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The influence of planting and harvesting times on the total phenolic content and antioxidant activity of *Talinum triangulare* (Jacq.) Willd

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ABSTRACT. The purpose of this study was to evaluate the influence of planting and harvesting times on the polyphenol content and antioxidant capacity of *Talinum triangulare* cultivated during two different seasons (winter or summer) and harvested 30 or 60 days after seedling establishment. Polyphenol content was quantified with the Folin-Ciocalteu method, and antioxidant activity was quantified with free-radical DPPH (1,1-diphenyl-2-picrilhidrazina). The highest levels of polyphenols were obtained from winter planting and from harvesting at 30 days. Antioxidant activity differed significantly in response to variation in planting and harvesting times, reaching 56.97% in extracts of plants produced in the winter and harvested at 30 days after planting. The times of planting and harvesting markedly influenced the content of polyphenols, and thus the antioxidant activity, of *T. triangulare*.

Keywords: portulacaceae, waterleaf, DPPH, polyphenol.

Efeito da época de plantio e colheita sobre o teor de fenólicos totais e atividade antioxidante de *Talinum triangulare* (Jacq.) Willd

RESUMO. O objetivo deste estudo foi avaliar a influência das épocas de plantio e colheita no teor de polifenóis e capacidade antioxidante de *Talinum triangulare* cultivadas em duas estações do ano (inverno e verão) e coletadas 30 e 60 dias após plantio. A quantificação dos polifenóis foi realizada pelo método de Folin-Ciocalteu e a atividade antioxidante por DPPH. Níveis elevados de polifenóis foram obtidos na semeadura de inverno e colheita em 30 dias. A atividade antioxidante variou significativamente entre as épocas de plantio e colheita de plantas, chegando a 56,97% nos extratos das plantas produzidas no inverno e colhidas aos 30 dias após a semeadura. Os dados mostraram que a época de plantio e de colheita influenciou o teor de polifenóis e, por conseguinte, a atividade antioxidante de *T. triangulare*.

Palavras chave: portulacaceae, beldroega-graúda, DPPH, polifenóis.

Introduction

Phenolic compounds exhibit different biochemical and pharmacological properties (KANG et al., 2010), and the total antioxidant activity of fruits and vegetables is related to their phenolic content (OBOH; ROCHA 2007). Natural polyphenols produce health benefits through their antioxidant activity. These compounds are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, reducing α-tocopherol radicals and inhibiting oxidases (AMIC et al., 2003). Interest in the benefits produced by polyphenols has increased in recent years because of the proven antioxidant capacity of these compounds (JACOBO-VELAZQUEZ; CISNEROS-ZEVALLOS, 2009). The literature reports beneficial effects of antioxidants in preventing various diseases caused by or related to excess free radicals in the human body. These diseases include cancer, hypertension, heart disease and diabetes (VALKO et al., 2007). Antioxidants are able to stabilize or deactivate free radicals before they attack biological targets in cells, reducing oxidative stress and subsequent tissue destruction (ATOUI et al., 2005).

Many researchers have studied the content of phenolic metabolites in medicinal plants, and the results of these studies have confirmed the hypothesis that these compounds contribute significantly to the antioxidant activity (BOUAYED et al, 2007; KATALINIC et al., 2006, OBOH, 2006) and nutritional properties (IWALEWA et al., 2005; ODUKOYA et al., 2007) of the plants. Studies have also shown that the content of phenols may vary according to the growth stage, the part of the plant

and the characteristics of the environment (FREITAS et al., 2004; PEIXOTO SOBRINHO et al., 2008; YARIWAKE et al., 2005). Antioxidant enzymes (produced by the body) and antioxidant nutrients (found in foods) can scavenge/deactivate reactive free radicals, transforming them to harmless substances (CHU et al., 2002). The most likely and practical way to fight degenerative diseases is to improve the body's antioxidant status. This goal can be achieved through the increased consumption of vegetables and fruits (OBOH; ROCHA 2007).

