COMMUNICATIONS IN PLANT SCIENCES

RESEARCH ARTICLE

Effect of rosemary, garlic, pepper and lemongrass extracts in postharvest of organic *Brassica oleracea* L. leaves

Mayeve Didomenico Melo[1]*, **William Gustavo Sganzerla**[2], **Guilherme Cruz Duarte**[1], **Jocleita Peruzzo Ferrareze**[2], **Ana Paula de Lima Veeck**[2], and **Paula Iaschitzki Ferreira**[2]

[1]Santa Catarina State University, Lages, SC, Brazil.
[2]Federal Institut of Santa Catarina, Lages, SC, Brazil.
Author for correspondence: maydomenico@hotmail.com.

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The growing concern about food safety makes necessary to search for alternative methods of food conservation. The objective of this work was to evaluate postharvest conservation of kale leaf using aqueous vegetal extracts. The experimental design was completely randomized, with the concentrations of 5, 10, 15, 20 and 25% of *Rosmarinus officinalis*, *Allium sativum*, *Capsicum baccatum*, *Cymbopogon citratus* and the control treatment using deionized water. The extracts were sprayed on kale leaves with manual spray over the entire adaxial and abaxial epidermis. The evaluated parameters were chlorophyll a and b, weight loss, total phenolics compounds, antioxidant activity and a visual analysis. The leaves remained at ambient temperature for six days. The results indicate that there was no significant conservation in the content of chlorophyll a, b and visual analysis when compared to the control, and the extracts did not have influence in the weight loss. There was an increase of total phenolic content and antioxidant activity with rosemary, lemongrass and pepper extracts, the garlic extract reduced the concentration of these bioactive compounds when compared to the control. In general, the application of the vegetal extracts did not influence in the postharvest conservation of kale leaves.

Highlighted Conclusion
The application of aqueous extracts in general does not influence positively postharvest conservation of kale leaves.

In recent years we had an increasing demand for functional, healthy food, and it has become necessary to produce food, free of chemical additives that may cause harm to the consumer's health. Functional foods should have beneficial properties in addition to the basic nutrients and are presented in the form of common foods. They are consumed in conventional diets, and demonstrate the ability to regulate body functions in order to protect against diseases such as hypertension, diabetes, cancer, osteoporosis and coronary diseases (Souza et al. 2003).
Kale leaf (*Brassica oleracea*) consumption in Brazil has gradually increased, due to the new ways of uses in the culinary and the recent discoveries about nutraceuticals properties (Novo et al. 2010). Kale leaf is a vegetable rich in proteins, carbohydrates, fibers, calcium, iron, vitamin A, niacin and vitamin C, besides being also a great source of antioxidants and phenolic compounds (Rigueira et al. 2016).

The organic cultivation of this vegetable is an economically viable option, replacing the use of agrochemicals with less aggressive practices to the environment and to the health of producers and consumers (Shingo and Ventura 2009). The growing concern with the diet-health binomial has led to an increase in the number of people who consume organic food, since harmful chemicals are generally used in conventional systems (Azevedo 2012).

Due to its metabolism, kale leaf has high perishability, having as indicators yellowing and loss of turgor causing a consequent short postharvest period (Sanches et al. 2016). For this reason, methods that increase shelf life without changing the taste and nutritional qualities and at the same time are health and safe have been sought.

In order to improve the defense and resistance of plants, studies involving the use of plant extracts have increased in recent years. The use of extracts of rosemary (*Rosmarinus officinalis*), garlic (*Allium sativum*), pepper

(*Capsicum baccatum*) and lemongrass (*Cymbopogon citratus*) have been tested for their ability to protect other cultures (Cruz et al. 2013, Poonpaiboonpipat et al. 2013, Peng et al. 2015, Bina et al. 2016).

Plants produce a series of metabolites that in many cases are biologically active, and a rich source of antimicrobial, allelopathic, antioxidant and bioregulatory properties also performing an important role in the defense of plants against damages caused by UV-B rays (Li et al. 1993, Fumagali et al. 2008). Among these metabolites we can mention jasmonic acid which was first isolated in 1962 from *Jasminum grandiflorum* and *Rosmarimus officinalis* (Demole et al. 1962). Jasmonic acid (JA) and its derivatives (jasmonates) are related to vegetables defense mechanisms. They induce the expression of genes encoding specific proteins, such as protease inhibitors, enzymes involved in production of flavonoids, and different disease-related proteins (Cortês 2000, Linares et al. 2010).

