formula:

$$(\%)\ \text{Reduction} = \frac{\text{PCEMN frequency in A} - \text{PCEMN frequency in B}}{\text{PCEMN frequency in A} - \text{PCEMN frequency in C}} \times 100$$

Where “A” is the group of cells treated with cyclophosphamide (positive control); “B” is the group of cells treated with *C. sativum* extract and “C” is the negative control group (0.9% NaCl).

2.7. Statistical analysis

Data were evaluated a priori by normality test. For statistical analysis were used the Kruskal-Wallis test, with significance level of 5%. All statistical tests were performed with the aid of Assistat 7.6 beta software.

3. Results

3.1. Soil chemical analysis

According to the soil chemical analysis (Table 1), it was found that all treatments presented high levels of the nutrientes phosphor (P), potassium (K), calcium (Ca) and organic matter (OM) when compared to reference values. The registered acidity also varied from

Table 1

Chemical analysis of soils under organic and chemical fertilizing conditions used for growth of *C. sativum* plants. (Source: FULLIN - Agronomic and Environmental Analysis Laboratory).

Parameter	Vegetative *C. sativum*		Flowering *C. sativum*		Reference value*
	Organic fertilizing	Chemical fertilizing	Organic fertilizing	Chemical fertilizing	
Phosphor - Mehlich (mg/dm^3)	104.0	60.0	73.0	150.0	30.0- 60.0
Potassium (mg/dm^3)	440.0	190.0	280.0	440.0	80.0-200.0
Calcium ($cmol_c/dm^3$)	7.0	4.0	4.0	8.8	1.5-4.0
Magnesium ($cmol_c/dm^3$)	1.7	0.6	0.6	0.8	0.6-1.0
Aluminum ($cmol_c/dm^3$)	0.1	0.1	0.1	0.5	0.4-1.0
H+Al ($cmol_c/dm^3$)	4.2	5.2	5.0	6.8	2.6-5.0
Organic matter (dag/dm^3)	3.7	4.1	3.7	3.5	1.6-3.0
pH measured at water	5.6	5.6	5.6	5.0	High acidity ≤ 5.0

*Reference values within the range described were considered middling. Lower and higher reference values were considered low and high, respectively.

medium to high. Therefore, *C. sativum* chemically fertilized showed higher content of the analyzed nutrients and the higher content of OM was observed in the cultivation with organic fertilizer.

3.2. Phytochemical analysis

The results obtained from the phytochemical analysis of the hydroalcoholic extract of the *C. sativum* leaves indicated the presence of flavonoids, coumarins and steroids in all treatments. Tannins, saponins, alkaloids, triterpenes and anthraquinones were not found.

3.3. Quantification of flavonoids

The absorbance values from samples of the hydroalcoholic extract of *C. sativum* were compared with the rutin calibration curve 0.5 mg/mL. Flavonoid contents of the *C. sativum* crude hydroalcoholic extracts in vegetative stage were (11.81 ± 0.32) µg/mL under organic fertilizing and (9.83 ± 0.25) µg/mL under chemical fertilizing. Besides, in flowering stage the contents of flavonoid were (8.26 ± 0.28) µg/mL under organic fertilizing and (8.34 ± 0.63) µg/mL under chemical fertilizing.

The results showed that the concentration of flavanoids found in the analysed *C. sativum* extracts were higher in the plants that were cultivated in the vegetative stage, regardless of the type of fertilization applied. In regard to fertilization, the highest flavanoids content were observed in the plants cultivated with organic fertilizers. In regard to the flowering stage with organic fertilization, lower flavanoids concentration were found, however the *C. sativum* cultivated with chemical fertilizers in this same stage showed a difference smaller than 0.1 µg/mL.

3.4. Mass spectometry

The Figure 1 showed the mass spectra in the mode ESI(-)FT-ICR MS of the *C. sativum* hydroalcoholic extracts that were cultivated with organic and chemical fertilization in different development stages (vegetative and flowering). The common result in all mass spectra obtanied were the signals of *m/z* 277.217 08-339.232 64 (Figure 1).

