

Quantification of bioactive compounds in crem (*Tropaeolum pentaphyllum* Lam) tubers: fibers, phenolic compounds and evaluation of its antioxidant activity

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Article history

Received: 22 March 2017

Received in revised form:

1 April 2017

Accepted: 3 April 2017

Abstract

Crem (*Tropaeolum pentaphyllum* Lam) is a tuber that is well-known in the south of Brazil, particularly in the states of Santa Catarina and Rio Grande do Sul. It is used as a condiment in food and is normally prepared by being grated and pickled in red vinegar. This study aimed to chemically characterize crem and to quantify its bioactive compounds. The following were evaluated: chemical composition; phenolic compounds by the Folin-Ciocalteu method; antioxidant capacity by scavenging of the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical; and flavonoids by high-performance liquid chromatography (HPLC). The results indicated that the tuber was a good source of fiber (16.79%); the values found for total phenolic compounds were 32.98 ± 0.57 mg GAE/100 g and DPPH free radical scavenging activity was $22.49 \pm 0.74\%$. In the quantification of phenolic compounds the following five flavonoids were identified: kaempferol (15.62 ± 0.01 mg/g); luteolin (12.17 ± 0.03 mg/g); quercetin (10.45 ± 0.01 mg/g) coumarin (6.38 ± 0.02 mg/g) and rutin (0.84 ± 0.01 mg/g). Other components found in crem were caffeic acid (6.15 ± 0.02 mg/g), gallic acid (4.97 ± 0.03 mg/g) and chlorogenic acid (4.73 ± 0.01 mg/g). The crem has good fiber supply, but presents low amount of phenolic compounds and antioxidant activity when compared to other condiments. Some flavonoids stand out in relation to other condiments, making it a culinary alternative.

Keywords

Crem (*Tropaeolum pentaphyllum* Lam)
Phenolic compounds
Antioxidant activity
Flavonoids

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Introduction

Natural resources remain important sources of bioactive substances, with great therapeutic potential, not only because of the vast number of plant species with untapped medicinal properties, but mainly because of the variety of primary and secondary metabolites which are synthesized by those plant species. A considerable percentage of people living in developing countries make use of traditional forms of medicine, including supporting and complementary therapies. Supporting therapies can include foods, and there has been much research into innovations in this area, including spices and medicinal plants which can be used in primary health care (Newman, 2003; Opara and Chohan, 2014). The Mediterranean diet is considered to be one of the best available in terms of its beneficial health effects, which are attributed to the consumption of low levels of fat, red wine, and herbs and spices. The latter are known to have beneficial health effects (Bower *et al.*, 2015). The major bioactive compounds include antioxidants,

which are present in plants in the form of phenolic compounds; the most studied phenolic compounds are flavonoids, vitamins A, C and E, and carotenoids (Moreno *et al.*, 2010).

Such foods include the plants from the Tropaeolaceae family, which includes *Tropaeolum pentaphyllum* Lam. The latter is recognized worldwide due to its pharmacological properties as an expectorant and for its digestive, dermatological, purgative, antiscorbutic, antimicrobial and anti-inflammatory properties (Luft *et al.*, 2014).

Crem (*Tropaeolum pentaphyllum* Lam) is a tuber which is popularly known in the southern states of Brazil (mainly Santa Catarina and Rio Grande do Sul) as batata-crem, cipó-crem, crem do mato or cinco-chagas. It is typical found in the borders of shrubs, forests, roadsides and clearings. Crem tubers are prized in traditional Italian cuisine; they are preserved by grating the tubers and immersing them in red vinegar; they are marketed for use as seasoning in soups and also to accompany meat. Apart from their culinary use, little is known

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about their chemical composition (Kinupp, 2007). Phytochemical analysis conducted by Gerhardt and Linares (2014) demonstrated the presence of flavonoids, saponins and cardiac glycosides. Tests for antimicrobial activity in relation to the crude extract demonstrated that *Tropaeolum pentaphyllum* Lam has potential activity regarding species of *Bacillus cereus* and *Enterococcus faecalis* with minimum inhibitory concentration (MIC) of 250 µg/mL.