Rosemary has antimicrobial properties and the use of essential oils and plant extracts have gained increasing attention as natural additives for extending the shelf life of food products (Tongnuanchan and Benjakul 2014). Garlic (*Allium sativum*) is rich in allicin which has antiviral, antifungal and antibiotic action (Corzo-Martínez et al. 2007). According to (Xing 2005), capsaicin is the main active component of pepper, the extract exhibited varying degrees of inhibition against microorganisms. Lemongrass (*Cympopogon citratus*) is characterized by a high content of citral (Paviani et al. 2006). Several studies have reported antimicrobial activities by lemongrass oil (Tzortzakis and Economakis 2007).

Based on these bibliographic data and to our knowledge, there are few researches withaqueous extracts of fresh vegetables since most of the studies are being performed with essential oils. The objective of this work was to test the effects of aqueous extracts of rosemary, garlic, pepper and lemongrass in the post-harvest of organic kale leaf.

MATERIAL AND METHODS

The experiment was conducted at Federal Institute of Santa Catarina. Kale was cultivated in an organic system. Leaves (*Brassica oleracea*) were collected by the end of day from four months old plants. Rosemary (*Rosmarinus officinalis*), pepper (*Capsicum baccatum*) and lemongrass (*Cymbopogon citratus*) were collected in a private production site and garlic (*Allium sativum*) was obtained from a local market.

Extracts of rosemary and lemongrass leaves, garlic bulbs and pepper fruits were prepared according to Brand et al. (2010) with modifications. We used concentrations of 0, 5, 10, 15, 20, 25% and the control treatment was deionized water. Samples were weighed and deionized water was added to reach the volume and samples were homogenized using blender for one minute. The extracts were then sifted twice.

The extracts were immediately sprayed on kale leaves with manual spray over the entire adaxial and abaxial surface, allowing to act for 5 min. The extract excess was gently removed with paper towels. Leaves were stored in transparent plastic bags for food RoyalPack® with no control of temperature and relative humidity during six days.

The evaluated parameters were weight loss, chlorophyll content a, b, total phenolics compounds, antioxidants activity and visual analysis.

The weight loss was measured with the aid of a precision scale Bel®, where the leaves were weighed individually. Chlorophyll a and b were measured with a portable chlorophyllometer ClorofiLog Falker®. Considering the large surface of the kale leaf, two measurements were made on each side of the leaf. The evaluations were performed on day zero, two, four and sixth days.

To perform the analysis of total phenolic compounds and antioxidant activity, extracts from each treatment of the experiment were prepared using a 1:10 (m/v) dilution, that is, 1 gram of homogenized sample was diluted in 10 mL of 70 ºGL hydroethanolic solution (70:30 - 70 mL of absolute ethanol and 30 mL of distilled water). The extracts were allowed to stand for one week in a dark amber bottle and under refrigeration for the best extraction of the compounds. The extracts were then filtered on qualitative filter paper for analysis according to Sganzerla et al (2018).

The concentration of Total Phenolic (TPC) was determined by Swain and Hillis (1959) with modifications. It was added 104 µL of the sample extract and 1667 µL of distilled water in a test tube. After, it was added 104 µL of Folin-Ciocalteau 0.25 N reagent. Incubated the reaction during 3 minutes, and added 208 µL of sodium carbonate – Na_2CO_3 – 1N. After 2 hours, the absorbance was measured in Spectrophotometer UV - Vis 752D, Labman® at 725 nm and the standard curve was performed using gallic acid.

The antioxidant activity of the extracts was determined by the ability to remove the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, according to methodologies adapted from Brand-Williams et al. (1995), using the wavelength of 515 nm. For this, 150 µL of each extract was used in 1850 µL of 0.1 mM DPPH and the reading was

performed after 24 hours of incubation at ambient temperature. Trolox was used for the calibration curve. The evaluation of phenolics and antioxidant activity was performed for day zero and six of storage.

The visual analysis was performed on the sixth day of the experiment and was done by attributing notes from 1 to 3, with note 1 being attributed to green and tender leaves, note 2 for light yellowed, but under consumption conditions and 3 for completely yellowed and showing visible rot.