In Figure 2, the distribution profile of oxygenated substances in the hydroalcoholic extracts of *C. sativum* was represented hierarchically (O_1 to O_{16}).

Figure 3 showed the percentage distribution of the DBE in the molecules found in hydroalcoholic extract of *C. sativum* and a relationship with the presence of oxygen molecules.

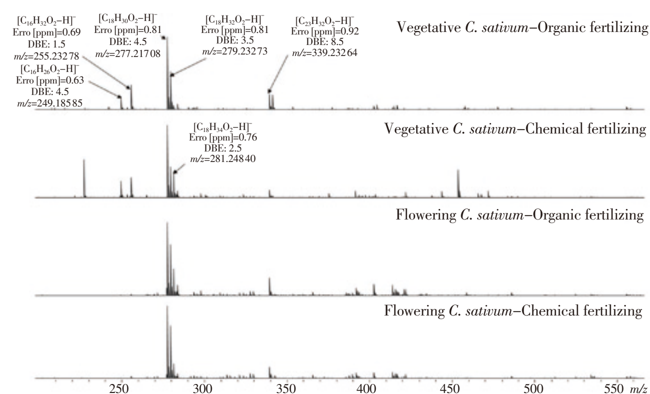

Figure 1. Mass spectra in mode ESI(−)FT−ICR MS of hydroalcoholic extracts of *C. sativum* grown under organic fertilizer and chemical fertilizer at different stages of development.

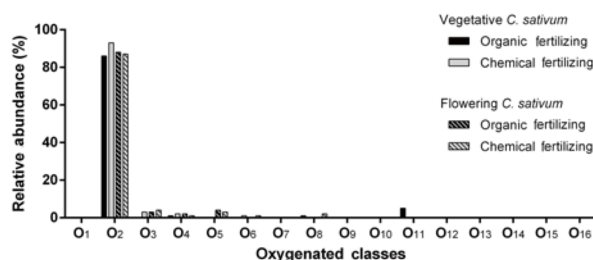

Figure 2. Profile of oxygenated substances classes in hydroalcoholic extracts of *C. sativum*.

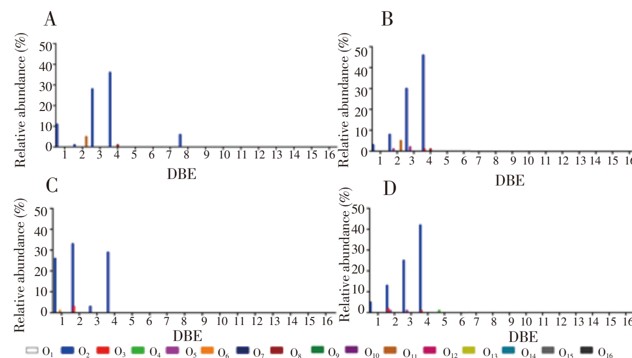

Figure 3. Correlation of DBE with oxygenated organic substances in hydroalcoholic extracts of *C. sativum*.

A: vegetative *C. sativum* grown under organic fertilizer; B: vegetative *C. sativum* grown under chemical fertilizer; C: flowering *C. sativum* grown under organic fertilizer; D: flowering *C. Sativum* grown under chemical fertilizer.

3.5. Antioxidant activity

The highest percentages of inhibition of radical DPPH were found in the *C. sativum* cultivated with chemical fertilization, both at the vegetative and flowering stage (Figure 4). The *C. sativum* extract at the vegetative stage cultivated with chemical fertilizer was the one that showed better antioxidant actvity, it reached 90% inhibition rate of the radical (DPPH) at a concentration of 1 000.00 µg/mL (Figure 4), with a tendency of reducing antioxidant capacity as there was a reduction of the concentration of the extract. When the same fertilization condition was considered, it was observed that the

Table 2

Frequency of PCEMNs in 1 000 PCE, ratio between number of polycromatic and normocromatic erythrocytes and percentage of reduction of CHEs of *C. sativum* against cyclophosphamide damage induction by simultaneous and pre–treatment protocols.