There is very little research in the literature regarding crem (*Tropaeolum pentaphyllum* Lam), and thus the present study is important because it aims to provide a chemical characterization, to quantify the phenolic compounds and antioxidant activity, and also to identify the flavonoids present in this tuber.

Materials and Methods

Raw material

The crem tubers were purchased in the city of Antônio Prado, in the state of Rio Grande do Sul, Brazil (latitude 28° 51' 30 and altitude of 658 meters), collected 5 days after the maturation phase. First, the tubers were cleaned under running water and peeled with a stainless steel knife. Then, they were grated (conventional grater, 2 mm x 2 mm) and dried in an oven with forced air circulation at 60°C for 16 hours. They were subsequently ground in a micro-mill (Marconi®, MA-630 model, < 1 mm) for 40 seconds and then packed in glass with a metal lid until the time of analysis.

Chemical composition

To assess the proximate composition and calorific values, the following analyses were performed: moisture in an oven at 105°C; ash in a muffle furnace at 550°C; protein content using the Kjeldahl method; dietary fiber by an enzymatic-gravimetric method. All these analyses were performed according to the AOAC (2005). Lipids were determined according to Bligh Dyer (1959); carbohydrates were calculated by difference: (100g - total fiber, proteins, lipids, ash and moisture); and the calorific value was calculated by multiplying the results of lipid analysis, soluble proteins and carbohydrates by their caloric values: 9, 4 and 4 kcal - Atwater conversion factors.

Extraction procedure

The extract was prepared by adding 50 mL 95% ethanol into 5 g of crem, which was stirred at 700 rpm at room temperature (25°C) for 60 minutes (Tomsone *et al.*, 2012).

Quantification of total phenolic compounds

The content of phenolic compounds was based on the colorimetric Folin-Ciocalteu method described by Singleton *et al.* (1999), with the modifications of Chandra and De Mejia (2004). To 0.5 mL of extract was added 2.5 mL of Folin - Ciocalteu reagent (diluted 1:10 v/v) followed of 2 mL of sodium carbonate (Na₂CO₃) (75 g/L). The sample was mixed. The control sample contained all the reagents of the reaction except the extract. After 2 hours incubation at room temperature, the absorbance was measured at 765 nm on a spectrophotometer (Bel, model SP 1105). The results of total phenolics content was expressed as gallic acid equivalents (GAE mg/100 g wet sample), calculated using a calibration curve: $Y = 0.0114x - 0.0154$, $R^2 = 0.9942$ where Y is the absorbance former concentration is constructed with concentrations ranging from 0 to 100 mg/100 g.

Antioxidant activity

The determination of antioxidant activity followed the methodology described by Boligon *et al.* (2009), based on the scavenging capacity of the 2,2- diphenyl-1-picryl-hydrazyl (DPPH) radical. The crem extract was tested at 7.81, 15.62, 31.25, 62.50, 125 and 250 µg/mL. Briefly, 2.5 mL of each sample was mixed with 1.0 mL of 0.3 mM DPPH ethanolic solution, the mixture was allowed to stand for 30 min. After that, the absorption was measured at 518 nm on a Shimadzu-UV-1201 (Shimadzu, Kyoto, Japan) spectrophotometer. A solution of DPPH in ethanol was used as negative control, and ascorbic acid as positive control. The tests were performed in triplicate, and the scavenging capacity was calculated as the equation below:

$$\% \text{ Inhibition} = 100 - \left[\frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100}{\text{Abs}_{\text{control}}} \right] \quad (1)$$

Where: Abs_{sample} is absorbance of crem extract; Abs_{blank} is absorbance of crem extract without adding the DPPH; Abs_{control} is absorbance the solution of ethanol in DPPH. The values of half-maximal inhibitory concentration (IC₅₀) were calculated using the equation obtained from a curve prepared with different concentrations of the sample.

Quantification of flavonoids and phenolic acids

All the reagents used for the quantification of flavonoids were of analytical grade. Methanol, acetic acid, gallic acid, chlorogenic acid and caffeic acid were purchased from Merck (Darmstadt, Germany). Quercetin, rutin, luteolin, kaempferol and coumarin

were purchased from Sigma (St. Louis, MO, USA). A high-performance liquid chromatography system (HPLC, Shimadzu, Japan) was used with an autosampler (SIL-20A model) equipped with alternating pump (LC-20AT model) degassing system (DGU 20A5 model) CBM 20A integrator and diode array detector (DAD, SPD-M20A model). All the commands were performed using LC solution 1:22 SP1 software.

