

REGULAR ARTICLE

Chemical composition and antioxidant ability of the crude extract of *Sedum praealtum* flowers

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Abstract

As part of our screening of antioxidant extracts from spices and food plants, the antioxidant activity of the EtHO extract of the flowers of aerial parts of *Sedum praealtum* was tested. Since the extract showed significant activity, a bioassay-directed fractionation of it was carried out. This investigation resulted in the isolation of kaempferol and quercetin as the principle responsible for the antioxidant activity. The antioxidant ability was quantified using the Fogliano method, which is known as DMPD. Flavonols which were characterized by NMR. This similar antioxidant effect has been obtained on some fruits studied by different authors using this same method.

Key words: Antioxidant activity, DMPD, *Sedum praealtum*

Introduction

During the history, the use of plants has had important variations. Their efficiency as therapeutic agents has been demonstrated to be base of the manufacturing of drugs utilized in medicine. In anaerobic organisms it is necessary and essential to reduce the doses of oxidants in different process as: growth cellular differentiations, immune response among others. When oxidative stress is created, it can cause cell damage that can lead to death. There is a growing interest in relation to health care, and in the search for new potential sources of natural antioxidants that would be able to stop or delay the oxidation of substrates in a chain reaction, and

which are very important to increase the resistance of many diseases, such as: phenolic compounds, occurring in most vegetable (Halliwell et al., 1995). Antioxidants play a significant role in the prevention of diseases, and have a capacity to reduce oxidative stress by chelating trace elements or scavenging free radicals and protecting antioxidant defenses (Prior et al., 1998; Kaur and Kapoo, 2002). For this reason, it is important to search for alternatives with antioxidant activity, as spices with secondary metabolites with antioxidants activity.

The flowers of *Sedum praealtum* D.C. are used in traditional medicine as an anti-inflammatory agent. The antioxidant ability was quantified using the Fogliano method, which is known as DMPD. Alkaloids, flavonoids, and sugars were identified in the ethanolic extract, and kaempferol and quercetin were isolated and characterized by NMR.

Material and Methods

Plant material

Flowers of *Sedum praealtum* were collected (10 kg) in December 2012 in the city of Texcoco,

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Mexico State, Mexico, from which it was obtained a crude extract and were identify secondary metabolites (Domínguez, 1973).

General

^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury-300 (300 MHz and 75.4 MHz) (Varian, Palo Alto, CA, USA) instrument, using CD_3OD , as a solvent and trimethylsilane (TMS) (Aldrich Chemical Co., Milwaukee, WI, USA) as internal standard.

Isolation of the compounds

The ethanol extract (7.5 g) of *S. praealtum* was applied to a Silica gel G 60 column (100 g) using methanol-acetone eluent as a flow rate of 1 mL. It was collected 42 fractions of 10 mL, and grouped into ten major fractions (1-10); by a thin layer chromatography analysis on Silica gel F₂₅₄ gel coated glass sheds development with methanol acetone 80:20. Fraction 7 (60.8 mg) and 8 (52.5 mg) contained kaempferol and quercetin, and were analyzed by mass spectrometry and NMR.

Total phenolics

A mashed sample of flowers crude extract was extracted in 2% HCl in methanol for 24 h in the dark and at room temperature. The extract was diluted with the same solvent used for extraction, to a suitable concentration for analysis. Total phenolics were measured according with to the Folin-Ciocalteu reagent method (Singleton and Rossi Jr., 1965; Pastrana-Bonilla et al., 2003). Two hundred microlitres of sample extract was introduced in a test tube, 1.0 mL of Folin-Ciocalteu reagent and 0.8 mL of sodium carbonate (7.5%) were added, and the contents were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of sample, used a standard curve generated with 100, 200, 300, and 400 mg/L of gallic acid.

Measurements of antioxidative ability by the DMPD method

DMPD, 100 mM, was prepared by dissolving 209 mg of DMPD in 10 mL of deionized water; 1 mL of this solution was added tom 100 mL of 0.1 M acetate buffer, pH 5.25 and the colored radical cation ($\text{DMPD}^{\bullet+}$) was obtained by adding 0.2 mL of a solution of 0.5 M ferric chloride (final concentration 0.1 mM). One milliliter of this solution was directly placed in a 1-mL plastic cubette and its absorbance al 505 nm was measured. An optical density of 1.022 unit of absorbance, which represents the inhibited signal,

was obtained. Standard solutions of the different antioxidant compound were prepared as follows: 1 mg/mL of ascorbic acid was prepared by dissolving 0.1 g of ascorbic acid in 100 mL of deionized water; 1 mg/mL de TROLOX was prepared by dissolving 0.1 g de TROLOX in 100 mL of methanol.