Protocol	Fertilizing condition	Treatment groups	PCEMN(/1 000 PCE)	Ratio (PCE/PCE+NCE)	Reduction (%)
Simultaneous treatment	Chemical fertilizing	Negative control	6.50 ± 3.42[a]	0.540 ± 0.022[c]	–
		Positive control	47.50 ± 7.02[b]	0.130 ± 0.027[a]	–
		CHE 50 mg/kg bw	29.75 ± 6.69[b]	0.270 ± 0.028[ab]	43.29
		CHE 100 mg/kg bw	32.63 ± 4.23[b]	0.330 ± 0.033[b]	36.28
		CHE 200 mg/kg bw	36.13 ± 1.72[b]	0.250 ± 0.024[ab]	27.74
	Organic fertilizing	Negative control	6.50 ± 3.42[a]	0.540 ± 0.022[c]	–
		Positive control	45.79 ± 7.02[b]	0.140 ± 0.027[a]	–
		CHE 50 mg/kg bw	37.33 ± 7.12[b]	0.290 ± 0.033[ab]	21.53
		CHE 100 mg/kg bw	31.92 ± 4.23[b]	0.340 ± 0.033[b]	35.31
		CHE 200 mg/kg bw	30.96 ± 6.87[b]	0.220 ± 0.027[ab]	37.75
Pre–treatment	Chemical fertilizing	Negative control	6.50 ± 3.42[a]	0.540 ± 0.022[b]	–
		Positive control	51.63 ± 7.02[c]	0.140 ± 0.027[a]	–
		CHE 50 mg/kg bw	30.29 ± 5.11[b]	0.230 ± 0.040[a]	44.98
		CHE 100 mg/kg bw	25.54 ± 7.60[b]	0.290 ± 0.052[a]	55.90
		CHE 200 mg/kg bw	38.54 ± 4.87[bc]	0.330 ± 0.020[a]	25.86
	Organic fertilizing	Negative control	6.50 ± 3.42[a]	0.540 ± 0.022[c]	–
		Positive control	49.67 ± 7.02[c]	0.090 ± 0.027[a]	–
		CHE 50 mg/kg bw	26.92 ± 5.86[b]	0.270 ± 0.012[ab]	52.00
		CHE 100 mg/kg bw	29.54 ± 9.66[b]	0.290 ± 0.034[b]	44.79
		CHE 200 mg/kg bw	39.88 ± 8.47[bc]	0.340 ± 0.033[bc]	22.77

Values followed by different lowercase letters [(a,b,c)] in the column differ statistically. Kruskal–Wallis test (P< 0.05).
Values are expressed as mean±SD.

best antioxidant capacity was always obtained in the extracts at the vegetative stage.

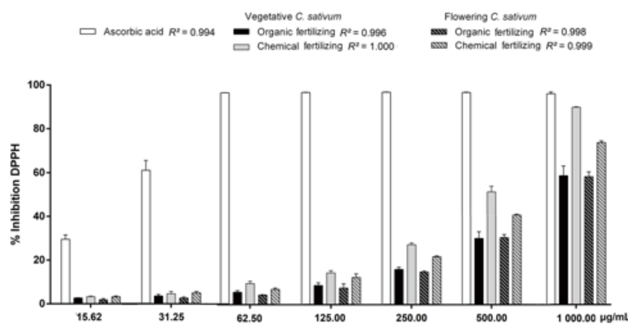

Figure 4. Antioxidant activity of vegetative and flowering *C. sativum* extracts grown under organic and chemical fertilizer.

3.6. Antimutagenic assay

3.6.1. Silmultaneous treatment

The best antioxidants results were found for the CHE in the vegetative condition, thus studies regarding the antimutagenicity and cytotoxicity were done with these samples.

The number of PCEMNs and the ratio with PCE and NCE observed in the simultaneous treatment were shown in Table 2.