Talinum triangulare (Jacq.) Willd (Portulacaceae) is an herb with fleshy green leaves, a succulent stem and pink flowers, known in Brazil as "*beldroega-graúda*", "*major-gomes*", "*maria-gorda*" and "*erva-gorda*" and by other common names (KISMANN; GROTH, 1995). It is an all-season vegetable. In West Africa, it is grown primarily from seed or by vegetative propagation (FASUYI, 2007; IMOH; JULIA, 2000). It is widely cultivated as a medicinal and food crop in West Africa, Asia, and South America, especially in Nigeria, where it is known as waterleaf (its common English name). It is presently one of the most important sources of employment opportunities for the majority of the unemployed youths and women in the rural population. During the past two decades, its cultivation has attracted considerable attention because of its general acceptability by consumers and its export potential (NYA et al., 2010). The whole plant is used to treat a variety of diseases, including hepatic ailments (LIANG et al., 2011). *T. triangulare* leaves are used in folk medicine to treat diuretic and gastrointestinal disorders (MENSAH et al., 2008), measles and diabetes, as a laxative and in healing (AGRA et al., 2008). In Africa, the plant is used intensively and concomitantly with allopathic medicines in the opportunistic treatment of diseases by patients and is used by healthy people to prevent disease, based on its ability to increase stamina and to act as an immunostimulant (AGBONON et al., 2009). A previous study has shown that aqueous extracts of *T. triangulare* possess remarkable antioxidant activity, and the flavonoid content of extracts of the plant has been measured (ANDARWULAN et al., 2010; ODUKOYA et al., 2007; YANG et al., 2006).

Research and study of plants that may be of interest for the production of compounds with therapeutic properties are required to facilitate preservation, cultivation and pharmacognostic validation. In view of the potential of *T. triangulare* as food and in medicine, the objective of this study was to evaluate the influence of planting and harvesting times on the total polyphenol content and antioxidant capacity of the aerial parts of this plant.

Material and methods

Collection of plant material

Samples of *T. triangulare* (Jacq.) Willd (Portulacaceae) were obtained from plants propagated by seed and grown in a greenhouse in two seasons: winter (June-September 2007) and summer (October 2007-January 2008). The trials were conducted in a completely randomized design with four replications in a 2x2 factorial layout (two seasons of planting and two harvest times). The harvest of the plants during the two planting seasons occurred at 30 and 60 days from the establishment of seedlings, i.e., when they were approximately 5 cm in height. The plants were cut close to the ground and the aerial parts dried in a ventilated oven at a temperature below 45°C and then ground in a knife mill to obtain a total of 16 samplecs. A voucher specimen, number 6814, was deposited in the Federal University of Viçosa at the Herbarium VIC.

Quantification of total phenolics

The determination of total phenols in the samples was performed using the Folin-Ciocalteu method according to the methodology described by Andrade et al. (2007), with certain modifications. Powdered samples (1.0 g) were extracted with 200 mL of distilled water and heated under reflux for 30 minutes. The extract was decanted and filtered, and a 10.0 mL aliquot of the filtrate was transferred to a 50 mL volumetric flask. The aliquot was diluted with water to achieve a total volume of 50 mL. Subsequently, 1.0 mL of this dilution was transferred to a test tube, and 7.5 mL of water was then added. The test tube was shaken, and 0.5 mL of Folin-Ciocalteu reagent was added. After three minutes, 1.0 mL of sodium carbonate (15% w/v) was added. After homogenization, the solution was allowed to stand for 30 minutes, and the absorbance was determined at 760 nm in a spectrophotometer. A blank test was performed with distilled water in place of the aqueous extract. All tests were performed in triplicate.

The total content of phenols was determined by interpolating the absorbance of the samples, based on a calibration curve constructed with standard tannic acid (0.05 to 0.30 mg mL^{-1}). The results were expressed in mg g^{-1} extract, in tannic acid equivalents (TAE). The equation of the calibration curve of tannic acid was C = 0.4663 +0.0151 A, with a squared correlation coefficient of r^2=0.998, where C is the concentration of tannic acid and A is the absorbance at 760 nm. All tests were performed in triplicate.

Determination of antioxidant activity

The determination of antioxidant activity followed the DPPH (1,1-diphenyl-2-picrilhidrazina) method according to the methodology described by Andrade et al. (2007), with certain modifications. Extracts from each sample were prepared by maceration in methanol using 10 g of dry sample and 150 mL of methanol, held at room temperature for 48 hours with periodic agitation and then filtered and concentrated in a rotary evaporator under vacuum. The determination of the effective concentration (EC_{50}), the amount of extract needed to reduce 50% of the DPPH radical after the reaction equilibrium, was performed with the extracts obtained from different samples of *T. triangulare*. In this test, dilutions of 10, 30, 50, 80 and 100 μg mL^{-1} of the representative sample were prepared. The EC_{50} was determined from a linear regression of the graphically plotted points. On this plot, the ordinate represented the average antioxidant activity (%) of the samples, and the abscissa represented the concentration of the extract (mg mL^{-1}).