The experimental design was completely randomized, and the treatments were arranged in a 6x4 double factorial scheme in which the concentration of the extract (0, 5, 10, 15, 20 and 25%) and storage periods (0, 2, 4, and 6 days) were studied for chlorophyll a, b and weight loss, 6x2 double factorial scheme in which the concentration of the extract (0, 5, 10, 15, 20 and 25%) and storage periods (0 and 6 days) were studied for bioactive compounds and antioxidant activity, and means analysis for de visual analysis, with seven replicates. The results were submitted to analysis of variance and the means of the seven replicates were compared by the Tukey test at 5% probability using ASSISTAT® 7.0 software. The correlation analysis between bioactive compounds and antioxidant activity were made using ASSISTAT® 7.0 software.

RESULTS AND DISCUSSION

According to Table 1, there was statistical variation for chlorophyll a in the different concentrations tested for rosemary, pepper and lemongrass extracts, while garlic extract, did not present statistical difference. The extract of pepper at the concentration of 15% acted negatively in comparison to the 10% concentration but did not differ statistically from the control treatment. Rosemary and lemongrass extracts at the concentration of 20% acted negatively, since there was a chlorophyll a reduction. Some studies have shown that the methyl jasmonate molecule acts in the process of plant senescence, promoting chlorophyll degradation Zhu et al (2017). In this way, the methyl jasmonate present in rosemary leaves may have contributed to the reduction of chlorophyll a when compared to the control.

Table 1. Chlorophyll a in kale leaves treated with different plant extracts.

Concentration	Extract			
	Rosemary	Garlic	Pepper	Lemongrass
0	36.68 a	35.11 a	35.12 ab	36.68 a
5%	34.94 ab	35.37 a	35.60 ab	34.94 ab
10%	34.83 ab	35.06 a	36.81 a	34.83 ab
15%	35.51 ab	32.85 a	34.86 b	35.51 ab
20%	33.62 b	35.24 a	36.55 ab	33.62 b
25%	34.88 ab	34.43 a	35.42 ab	34.88 ab

Mean with same letters in the column is not significantly different using the Tukey test ($p < 0.05$). CV% = 9.59 (*Rosmarinus officinalis*) CV% = 10,28 (*Allium sativum*) CV% = 6,73 (*Capsicum baccatum*). CV% = 9,59 (*Cymbopogon citratus*).

Similar behavior was observed for chlorophyll b (Table 2) where the rosemary extract did not show statistical difference and the other extracts showed statistical difference among concentrations. The extract of pepper at the concentration of 15% and 25% acted negatively in comparison to the 10% concentration but did not differ statistically from the control treatment. The garlic extract at 15%, and lemongrass at 10, 20, 25% acted negatively, decreasing chlorophyll b when related to the control. These results can be related to the allelopathic interaction between the molecules present in the extracts and the mechanisms of synthesis or degradation of chlorophylls Einhellig (1986). Peng at al. (2016) tested 0.2 and 1% allicin in postharvest of *Lactuca sativa* var. *angustana Irish* and verified that the total chlorophyll content in the concentration of 1% was 76% higher when compared to the control and 16.6% higher than the 0.2% concentration.

Poonpaiboonpipat et al. (2016) tested the phytotoxicity of lemongrass essential oil on *Echinochloa crus-galli* seed germination and post-emergence foliar application at concentrations of 1.25, 2.5, 5 and 10% and verified a decrease in chlorophyll a and b content with increasing concentration. After 6h of the treatments, chlorophyll a and b contents remained at 100% in the control treatment, while at 10 % concentration chlorophyll a and b were 12.06 and 16.16%, respectively. A similar result was observed by Carmo et al. (2007) studying the allelopathy of aqueous extracts of cinnamon-sassafras in the sorghum culture, in the concentration of 10%, where extracts of leaves and branches inhibited the chlorophyll production of the seedlings.

Table 2. Chlorophyll b in kale leaves treated with different plant extracts.