There was no significant reduction of the frequency of PCEMNs under the condition of simultaneous treatment with organic vegetative *C. sativum* extract induced by cyclophosphamide when compared to the positive control (Table 2). Regarding cytotoxicity, the dose of 100 mg/kg showed greater protective effect, but when compared to other doses studied, no significant difference was observed.

Similar results were found for the chemical fertilizer condition regarding the PCEMNs number and there was reduction in the values of the ratio [PCE/(PCE + NCE)]. The higher reduction was observed in the dose of 100 mg/kg (Table 2).

When fertilization conditions were compared among them, using the Mann-Whitney test for paired verification of organic and chemical treatments, no statistical differences were found between them. The fertilization factor therefore seemed not to interfere significantly in the chemical protector effects of *C. sativum* extracts analysed. Also, it was not observed changes in their chemical composition.

3.6.2. Pre–treatment

Under the condition of pre-treatment, the best antimutagenic effect observed for the chemical *C. sativum* extract, was at the dose of 100 mg/kg, with a reduction level in the frequency of PCEMNs compared to the negative control. As for the cytotoxicity, it was found that none of the doses of *C. sativum* extract cultivated with chemical fertilizers differed statistically from the positive control. The highest dose (200 mg/kg) was the one that showed greater reduction in levels of cytotoxicity caused by cyclophosphamide. It did not statistically differed from the others (Table 2).

The *C. sativum* extract fertilized organically showed similar behaviour to the chemically fertilized extract. In the latter, the two lower doses showed better antimutagenic potential, because there was a reduction in the number of PCEMNs when compared to the positive control. Although not very significantly, there was a tendency of a reduction in the level of cytotoxicity when the dose

was increased (Table 2).

By analysing the antimutagenicity assay, we noted a greater reduction PCEMNs in the pre-treatment condition compared to simultaneous treatment, but even though the extract had been observed for seven days, this time was not sufficient to show statistically different effects among the doses.

4. Discussion

4.1. Soil analysis

The type and availability of nutrients in the soil are factors that can interfere with the production of secondary metabolites such as flavonoids, coumarins and tannins[2], According to Maia *et al*.[24], the lowest nutritional support can direct the metabolism of the plant for the production of secondary metabolites. Also, according to the authors, although the organic fertilizer makes available less nutrients in the soil, it provides greater diversity of nutrients in relation to mineral fertilizer, which can also interfere with the plant secondary metabolism.

4.2. Phytochemistry

Studies show that the production of secondary metabolites, such as flavanoids, coumarins and tannins, can be influenced by the enviroment mainly in situations in which there is lack or excess of a production factor[2]. In this study, through the preliminary phytochemical analysis, no differences were found in the composition of *C. sativum* cultivated under different fertilization conditions. A similar situation was observed by Wong and Kitts[25], in a study on *C. sativum* stems which the presence of flavonoids and coumarins was verified, as predominant in all treatments tested under different irrigation regimes. This also confirms the results presented in this article, Costa *et al*.[26], in studies of *Cymbopogon citratus* (lemon grass) submitted to organic and chemical fertilization, noted that although the plants had different biomass production, they did not show differences in essential oil content.

Several studies point to a close relationship between the concentration of flavonoids in the plant and its antioxidant activity[27], so we continued with the quantification of these compounds. It was found that organic fertilization presented higher amount of flavanoid and less availabilty of almost nutrients evaluated. Bortolo *et al*.[28] suggested that the decrease in the availability of resources, as the nutrients evaluated in our study, can redirect the carbon in the primary metabolites production to the secondary metabolite production such as flavanoids and others phenolic compounds.