The reverse-phase chromatographic analyses were performed by gradient using a C18 column (4.6 mm x 250 mm) packed with 5 µm diameter particles; the mobile phase consisted of water containing 1% acetic acid (A) and methanol (B). The elution program was: 5% B for 2 min; 20% B until 10 min; 40, 50, 60, 70 and 80% B every 10 min (Barbosa Filho *et al.*, 2014). Crem (*Tropaeolum pentaphyllum* Lam) extract and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use, the extract was analyzed at a concentrations of 25 mg/mL. The flow rate was 0.6 ml/min and the injection volume was 50 µl. All the chromatographic procedures were performed at room temperature.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of responses and the slope using three independent calibration curves, as defined by Boligon *et al.* (2012). The LOD and LOQ were calculated as 3.3 and 10σ/S respectively, where σ was the standard deviation of the responses and S was the slope of the calibration curve.

Statistical analysis

The analyses were performed in triplicate. The results were expressed as mean ± standard deviation and subjected to analysis of variance (ANOVA); the means were compared by Tukey's test, considering a significance level of 95% (p<0.05). The results were analyzed using the SPSS version 7.0 program.

Results and Discussion

The results for chemical composition are shown in Table 1. The moisture content was considered to be high (75.41%). Melo Filho and Vasconcelos (2011) noted that food with high levels of moisture (over 40%) are more susceptible to chemical and microbiological reactions and they require more care in terms of preservation. The ash content was 1.39%, which corresponds to minerals or impurities contained in the food (Araújo *et al.*, 2006). These values were higher than those reported by Brito *et al.* (2011), who studied yam (*Dioscorea* sp.) and found values of

65.62% and 0.96% for moisture and ash respectively. In the present study the protein content was 2.56%, which was lower than the values found by Brito *et al.* (2011) of 4.13% to 6.35% in yam (*Dioscorea* sp.). The lipid content in the present study was 0.41%, which was similar to the value found by De Paula *et al.* (2012), who analyzed the chemical composition of six varieties of yam tuber (*Colocasia esculenta* L), and found values that ranged from 0.25% to 0.45% for lipids. The crem presented a value of 16.79% for fiber. This was considered to be a high level when compared to the study by De Paula *et al.* (2012), which analyzed fiber in different varieties of yam, and found values ranging from 1.43% to 2.73%. Thus, it was observed that the crem contained a good amount of fiber and consequently, if it is consumed in natura in the form of preserves it can add value to health.

The consumption of dietary fiber can reduce the risk of cardiovascular disease, colon cancer and obesity. Products rich in fiber are increasingly popular, and this has encouraged researchers to investigate new sources of fiber and to develop products with high fiber content (Chau and Huang, 2004).

The crem contained 3.44% carbohydrates and a caloric value of 27.9 Kcal; these were lower results than reported by Storck *et al.* (2013), who studied vegetables such as potatoes (*Solanum tuberosum* ssp. *Tuberosum*) and found 14.7% carbohydrates and 64 Kcal.

In terms of total phenolic compounds and DPPH free radical scavenging activity, the crem presented values of 32.98 ± 0.57 mg GAE/100g and IC₅₀ 148.15±0.13 µg/mL, respectively. Tomsone *et al.* (2012) found values of 23.02 to 334.29 mg GAE/100g, in extracts of horseradish extracts (*Armoracia rusticana*), which is similar to crem and which is also consumed in pickled form.

Kaur and Kapour (2002) quantified the phenolic compounds of 80% hydro-ethanol extracts of ginger (*Zingiber officinale*) and found 221.3 mg GAE/100g of total phenolic compounds, which were higher value than that found in the present study. Higher values for the antioxidant activity with IC₅₀ of 4.25 mg/mL were also reported in the study developed by Mošovská *et al.* (2015) using methanolic ginger extract. Simonovska *et al.* (2003) found lower values for phenolic compounds (0.35 mg GAE/100g) in yacon potato tuber (*Smallanthus sonchifolius*) using extraction with boiling water, and 0.032 mg GAE/100 g using Soxhlet for extraction with solvents such as methanol, petroleum ether and ethyl acetate. Oliveira (2011) found values for total phenolic compounds of 24.35 mg GAE/100g and 24.09 mg GAE/100g for Dedo-de-Moça pepper (*Capsicum baccatum* var.