A dose-response curve was derived for TROLOX, ascorbic acid, by plotting the absorbance at 505 nm as percentage of the absorbance of the uninhibited radical cation solution (blank) according to the equation

$$\text{Inhibition of } A_{505} (\%) = (1 - A_f/A_0) \times 100 \quad (1)$$

where A_0 is the absorbance of inhibited radical cation and A_f is the absorbance measured 10 min after the addition of antioxidant samples.

The antioxidant ability of the samples was expressed as TEAC (TROLOX equivalent antioxidant capacity) using the calibration curve plotted with different amounts of TROLOX (Figure 1), and as VCEAC (vitamin C equivalent antioxidant capacity) using the calibration curve plotted with different amounts of ascorbic acid Figure 2.

Spectrometric measurements were recorder by using a GenesysTM 10 spectrophotometer.

Treatment of the sample

5 g of crude extract of the flower of *Sedum praealtum* were added with 100 mL aqueous methanol (80%), it was allowed to soak at room temperature for 24 h, the methanolic extract was vacuum filtered through a Whatman filter paper # 1, it was taken an aliquot of 50 μL from the filtrate, the aliquot was added with 950 μL of the dye of $\text{DMPD}^{\bullet+}$, it was stirred for 10 min, and the absorbance was read at 505 nm.

Statistical analysis

Samples were analyzed in triplicate and results are given as average \pm SD. Statistical analysis was done with software SigmaStart to identify differences between groups, antioxidant capacity of crude extracts at $p < 0.01$ significant level.

Results

In the crude extract of the flowers of *S. praealtum* there were identified alkaloids, flavonoids, coumarins and reducing sugars, secondary metabolites found in the same genus and a high antioxidant activity was found (Pastrana-Bonilla et al., 2003; Fogliano et al., 1999). Two compounds were identified and characterized as kaempferol and quercetin. The structures of the isolated compounds were established by ^1H and ^{13}C

NMR data and confirmed by comparison with those reported in the literature, (Guvenalp, 2005; Chien-Chang et al., 1993).

The results of the quantification of the phenolic compounds in the crude extract of *S. praealtum* analyzed are shown in Table 1, expressed as gallic acid equivalents (GAE) in milligrams per 100 milligrams of extract.

Table 1. Total phenolics content in crude extract of *S. praealtum*.

<i>S. praealtum</i> extract	<i>S. praealtum</i> extract (mg GAE/100 mg extract)
1	718.03
2	688.03
3	659.21
Mean	688.42± 21.4

GAE = gallic acid equivalents

Antioxidant capacity of the crude extract of the flowers *S. praealtum* by the DMPD Method

The results of the dose-response curve obtained by using TROLOX are shown in Figure 1. The standard deviation is very low and the dose-response curve is highly reproducible.

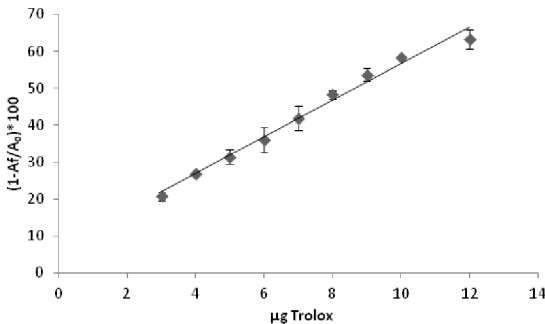


Figure 1. Degree of inhibition of the absorbance at 505 nm, as a function of the TROLOX concentration (n=3).

Inhibition of the absorbance at 505 nm is linear between 0.2 and 12 µg of TROLOX. The relationship calculated within this range of the standard compounds is

$$A_{505}(\text{inhibition}) = 4.6 (\mu\text{g of TROLOX}) + 7.0 \quad (2)$$
$$r^2 = 0.987$$

The results of the dose-response curve obtained by using ascorbic acid are shown in Figure 2.

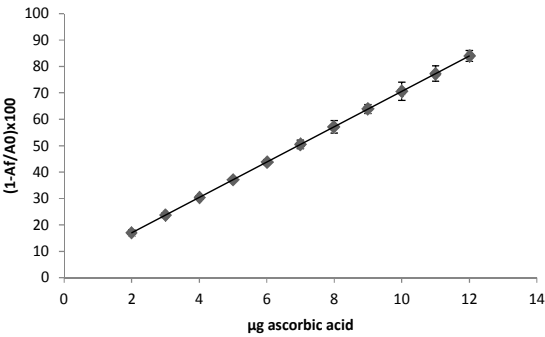


Figure 2. Degree of inhibition of the absorbance at 505 nm as a function of ascorbic acid concentration (n=3).