Concentration	Extract			
	Rosemary	Garlic	Pepper	Lemongrass
0	10.78 a	14.21 a	13.42 ab	15.05 a
5%	10.74 a	13.23 ab	13.12 ab	13.27 ab
10%	9.80 a	13.07 ab	14.40 a	13.16 b
15%	9.85 a	11.84 b	12.36 b	13.28 ab
20%	10.13 a	13.24 ab	13.54 ab	12.16 b
25%	11.12 a	13.73 ab	12.90 b	13.11 b

Mean with same letters in column is not significantly different using the Tukey test (p <0.05). CV% = 20,07 (*Rosmarinus officinalis*). CV% = 18,09 (*Allium sativum*). CV% = 13,11 (*Capsicum baccatum*). CV% = 17,84 (*Cymbopogon citratus*).

In the visual analysis (Table 3), the rosemary extract presented statistical difference, where the effect was negative, being the concentration of 15% yellower than the control. Higher averages represent the more decreased. No fungal attack was observed on kale leaves.

Table 3. Visual analysis of kale leaves treated with different plant extracts.

Concentration	Extract			
	Rosemary	Garlic	Pepper	Lemongrass
0	1.57 b	1.57 a	1.43 a	1.14 a
5%	1.86 ab	1.43 a	1.43 a	1.71 a
10%	2.14 ab	1.43 a	1.29 a	1.57 a
15%	2.43 a	2.29 a	1.71 a	1.57 a
20%	2.29 ab	2.14 a	1.00 a	1.71 a
25%	2.29 ab	2.00 a	1.57 a	1.29 a

Mean with same letters in column is not significantly different using the Tukey test (p <0.05). CV% = 22,5 (*Rosmarinus officinalis*). CV% = 36,18 (*Allium sativum*). CV% = 33,5 (*Capsicum baccatum*). CV% = 36,12 (*Cymbopogon citratus*).

According to Table 4, no extract statistically differed for the percentage of weight loss. As in Peng et al. (2016), there was no statistical variation for fresh weight loss between the control and the concentrations of 0.2 and 1% allicin. Bina et al. (2016) observed a lower weight reduction in onions stored at 25 °C and 30 °C with rosemary branches and leaves when compared to storage for the same period and temperature conditions, without the rosemary branches and leaves.

Table 4. Percentage of mass loss in kale leaves treated with different vegetable extracts.

Concentration	Extract			
	Rosemary	Garlic	Pepper	Lemongrass
0	15.36 a	17.21 a	17.25 a	15.36 a
5%	14.30 a	15.27 a	16.73 a	14.30 a
10%	15.65 a	16.77 a	16.49 a	15.65 a
15%	16.29 a	15.01 a	13.41 a	16,29 a
20%	18.02 a	15.05 a	16.64 a	18.02 a
25%	14.50 a	18.68 a	17.57 a	14.51 a

Mean with same letters in column is not significantly different using the Tukey test (p <0.05). CV% = 26,33 (*Rosmarinus officinalis*). CV% = 24,76 (*Allium sativum*). CV% = 22,07 (*Capsicum baccatum*). CV% = 20,05 (*Cymbopogon citratus*).

The application of garlic extract decreased the antioxidant activity and total phenolics (Figure 1A) whereas for the pepper extract it was observed an increase in the antioxidant activity and total phenolics at concentrations of 5, 15, 20 and 25% (Figure 1B), this behavior was similar to Cao et al. (2009) with an increase of total phenolics during storage of *Eriobotrya japonica* when tested methyl jasmonate, an elicitor of plant defense.