The antioxidant activity of different samples of *T. triangulare* was evaluated by monitoring the consumption of free radical DPPH in contact with solutions of these samples, using concentrations near the previously established EC_{50}. Measurements of the absorbance of the reaction mixtures (2.5 mL of sample or control, with 1.0 mL of DPPH at a concentration of 0.06 mM) were performed at 515 nm 30 minutes after adding the DPPH solution. A mixture of methanol (1 mL) and methanol extract solution (2.5 mL) was used as a blank. All tests were performed in triplicate.

The absorbance values at all concentrations tested were converted to percentage antioxidant activity (AA) with the following equation:

$$\%AA = \frac{ABScontrol - (ABSsample - ABSblank)}{ABScontrol} \times 100$$

where ABScontrol is the absorbance of the methanolic solution of DPPH and ABSsample is the absorbance of the reaction mixture (DPPH + sample).

Statistical analysis

The data were evaluated with an analysis of variance, and the means were compared using a Tukey test at a probability level of 5%.

Results and discussion

Quantification of phenolic compounds

An analysis of variance showed that there was a significant effect ($p \leq 0.01$) of the planting and harvesting times on the content of total polyphenols in *T. triangulare*. The highest levels resulted from winter planting and from harvest at 30 days. Under these conditions, the average phenol content reached 0.192 and 0.186 mg g^{-1} extract, respectively, expressed in tannic acid equivalents (TAE) (Table 1).

Table 1. Content of total polyphenols (mg g^{-1} extract) of *T. triangulare* in different planting and harvest seasons.

Harvest season	Harvest time		Mean
	30 days	60 days	
Winter	0.204	0.180	0.192 ± 0.015[a]
Summer	0.168	0.145	0.157 ± 0.027[b]
Mean	0.186 ± 0.020[A]	0.163 ± 0.031[B]	

In column, means followed by the same lowercase letters and in the row, means followed by the same uppercase letters, are not significantly different at 1% probability by the F test.

Seasonal fluctuations in the chemical composition of *T. triangulare* have not been previously addressed in the literature. The production of polyphenols by *T. triangulare* appeared to be related to not only the season but also the harvest time. Although the extracts from the crop planted in winter showed higher levels of phenols, significant differences were also found for different harvest times, with the levels of phenols higher in plants harvested after 30 days (Table 1).

Similar results have been obtained for *Smilax campestris*, in which phenolic compounds present in the leaves and rhizomes showed well-defined production rates according to the growth stage, with maximum production during the month of July, which corresponds to flowering (RUGNA et al., 2007). Additionally, Rugna et al. (2008) compared young and adult leaves of *S. campestris* and found quantitative differences in the concentration of polyphenols. The concentration for young leaves was 15% higher than that for adult leaves.

Studies of forest plant species in South Africa showed that, in general, the levels of tannins and total phenols were higher in all species during the growing season (SCOGINGS et al., 2004). The mechanism of increased biosynthesis of particular active principles in young leaves is most likely the result of a programmed strategy of plant defense. This defense strategy evolves to produce secondary metabolites to protect the leaves during their normal ontogeny. The tender young leaves are vulnerable to pests and diseases (SCOGINGS et al., 2004; LIU et al., 1998).

Differences in the levels of phenolic compounds relative to environmental characteristics have also been found in other medicinal species. Silva et al. (2007) studied populations of wild and cultivated *Baccharis trimera* and concluded that higher levels of total phenols were found between May and October

in the cultivated population and between June and September in the wild population. Lower levels of total phenols occurred during the wettest season, with higher temperatures and during the period of intense vegetative growth.

Yariwake et al. (2005) concluded that in *Maytenus aquifolium*, phenolic compounds were produced in lower concentrations in seasons with well-defined photoperiods: winter (short days and long nights) and summer (long days and short nights). In contrast, the production of higher levels of secondary metabolites was correlated with the seasons having less well-defined differences between the duration of day and night (spring and autumn). This trend is consistent with the photoprotective role of phenolics in plant defense (HARBORNE; WILLIAMS, 2000).

Seasonal variations in photoperiod, light intensity and temperature can significantly alter the levels of various phenolic groups in different seasons (YAO et al., 2005), as shown by the results of this work with *T. triangulare*.