Inhibition of the absorbance at 505 nm is linear between 0.2 and 12 µg of ascorbic acid. The relationship calculated within this range of the standard compounds is:

$$A_{505}(\text{inhibition}) = 6.70 (\mu\text{g of ascorbic acid}) + 3.63 \quad (3)$$
$$r^2 = 0.997$$

Table 2 shows the antioxidant capacity data of the crude extract of *S. praealtum* expressed as TEAC (TROLOX equivalent antioxidant capacity) and as VCEAC (vitamin C equivalent antioxidant capacity) in crude extract of *S. praealtum* flowers

Table 2. Antioxidant capacity of the crude extract of the flowers *S. praealtum* expressed as TEAC and as VCEAC by the DMPD method.

<i>S. praealtum</i> crude extract	TEAC (µM TE/g extract) A ₀ = 1.022	VCEAC (µM VCE/ g extract)
1	97.74	108.57
2	100.01	110.96
3	100.70	111.70
Mean	99.48 ± 1.55	110.40 ± 1.62

TEAC (TROLOX equivalent antioxidant capacity), VCEAC (vitamin C equivalent antioxidant capacity), TE (TROLOX equivalent), VCE (vitamin C equivalent)

The antioxidant activity founded in the *Sedum praealtum* (0.90 µM TROLOX/g FF) are in the same order of activity reported in some plant extracts such as mango 1.57, eucalyptus 0.69, and potato 0.80 µM (Pellegrini, 2003; Pretel, 2007).

Discussion

The present study was carried out to evaluate the antioxidant effect of ethanolic extract of flowers of *Sedum praealtum* DC (crude extract), from the extract, two flavonols were isolated which were

characterized by NMR as kaempferol and quercetin.

In the crude extract of the flowers of *S. praealtum* there were identified alkaloids, flavonoids, coumarins and reducing sugars, secondary metabolites found in the same genus and a high antioxidant activity was found (Pastrana-Bonilla et al., 2003; Fogliano et al., 1999).

The structures of the isolated compounds were established by ^1H and ^{13}C NMR data and confirmed by comparison with those reported in the literature. The two flavonoids characterized are: 3,5,7-trihydroxy-2-(4-hydroxy-phenyl)-chromen-4-one (kaempferol) and 2-(3,4-dihydroxy-phenyl)-3,5,7-trihydroxy-chromen-4-one (quercetin). The signals of NMR are as follows: Signals at 6.18 (d, $J = 1.7\text{ Hz}$, 1H, H_6) and 6.40 (d, $J = 1.9\text{ Hz}$, 1H, H_8), are typical of flavonoid nucleus so they are assigned to meta-coupled protons of A-ring (H-6 y H-8) of a flavonoid nucleus, while chemical shifts at 6.89 (d, $J = 8.4\text{ Hz}$, 1H, H_5), 7.55 (d,d, $J = 8.3\text{ Hz}$, 2.2 Hz , 1H, H_6), and 7.68 (d, $J = 2.2\text{ Hz}$, 1H, H_7) are due to H-5 , H-6 y H-7 of the C ring.

The spectra of ^{13}C NMR shows a low field signal at 176.5 due to the carbonyl group at C-4, complete assignment was aided by comparison of the observed NMR spectral data with that reported in literature (Guvenalp, 2005; Chien-Chang et al., 1993).

Quercetin is a plant pigment, has the structure to act as powerful antioxidant. It is found in many plants and foods. People use quercetin as a medicine (UMMC, 2012). It could be key in fighting several chronic degenerative diseases, circulation problems and preventing cancer (Bouktaib et al., 2002; Janisch, 2004). The oxidation of LDL is believed to play an important role in the pathogenesis of atherosclerosis and hence the risk of cardiovascular disease. Chopra et al. (2000) studied the effect of nonalcoholic red wine extract and one of its constituents, quercetin, on the oxidative resistance of LDL. Quercetin, when given on its own also inhibited LDL oxidation. The red wine extract and quercetin supplements had the same effect.

Previous studies have shown that flavonoids, in particular quercetin, can lower lipid concentrations in hyperlipidemic rats (Yugarani, 1992). It has been reported that quercetin acts like an antihistamine and anti-inflammatory, and may help protect against heart disease. It is also used for diabetes, cataracts, hay fever, peptic ulcer, asthma, schizophrenia, viral infections, chronic fatigue syndrome, and for treating chronic infections of the prostate. The antioxidant activity of quercetin's

metabolites and the pathways of metabolic conversion need to be identified and evaluated to accurately determine the effect quercetin has *in vivo* and its effectiveness in preventing diseases arising from oxidative damage (Bentz, 2009; Bouktaib et al., 2002). An 8-year study found the presence of three flavonols: kaempferol, quercetin, and myricetin, in a normal diet were associated with 23% reduced risk of pancreatic cancer, a rare but frequently fatal disease, in tobacco smokers (Nöthlings et al., 2007). There was no benefit in subjects who had never smoked or had previously quit smoking.