The prossessing of the spectra through the software Data Analysis, the signal *m/z* 277.217 08 has a molecular formula $C_{18}H_{30}O_2$ (error 0.81 ppm, DBE 4 and *m/z* 277. 217 30), suggesting α-linolenic acid. The signal of *m/z* 339.232 64 has a molecular formula $C_{23}H_{32}O_2$, (error 0.92 ppm, DBE 8 and *m/z* 339.232 95) which is shown to be a chemical substance core cyclofenatrene. The mass spectra profiles between the extracts of *C. sativum* cultivated with organic and chemical fertilization in the flowering stage are similar in regard to the major signals and some visible minor signals, which indicates that there are similar components between them. Carvalho *et al*.[29], who worked with samples of *Cannabis sativa* (marijuana) seized by the Civil Police of the Espírito Santo state, also noted by ESI(±)-FT-ICR MS similar signals in the samples which allowed them to elaborate a chemical profile.

In regard to the vegetative stage, the signal of *m/z* 249.185 85 and 255.232 78 were detected both in the treatment of the plants with chemical and organic fertilization. The prossessing of the spectra showed that the signal *m/z* 249.185 85 has a molecular formula $C_{16}H_{26}O_2$ (error 0.63 ppm, DBE 4 and *m/z* 249.186 00) and the signal 255.232 78 has the molecular formula $C_{16}H_{32}O_2$ (error 0.81 ppm, DBE 4 and *m/z* 255.232 95) which may be the palmitic acid[30]. Fasciotti *et al*.[31] studied chemical profiles of *Swietenia macrophylla* (Brazilian mahogany) and *Khaya ivorensis* (African mahogany) and they also detected different signals between the species, which shows the importance of this technique for comparing samples.

The chemical species with two oxygens have DBE that preferably equals 3, indicating that they can be substances with double bonds and the presence of carbonyl group in its structure. DBE equals 4 can be related to the aromatic ring bonded to hydroxyl groups, which indicates the presence of phenolic compounds.

The extract that was different from the others in regard to the DBE was the one cultivated with chemical fertilizers in the vegetative stage, DBE equals 1 for compounds with two oxygen molecules in its structure, which may indicate the presence of carboxylic acid derivatives and such palmitic and α-linolenic acids. The presence of phenol and double bonds in organic molecules gives them various biological properties, such as antioxidant ability.

4.3. Antioxidant activity

Melo *et al*.[32] studied 15 different plants of the cerrado. The authors stated that substances that present the results of antioxidant activity higher than 60% are important to reduce free radicals and help prevent atherosclerotic-related diseases. *C. sativum* aqueous and etheric extracts have antioxidant capacity against lipid oxidation. This action can be attributed to its phenolic compounds. Within this group are the flavonoids, which are commonly suggested as responsible for the antioxidant activity of various plant extracts[27]. However, in our study such a direct relation was not observed, since the highest

elimination of the radical (DPPH) we found (chemical vegetative *C. sativum*) was not directly related to the extract that had the highest number of flavonoid content (vegetative *C. sativum* grown under organic fertilization).

Wong and Kitts[25] analysing methanolic and aqueous extracts of leaf and stem of *C. sativum*, also found similar results to the ones presented here. Also, in this study the methanolic extract of the stem had a higher concentration of flavonoids, but showed less capacity to inhibit the hydrophobic radical DPPH when compared to aqueous extract of the stem. The phenolic concentration detected was smaller. This may be explained due to chemical structures of flavonoids found in each specific situation.

The antioxidant activity of phenolic compounds is attributed to their reductive properties and their chemical structure. The presence of a catechol group, which is formed by a benzene ring bonded to a two hydroxyl groups is reported as important for the antioxidant activity[33]. The common structure of a flavonoid has three aromatic rings A, B and C. The rings A and B contain two hydroxyl groups at the positions 7 and 8 and at the positions 3 and 4, respectively. The third ring (C) is formed of a double bond C2 = C3 conjugated with the function ketone, responsible for the electron displacement of the ring B and for the double bonds with the hydroxyl groups at the positions 3 and 5, which also gives antioxidant activity to the flavonoid[33]. Flavonoids that looses hydroxyl groups have their antioxidant activity reduced[34].

Mendes *et al*.[35], studying seasoning mixes, also reported that *C. sativum* and *Petroselinum crispum* (parsley) have good antioxidant activity when combined with other seasonings like *Allium sativum* (garlic) and *Allium cepa* (onions). The combination exceeded 70% of inhibition of the radical DPPH. In this same study, the authors also found that coriander obtained antioxidant capacity higher than parsley.