Phenolic compounds can protect the human body from free radicals. The formation of free radicals is associated with the normal natural metabolism of aerobic cells. The antiradical activity of flavonoids and phenols is principally based on the structural relationship among the different parts of their chemical structure (RICE-EVANS et al., 1987). The concentration of phenolic compounds in plant tissues varies with the rate of metabolic catabolism and biosynthesis. It can be influenced by hormonal balance, or it can be directly controlled by enzymes and by the balance of the enzyme substrates (SIQUEIRA et al., 1991). Other factors, such as carbohydrates, nutrition and water quality, may influence the biosynthesis of phenolics and the differences in the concentrations and types of phenolic composition of plant species. These effects depend on the growth stage and on the plant part assessed. Stress can also influence the release of polyphenols from vacuoles as well as the new synthesis of phenols (McCONCHIE et al., 1994). For example, groups of phenolics such as ferulic acid and *p*-coumaric acid are commonly found in the roots, while isoflavonoids occur primarily in the leaves and are less prevalent in the petioles, stems and roots (SIQUEIRA et al., 1991).

Antioxidant Activity

The EC_{50} value obtained from the linear equation $y = 0.6309\ x + 10.249$ ($r^2 = 0.9958$) was 63 μg mL^{-1}. This value represented the overall content for the plant regardless of the growing season and harvesting time because it was obtained from an equal mixture of all samples. This value expresses the amount of antioxidant needed to reduce the initial concentration of DPPH by 50% and was used as the basis for the choice of the concentrations of the samples used in the tests. Previous studies (ADEFEGHA; OBOH, 2011; ANDARWULAN et al., 2010; ANYASOR et al., 2010; ODUKOYA et al., 2007; YANG et al., 2006) have demonstrated that *T. triangulare* (Jacq.) Willd. shows antioxidant activity. Liang et al. (2011) have concluded that *T. triangulare* extracts show strong reducing power, hydroxyl radical scavenging activity and superoxide anion scavenging activity.

The antioxidant activity of the plants varied significantly between different planting times and between different harvesting times, reaching 56.97% in extracts of plants produced in the winter and harvested at 30 days after planting. An extract was used with a concentration of 80 μg mL^{-1}, near the EC_{50} obtained for the species (Table 2). This result implied that the profile of the polyphenols of *T. triangulare* may influence the antioxidant activity of the plants because the highest levels of phenolic compounds were obtained in the same samples (Table 1).

Table 2. Antioxidant activity (%) of extracts (80 μg mL^{-1}) of *T. triangulare* plants obtained from two planting dates and two harvest seasons and tested with stable free-radical DPPH methodology.

	Harvest season	
	30 days	60 days
Winter	56.97± 2.754 ^{aA}	34.87± 1.985 ^{bA}
Summer	43.13 ±6.812 ^{aB}	40.23 ± 3.652^{aA}

In columns, means followed by the same lowercase letters, and in the rows, means followed by the same uppercase letters, are not significantly different at 5% probability by the Tukey test.

Several authors have demonstrated a positive relationship between total phenolic content and antioxidant activity in fruits and vegetables (ABIDILLE et al., 2005; KAUR; KAPOOR, 2002), whereas other authors have not found such a correlation (ISMAIL et al., 2004).

Iwalewa et al. (2005) used the method of DPPH antioxidant activity to evaluate the antioxidant capacity of nine plant species consumed in Nigeria and classified *T. triangulare* as a pro-oxidant. The antioxidant and pro-oxidant effects of polyphenols have been described. These findings show contrasting effects of polyphenols on the physiological processes of the cell. As antioxidants, polyphenols may improve cell survival. As pro-oxidants, they can induce apoptosis and prevent tumor growth (LAMBERT et al., 2005). The antioxidant activities of plant phytochemicals result because these chemicals prevent the production of

free radicals, because they neutralize/scavenge free radicals produced in the body or because they reduce/chelate the transition metal composition of foods (MELO et al., 2006; OBOH; ROCHA, 2007).

Furthermore, the results of this study indicate that *T. triangulare* has secondary metabolites capable of capturing free radicals. A positive relationship between the content of polyphenols and antioxidant activity was observed, and higher levels of polyphenols and antioxidant activity were obtained from winter planting and from plants harvested at 30 days.

Conclusion

The biosynthesis of polyphenols in *T. triangulare* was influenced by the planting and harvesting times. A significant increase in the production of these compounds was observed as a result of winter planting and of harvesting at 30 days. These planting and harvesting times could be used to optimize the nutritional or medicinal properties of the plant. These results help to underscore the importance of evaluating the influence of cultivation and harvesting practices on the composition of secondary metabolites of plants. The results of this study demonstrate the possible modulation of antioxidant activity of the plant by agricultural practices because a positive correlation was observed between the content of polyphenols and the scavenging activity of extracts of *T. triangulare*.

As natural substances can confer protection against the risks associated with many disease processes caused by the action of free radicals, the results of this study support the continued study of the antioxidant action of the substances isolated from the ethanolic extract of *T. triangulare*.

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