Kaempferol is a yellow crystalline solid (Calderon-Montaña et al., 2011), who has been identified in many plant species commonly used in traditional medicine, and also it can be isolated from many foods, and it has been identified in many edible plants (Park et al., 2006).

Kaempferol gives the flowers of *Acacia decurrens* and *Acacia longifolia*, and lotus (*Nelumbo nucifera*), among others their color (Hosseinzadeh et al., 2007; Aung et al., 2002). The petal color of the *Sedum praealtum* flowers may be due to its content of kaempferol and quercetin (Deng, 2013).

Like quercetin, some epidemiological studies have found a positive association between the consumption of foods containing kaempferol and a reduced risk of developing several disorders such as cancer and cardiovascular diseases. Numerous preclinical studies have shown kaempferol and some glycosides of kaempferol have a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, neuroprotective, antidiabetic, anxiolytic, antiosteoporotic, estrogenic/antiestrogenic, and antiallergic activities (Klaus-Peter, 1999; Calderon-Montaña et al., 2011). Antidepressant properties have been reported in tests on animals (Hosseinzadeh et al., 2007; Hou et al., 2010).

Increased intake of flavonoids from food is associated with a reduced risk of developing cancer, cardiovascular disease and stroke. The crude extract from the flowers of *S. praealtum*, might have a role in the prevention of degenerative diseases in humans, especially in developing countries where economic resources are low.

Total phenolics content

The value of total phenolics of the extract obtained from flowers of *S. praealtum* was 688.42 mg GAE/100 mg, these results are comparable to those obtained in the crude extract of *S. sediforme*

which has a total phenolic content of 605.1 mg GAE/100 mg (Guvenalp, 2005; Chien-Chang et al., 1993; Sakar, 1993), so it should be noted that the values obtained in this genus of plants are high in phenolic content in its composition.

Total phenols found in some fruits are 527.2 mg GAE/100 g in cranberry, 417.56 mg GAE/100 g in blueberry (Sun, 2002; Sellapan, 2002), the apple has a phenolics content of 296.3 mg GAE/100 g; taking into account these values, we can consider that the extract of *S. praealtum* has an important amount of total phenolic content. The phenols found in the results, should be taken into account when considering its antioxidant capacity.

The value of total phenolics compounds of the extract obtained from flowers of *S. praealtum* (688.42 mg GAE/100 mg) was generally higher than those of fruits and seeds with high content of these compounds, since it is a concentrated extract.

The consumption of foods with high total phenolic compounds could provide a better protecting effect against free radicals than berries. The crude extract of the flowers of *S. praealtum* can be used to obtain a protective effect against free radicals, due to their high content of total phenolic compounds (688.42 mg GAE/100 mg).

Antioxidant capacity (AC) of the crude extract of the flowers *S. praealtum* by the DMPD Method

According to the results shown in Table 2, it can observe that the AC values expressed as TEAC in the crude extract of *Sedum praealtum* flowers are in the range of 99.74 to 100.70 $\mu\text{M TE/g}$ of crude extract.

Another way to express the AC is like the equivalent antioxidant capacity of vitamin C (VCEAC) that give higher values than those reported with TEAC so that vitamin C is more abundant in nature than vitamin E which is the TROLOX, and values vary between 108.57 to 111.7 $\mu\text{M VCE/g}$ of crude extract. Although it is more common to report the antioxidant capacity, as TEAC than, VCEAC.

In the scientific literature, there are publications which report the AC of various plants by various methods, in addition DMPD method, such as of DPPH and ABTS. It has been reported that the results obtained with these methods are very similar between each other, and it is valid to compare the results because there are no significant differences (Kuskoski et al., 2005).

There are reported studies of the antioxidant capacity of the fruits of grape and acerola 46.6 and 3.6 $\mu\text{M TROLOX/g}$ sample respectively (Kuskoski

et al., 2005), determined by the method of DMPD. The antioxidant capacity the flowers' extract (99.48 $\mu\text{ET/g}$) was greater than those of the fruits mentioned above: This is because it is part of an extract containing a higher concentration of metabolites, which are found in the whole fresh flower (FF). In the whole FF, those values would be 100 times lower (0.99 $\mu\text{M/g FF}$).