Table 1. Chemical characterization of crem (*Tropaeolum pentaphyllum* Lam) (n=3).

Constituents	Crem (<i>Tropaeolum pentaphyllum</i> Lam)
Moisture (%)	75.41±0.03
Ash (%)	1.39±0.04
Protein (%)	2.56±0.01
Lipids (%)	0.41±0.01
Dietary fiber (%)	16.79±0.82
Soluble fiber (%)	0.54±0.18
Insoluble fiber (%)	16.25±0.86
Carbohydrates (%)	3.44
Phenolic compounds (mg GAE/100g)	32.98±0.57
Antioxidant activity – IC50 (µg/mL)	148.15±0.13
Total Energetic Value (Kcal/100g)	27.69

Values expressed as mean ± standard deviation.

Pendulum) and Cheiro Ardidá pepper (*Capsicum chinense*), respectively, which were extracted in hydro-alcoholic medium (1:5). In the same samples, the author identified antioxidant capacity with IC50 values of 2559.45 µg/mL and 651.14 µg/mL, respectively, higher than crem. It is important to note that, according to Andreo and Jorge (2006), the solvent used in the extraction process, the species of plant that is studied, and the stage of maturation are all factors that decisively influence the amount of phenolic compounds that are obtained. Comparing the results in the present study with the contents of total phenolic compounds obtained from fruits such as guava (*Psidium guajava*) with 83.1 mg/100g; pineapple (*Ananas sativa*) with 21.7 mg/100g; and soursop (*Anona muricata*) with 84.3 mg/100g (Kuskoski *et al.*, 2005), it can be seen that crem represents a low source of phenolic compounds.

Bianchin and Carpes (2012), found higher values compared to crem in the evaluation of the antioxidant activity with IC₅₀ of 356.67 µg/mL in ethanol extracts of rosemary (*Rosmarinus officinalis*). Lower values IC₅₀ of 80.482 µg/mL were reported in study of Jain and Bhagia (2016) in aqueous extract of fresh oregano (*Origanum vulgare* L.) leaves. According with Roesler *et al.* (2007), an extract that has high potential in sequestering free radicals has a low IC50 value.

In table 2, the total phenolic compounds present are kaempferol, luteolin, quercetin, coumarin, rutin, caffeic acid, gallic acid and chlorogenic acid. The flavonoid found in the greatest quantity was kaempferol with 15.62 mg/g. A lower value than this (10.65 mg/g) was reported by Bae *et al.* (2012) in relation to hydroalcoholic extract of pepper (Habanero variety). Justesen and Knuthsen (2001) found 0.12 mg/g in fresh spring onion (*Allium schoenoprasum*). Kong *et al.* (2013) reported that

kaempferol was effective against atherosclerosis caused by high cholesterol levels in rabbits, possibly because it improved the antioxidant capacity of the molecules.

In a study by Rao *et al.* (2012), luteolin induced apoptosis in cancer cells without affecting the physiological function of the drug transporters in body tissues. Luteolin was evaluated by Kruma *et al.* (2008) and was found in lesser amounts in commercial oregano (6.4 mg/g) and thyme (4.7 mg/g) samples than the value for crem found in the present study (12.7 mg/g).

The content of quercetin in the present study was 10.45 mg/g. Hu *et al.* (2015) found that, in animal studies, quercetin can protect gastric epithelial cells from oxidative damage and also improve the production of reactive oxygen species. This may be attributed to the inhibition of oxidative stress, the regulation of mitochondrial dysfunction, the initiation of antioxidant defense, and the inhibition of apoptosis. Lower values for quercetin (0.34; 0.47 and 0.45 mg/g) were found in three samples of Mexican oregano (*Lippia graveolens* H.B.K) in a study by Lin *et al.* (2007).