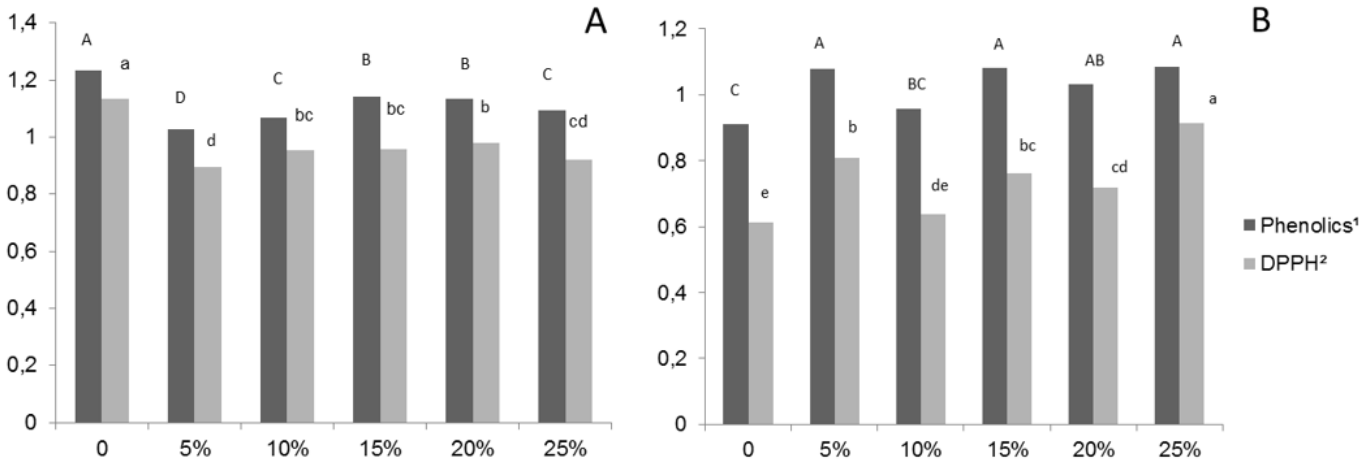

Figure 1. Total phenolics compounds and antioxidant activity in kale leaves treated with different concentrations of garlic (A) and pepper (B) extracts. ¹mg GAE g^{-1} of leaf. ²mg TEAC g^{-1} of leaf. Means with same letters is not significantly different using the Tukey test ($p < 0.05$).

Lemongrass extract, in the concentrations of 15 and 20% increased the antioxidant activity in relation to the control, while the concentrations of 5, 10 and 25% decreased in relation to the control. The behavior of total phenolics was similar to the antioxidant activity, where the concentrations of 15 and 20% increased in relation to the control, while the other concentrations were statistically the same as the control treatment (Figure 2A).

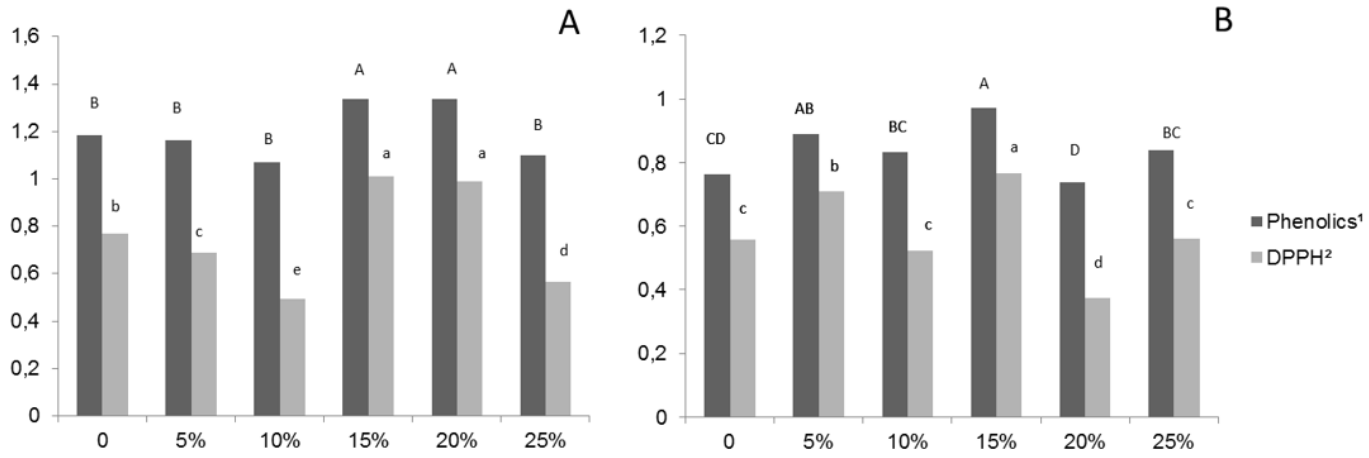

Figure 2. Total phenolics compounds and antioxidant activity in kale leaves treated with different concentrations of lemongrass (A) and rosemary (B) extracts. ¹mg GAE g^{-1} of leaf. ²mg TEAC g^{-1} of leaf. Means with same letters is not significantly different using the Tukey test ($p < 0.05$).