AC values of muscadine grapes (*Vitis rotundifolia* Michx.), following the ABTS method (Pastrana-Bonilla et al., 2003) were, on average, 2.4, 12.8, 281.3, and 236.1 $\mu\text{M TE/g}$ of FW (fresh whole fruit), for pulps, skins, seeds, and leaves respectively. Seed and leaves showed a higher AC, more than twice of those founded in the crude extract of flowers. Meanwhile the pulp and the skin antioxidant values were lower than the value of the crude extract of flowers. The value reported of the antioxidant capacity of the skins of muscadine grapes (12.8 $\mu\text{M TE/g}$ of FW) was very close to those obtained in the fresh flower of *S. praealtum* (0.90 $\mu\text{M TE/g FF}$).

The pitaya cactus (*Stenocereus stellatus* Riccobono) fruit was studied, as potential nutraceutical food. The antioxidant capacity (Fogliano, 1999), displayed by the four pitaya types studied, red, cherry, yellow and white, for the four pitaya types TEAC varied between 11 and 17.3 $\mu\text{mol/g}$ (Beltrán-Orozco, 2009) are similar to those reported for some fruits of the *Vaccinium* genus, regarded as the fruits having the highest antioxidant capacity (13.9-17.0 $\mu\text{mol TE/g}$ of edible sample) (Prior et al., 1998), it is comparable to: cabbage (*Brassica oleracea*) 17.7 $\mu\text{mol TE/g}$ of edible sample; strawberry (*Fragaria ananassa*) 15.4 $\mu\text{mol TE/g}$ edible sample; and spinach (*Spinacia oleracea*) 12.6 $\mu\text{mol TE/g}$ edible sample (Pellegrini, 2003). All these values of AC, were below those of the AC crude extract of *S. praealtum* (99.74 to 100.70 $\mu\text{M TE/g}$), but higher than the AC found in fresh flower (0.90 $\mu\text{M TE/g FF}$).

The antioxidant activity found in some plant extracts found values of *Mangifera indica* (mango) 1.57, and *Eucalyptus globules* (eucalyptus) 0.69 $\mu\text{M DPPH/dry sample}$, those values are in the same order than the antioxidant activity founded in the *Sedum praealtum* (0.90 $\mu\text{M TROLOX/g FF}$). Therefore, the flower has an acceptable antioxidant activity.

The AC of the oil obtained from four varieties of chia seed (*Salvia hispanica* L.) from the State of Puebla and the State of Colima, Mexico, as well as, the corresponding deffated fractions of chia seed.

The AC of chia oil was between 1.32 to 4.58 $\mu\text{mol TE/g}$ of sample, while the defatted fraction was between 45.56 to 98.73 $\mu\text{mol TE/g}$ of sample. The fraction defatted chia seed proved to be a rich source of antioxidants compared to certain fruits like cranberry and pomegranate (Beltrán-Orozco, 2011; González-Jiménez, 2012).

In summary the AC values of the crude extract of *Sedum praealtum* flowers are in the range of 99.74 to 100.70 $\mu\text{M TE/g}$ of crude extract. The values found in *Sedum praealtum* flowers are appropriate values, as they are higher than those reported in some common foods like potato that has a value of 0.80 $\mu\text{M TROLOX/g}$ per sample.

Most fruits ingested in the diet, have antioxidant capacity values in the range of 0.64 to 20.24 $\mu\text{M TROLOX/g}$ per sample (Pellegrini, 2003).

Most of the vegetables in the food ingested daily have antioxidant capacity values in the range of 0.30 to 8.49 $\mu\text{M TROLOX/g}$ sample, 76% of vegetables daily consumption does not stand up to 10 $\mu\text{M TROLOX/g}$ sample (Pellegrini, 2003).

The antioxidant capacity of a compound, mixture, or food, is given not only by the sum of the antioxidant capacities of each of its components, but also by the microenvironment in which the compound is located. The compounds interact with each other, and may produce synergistic or inhibitory effects (Kuskoski et al., 2005). The antioxidant capacity of *Sedum praealtum* flowers may be due to their content of ascorbic acid, and their phenolic compounds (quercetin and kaempferol).

Conclusion

The use of crude extract of the flowers could contribute in the prevention of the occurrence of most degenerative diseases due to its high content of total phenolics compounds, and, its high antioxidant activity, which provides a protecting effect against the proliferation of free radicals.

The antioxidant activity of fresh flowers of *Sedum praealtum* is between the range of most fruits and vegetables, thus *Sedum praealtum* flowers can be considered as a potential nutraceutical resource.

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