In the present study, the crem contained 6.38 mg/g of coumarin, which has been attributed with anticoagulant, estrogenic, photosensitizing, antimicrobial, vasodilatory and anthelmintic properties, among others (Hoult and Payá, 1996; Ojala, 2001). A study by Singh *et al.* (2014) found induction of apoptosis in the use of coumarin in cervical cancer cells. The coumarin content found in the present study was higher than that described in a study by Alvarenga *et al.* (2009) of green guaco leaves at a concentration of 0.52% (5.20 mg/g), which is used as a tea in folk medicine.

The rutin content found in the crem was 0.84 mg/g. Rutin has been found to possess various

Table 2. Phenolic compounds in crem (*Tropaeolum pentaphyllum* Lam).

Compounds	Crem (<i>Tropaeolum pentaphyllum</i> Lam)		LOD µg/mL	LOQ µg/mL
	mg/g	%		
Kaempferol	15.62 ± 0.01 ^a	1.56	0.015	0.049
Luteolin	12.17 ± 0.03 ^a	1.21	0.035	0.115
Quercetin	10.45 ± 0.01 ^a	1.04	0.013	0.042
Coumarin	6.38 ± 0.02 ^b	0.63	0.007	0.021
Rutin	0.84 ± 0.01 ^c	0.08	0.027	0.115
Caffeic acid	6.15 ± 0.02 ^b	0.61	0.018	0.059
Gallic acid	4.97 ± 0.03 ^a	0.49	0.023	0.076
Chlorogenic acid	4.73 ± 0.01 ^a	0.47	0.009	0.034

*Values expressed as mean ± standard deviation, n=3. Different letters in the column indicate significant differences (p < 0.05) by Tukey's test.

pharmacological effects, including antibacterial, antitumor, anti-inflammatory, anti-diarrhoeal, antiulcer, anti-mutagenic, immunomodulatory, hepatoprotective and vasodilatory properties, as well as providing myocardial protection (Janbaz *et al.*, 2002). Compared to the present study, a higher concentration of rutin (10.82 mg/g) was detected in a study of rosemary leaves (*Rosmarinus officinalis* L.) (Frescura *et al.*, 2013).

The phenolic acid that was identified in the greatest concentration in the crem was caffeic acid, followed by gallic acid and chlorogenic acid.

The concentration of caffeic acid found in the crem was 6.15 mg/g. This is very relevant considering that caffeic acid has fungicidal action and that some studies also report that it may have anticarcinogenic action (Olthof *et al.*, 2001; Vicente *et al.*, 2009). Caffeic acid also presents antioxidant, immunomodulatory and anti-inflammatory activity, which may act to reduce oxidative stress (Vicente, 2009). Higher values for caffeic acid (12.93 mg/g) were found by Frescura *et al.* (2013) in a study of rosemary leaves (*Rosmarinus officinalis* L.).

The content of gallic acid found in the crem was 4.97 mg/g. This was higher than the value found in marjoram (*Origanum majorana* L.) (2.90 mg/g) in a study by Roby *et al.* (2013). Gallic acid is attributed with anticarcinogenic, anti-microbial and anti-viral properties plus potent antioxidant activity; it also scavenges reactive oxygen species (Ow and Stupans, 2003; Savi *et al.*, 2005).

The content of chlorogenic acid found in the crem was 4.73 mg/g, which was a higher level than that found in sage (12.20 mg/g) and lower than found in marjoram (2.50 mg/g) (Roby *et al.*, 2013). Several studies have shown that chlorogenic acid has a preventive role in the development of human colon cancer and that it inhibits the proliferation of different

lineages of tumor cells (McCann *et al.*, 2007; Kurata *et al.*, 2007; Jaganathan *et al.*, 2009; Janicke *et al.*, 2011).

Conclusion

It could be concluded that the crem presents considerable fiber content. Regular consumption of this food in the diet or added to products can provide a healthier diet and attribute flavor once it is used as a condiment. From the results of phenolic compounds and antioxidant activity can be inferred that the crem presents low contents of the same, compared to other foods. Ingestion of crem can confer health benefits, which until then were unknown to the population. Although it exhibits low amounts of flavonoids, some stand out like kaempferol and caffeic acid compared to other condiments and it becomes an alternative as a condiment in cooking.

Acknowledgement

The authors wish to thank the Coordination of Improvement of Higher Education Personnel (CAPES) for supporting this research.

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