Extracts that have good antioxidant capacity may assist in performance of the enzymes involved in repairing damage caused by mutagen such as cyclophosphamide, by making more stable environment with less presence of free radicals.

4.4. Antimutagenicity

Pretreatment and the simultaneous protocols allow to evaluate the chemical or enzymatic inactivation of the mutagens promoted by the compounds tested. The pretreatment protocol is commonly used to evaluate prior protection promoted by the compound tested and the simultaneous treatment is used to assess the ability of the tested compounds to compete with the mutagen simultaneously and thus promote DNA protection.

By evaluating the influence of nitrogen sources and doses on the production of biomass and essential oil content of *Baccharis trimera* (Less) at different harvest stages, Palácio *et al*.[36] found no differences in both quantity of dry matter and essential oil content in different harvest times. Simili *et al*.[37] studied with *Sorghum bicolor* (sorghum cv. AG 2501C) and found that the chemical composition did not change significantly with nitrogenous and potassic fertilizers, concluding that they did not influence significantly in their composition, due to high soil fertility and low rainfall, in relation to the soil and the time of the experiment.

The cyclophosphamide is an alkylating agent. When metabolized in the liver, it also causes reactive oxygen species such as superoxide anion radicals, hydroxyl and peroxyl, singlet oxygen and hydrogen peroxide, highly reactive compounds that seek to stabilize their chemical structure, resulting in damage to cell structures and causing the aging of the organism[38]. One suggestion is that the *C. sativum* extract, due to its antioxidant activity, enters the cell, and creates a stable environment, which ends up contributing in reducing damage of the genetic material, and causes a reduction in the number of micronucleus in mice treated with the mutagen cyclophosphamide.

Cortés-Eslava *et al*.[39] also found a antimutagenic action in the aqueous extract of *C. sativum* leaves, inducing up to 80% of damage reversion against aromatic amines in hepatocytes *in vitro* through the Ames test, thus demonstrating that coriander can act as chemical protection, even from other mutagens.

During chemotherapy treatments, depletion of cellular antioxidants which increases the production of reactive oxygen species is observed. Thus, the presence of phenolic compounds with antioxidant capacity may increase the efficiency of chemotherapy not only by reducing the cytotoxicity because of its antioxidant properties, but also by inhibiting the topoisomerase DNA or tyrosine syntase enzyms[40].

Studies suggest that phenolic compounds also have positive biological effects on diets, in these cases they are related to the anti-carcinogenic activity, anti-microbial and anti-inflammatory[41]. They are indicated for treatment and prevention of cancer, cardiovascular and inflammatory diseases *in natura*[42]. Knowing that chronic diseases such as cancer, cardiovascular and neurodegenerative diseases are associated with the daily exposure to mutagens, a condiment used to season food that acts as an antimutagenic potential is a very useful agent in preventing these diseases.

Analyzing the results presented here, we can conclude that all treatments, both in simultaneous test and in the pre-treatment test, show reduction of micronucleus when compared to the positive control group (cyclophosphamide).

In conclusion, the preliminary phytochemistry indicates the presence of coumarins, flavonoids and steroids in all the extracts, no differences between them are found after the tests. The analysis by mass spectrometry reveals distinction in only two molecular peaks between the chemical vegetative *C. sativum* extract and the others.

This study also indicates the potential use of *C. sativum* as a source of natural antioxidants. The *C. sativum* extract in the vegetative stage and chemically fertilized presents the best antioxidant performance when compared to the others. As for the micronucleus assay, all treatments, whether under the simultaneous treatment or pre-treatment, show micronucleus reduction compared to the positive control group. These findings suggest the appropriate time of harvest, and growing condition can improve bioactive components for health benefits and reinforce roles of this culinary plant in health promotion and disease prevention.

Conflict of interest statement

The authors declare no conflict of interest.

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