For rosemary extract at 5 and 15%, the antioxidant activity increased, for the concentrations of 10 and 25% there was no difference and decreased at the concentration of 20%. Total phenolics increased at concentrations of 5 and 15%, while the concentrations of 10, 20 and 25% there was no statistical difference in relation to the control (Figure 2B). These results are similar to the results we found for the lemongrass extract and the results found by Mirdehghan and Valero (2016) where applications of thymol and *Aloe* in postharvest of *Solanum lycopersicum* at 8 days of storage under controlled temperature and treatments did not differ statistically from the control for the total phenolic content and antioxidant activity.

All extracts presented a similar behavior with increase of antioxidant activity (Figure 3A) and total phenolics compounds (Figure 3B) during the postharvest storage time, similar to the behavior observed for Esplá et al. (2017) with preharvest application of oxalic acid in *Cynara scolymus,* the content of total phenolic increased along storage, except antioxidant activity that did not change.

For all the extracts there was a high positive correlation between the antioxidant activity and total phenolics, being r = 0.86 for the rosemary extract, r = 0.88 for the garlic extract, r = 0.95 for the lemongrass extract and r = 0.81 for the pepper extract. A similar result was observed by Connor et al. (2002), where a strong correlation between these variables was observed when the postharvest of different blueberry cultivars was studied.

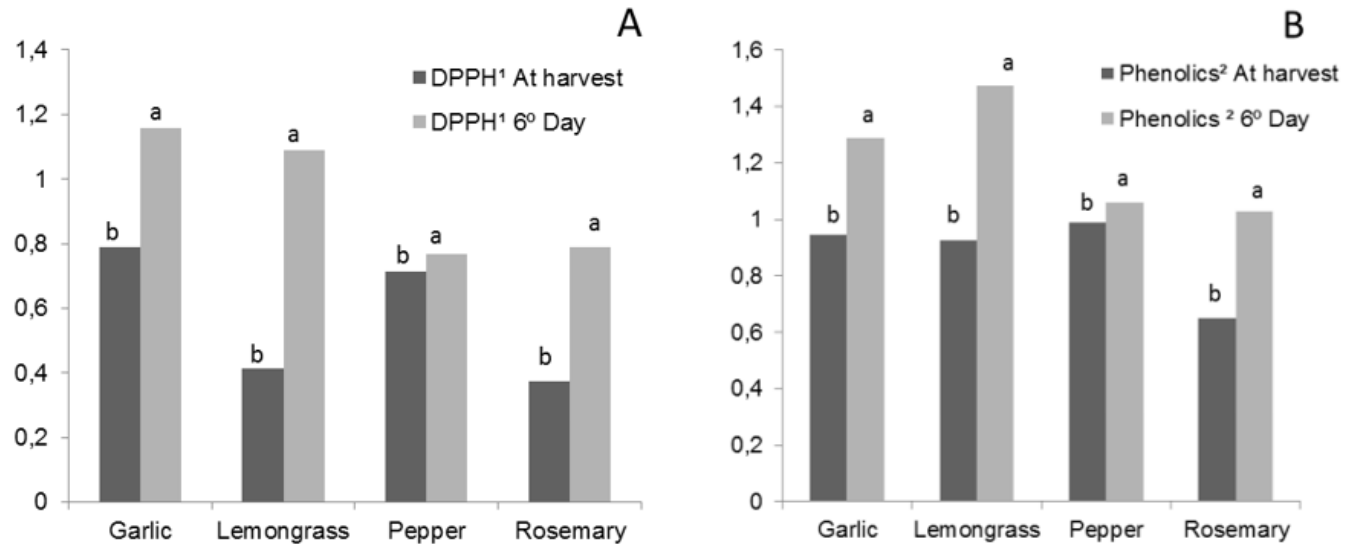

Figure 3. Comparison of antioxidant activity (A) and total phenolics compounds (B) at harvest point and at 6 days of storage. ¹ mg TEAC g-1 of leaf. ² mg GAE g-1 of leaf. Means with same letters is not significantly different using the Tukey test (p <0.05).

In conclusion, vegetable extracts did not contribute to the maintenance of chlorophyll a and b, also no reduction was observed in the parameter weight loss. Pepper, lemongrass and rosemary extracts increased total phenolic concentrations and antioxidant activity. Garlic extract decreased the content of these compounds. Total phenolics and antioxidant activity increased on the sixth day of storage when compared to the harvest time. The application of aqueous extracts in general did not influence positively postharvest conservation of kale leaves.

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